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Effect of fasting on feed intake, growth and mortality of chinook salmon, *Oncorhynchus tshawytscha*, during an induced *Aeromonas salmonicida* epizootic

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Abstract

Anorexia is a common sign of bacterial and viral diseases and is thought to be a negative consequence of the disease process. However, this inappetence may be an active portion of the host defence system. We tested this idea by withdrawing food for 32 days from juvenile chinook salmon (*Oncorhynchus tshawytscha*) during an *Aeromonas salmonicida* epizootic, induced by cohabitation. Disease-specific mortality was low (5.0% and 12.5% in fed and fasted groups, respectively); there was no mortality in uninfected control fish. While only very few fish had detectable *A. salmonicida* in the kidney, at the termination of the experiment, an average of 18.5% and 65.0% of the fish in fed and fasted groups, respectively, had this bacterium in or on mucus, but these mean values were not statistically different because of high variation between replicates. Feed intake was measured by X-radiography at days 16 (fed groups) and 32 (all groups). Feed intake as well as growth were unaffected by exposure to bacteria. However, food consumption was greater when fasted fish exposed to *A. salmonicida* were offered a meal than in those infected individuals that had been eating. This result may be relevant for application of medicated diet, as it seems possible that fasting

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of sick fish before administration of medicated ration could increase the probability that sick individuals would also eat.

Keywords: *Aeromonas salmonicida*; Fasting; Feed intake; Furunculosis

1. Introduction

Furunculosis, caused by *Aeromonas salmonicida*, is a troublesome and widespread disease of intensive fish farming, causing heavy mortality especially of salmonids. Fortunately, effective antibiotics and vaccines have been developed to control the disease. However, there are drawbacks of using chemotherapy, for example, the possibility of developing antibiotic-resistant strains of bacteria, reduced growth rates (Midtlyng and Lillehaug, 1998), increase of labour, cost of pharmaceuticals, possible decrease of the market value of the meat (Manning, 1998) and a risk of self-injection of the vaccine.

Another possibility for fighting the disease would be promotion of innate immunocompetence. In general, viral and bacterial infections are accompanied by loss of appetite, which is often regarded as an undesirable manifestation of sickness. However, evidence suggests that infection-induced loss of appetite can be an active defence mechanism of the host. This has been documented in mice (Murray and Murray, 1979; Wing and Young, 1980) and in humans (Murray et al., 1978, 1995). It has also been observed that after refeeding famine victims, incidence of infection increased, especially in those with the highest weight gain (Murray and Murray, 1977a,b; Murray et al., 1995). Parenthetically, the virus disease IPN is known to occur more severely in the largest and most rapidly growing fry and fingerlings (Wolf, 1989).

The mechanism of the reduction of the disease during anorexia is not known even though several have been proposed. In mice, starvation has been shown to increase the activation of macrophages (Wing and Young, 1980), number of bone marrow and spleen macrophage progenitors (Wing et al., 1986) and enhance natural killer cell activity (Wing et al., 1983; Nakamura et al., 1990). In addition, several other possible mechanisms of the action of anorexia in disease resistance have been proposed (Murray et al., 1978; Exton, 1997).

Effects of feed deprivation during bacterial infection have also been tested with fish. Fasting during epizootics has been shown to increase survival in some cases (Damsgård et al., 1998b; Wise and Johnson, 1998). Damsgård et al. (1998b) reported only 2% mortality in fasted groups of Atlantic salmon (*Salmo salar*), while fully fed groups had 36% mortality after infection with *Vibrio salmonicida*. Wise and Johnson (1998) found about 87% survival in fasted channel catfish (*Ictalurus punctatus*), while survival of their fed counterparts was 59% during an *Edwardsiella ictaluri* epizootic. On the other hand, decreased survival has been observed in feed-deprived channel catfish infected with *Flavobacterium columnare* (Klesius et al., 1999), while Pirhonen et al. (2000) reported that survival in chinook salmon (*Oncorhynchus tshawytscha*) naturally infected with *Renibacterium salmoninarum* was indifferent to ration level.

Wise and Johnson (1998) suggested that the feeding process itself may increase exposure of the host to bacterial pathogens through ingestion. Enger (1997) also suggested

that one way of enhancing transport of *A. salmonicida* to fish occurs through feed pellets. *A. salmonicida* is a highly hydrophobic bacterium, and high concentrations of the bacteria are usually encountered at the surface film (Enger and Thorsen, 1992). Thus, lipid-rich food pellets that are dropped through the surface will be covered with this film containing pathogens and thereby more readily bring the bacteria into contact with the fish (Enger, 1997).

Plasma or serum cortisol concentration has widely been used as an indicator of stress in fish. Some researches have found no consistent effect of fasting on cortisol levels (Milne et al., 1979; White and Fletcher, 1986; Reddy and Leatherland, 1995; Varnavsky et al., 1995), while others have reported decreased cortisol concentrations when fish have been fasted (Barton et al., 1988; Farbridge and Leatherland, 1992). As stressful situations affect the ability of fish to resist microparasites (Schreck, 1996), it is likely that fasting itself through modulation of stress response would not increase the susceptibility to diseases but rather increase the immunocompetence.

In this paper, we report our findings of the effect of fasting on subsequent feeding, growth and survival during *A. salmonicida* infection in juvenile chinook salmon.

2. Materials and methods

The experiment was carried out at the Salmon Disease Laboratory of Oregon State University in Corvallis between 19 December 1999 and 20 January 2000. Fifty juvenile chinook salmon were acclimated in each of twelve 100-l tanks for 1 month before the start of the study. Well water (13 °C) was supplied to the surface of the tanks at 2 l min^{-1}. Starting on 19 December, food was withdrawn from fish in six of the tanks until the end of the experiment and fish in the other six tanks were fed to satiation. Infection was induced by cohabitation. To ensure the virulence of the bacteria stored in liquid nitrogen, they were first injected into five fish in a separate tank. Three days after injection, these fish were killed and the bacteria from the head kidney were cultivated on agar at 17 °C. Two days later, the plates were washed with saline and a solution at a concentration of 10^9 bacteria ml^{-1} was prepared as estimated by photometry (OD = 1.0 at 540 nm) and used to infect the cohabitants. Thirty cohabitants were adipose fin clipped and bathed in 40 l of *A. salmonicida* solution at a concentration of 10^7 bacteria ml^{-1} for 24 h. On 21 December, five infected cohabitants were added into three of the fasted and three of the fed groups of fish. The naive fish in the experimental tanks will be later referred to as test fish. By these means, we had four different groups with three replicates each: (1) fasted control, (2) fasted infected, (3) fed control and (4) fed infected.

The fish that were fed were fed twice a day (once during the weekends) to satiation with a commercial dry food (Biodry 1000 by Bio-Oregon), which was ground into powder and repelleted. All food had to be repelleted to obtain identical food to that used in feed consumption estimations. A small batch of the ground feed was mixed with X-ray dense lead glass beads (0.8% of weight; ballotini size 9, Jencons, Leighton Buzzard, UK) for the estimation of individual feed intake by X-radiography (Jobling et al., 1993). This was done on days 16 (only fed groups) and 32 (all groups) by netting a sample of 10 fish from each tank right after feeding and killing them by an overdose of buffered tricainemethanesul-

fonate (MS-222), X-raying the fish and calculating the number of beads in each individual from X-ray plates. A blood sample was taken from these fish by caudal severance, and plasma was separated. A pooled sample of plasma from each tank (20 μl of plasma of each individual) was analysed for cortisol by radioimmunoassay (Redding et al., 1984) in duplicate. The lowest standard was 3.9 ng ml^{-1}, and all samples below this standard were designated to contain this same amount of cortisol.

All dead fish were tested for *A. salmonicida* by taking a 10-μl loopful of the head kidney on Coomassie brilliant blue agar plates, resulting in dark blue colonies that turned the medium dark brown, indicating *A. salmonicida* (Cipriano et al., 1992). On each sampling day for estimation of food consumption, all fish killed (10 individuals $tank^{-1}$) from infected tanks were also tested in the similar manner for possible infection. After sampling 10 fish for plasma, kidney and food consumption at the final sampling, the water level in the tank was lowered and the remaining animals were euthanized with an overdose of MS-222 in the tank. Then the tank was carefully drained and the fish were sampled for bacteria in or on the mucus (as described above for the kidney) as well as in the kidney.

As we found only low mortality rates after 3 weeks from the start of the infection, we attempted to induce higher mortalities by purposely stressing the fish on days 22 and 23 for 60 min by lowering the water level in all the tanks to 4–5 cm, so that the backs of fish were exposed to air.

Arcsin transformed data were used to test possible differences between means of percentual data with the Mann–Whitney *U*-test and nested ANOVA was used to assess differences in feed intake and fish weight between the treatments. ANOVA was used to test possible differences in cortisol data between the treatments.

3. Results

We induced furunculosis with low mortality and low level—if any—infection in the kidneys of survivors. None of the fish (total $n = 60$) sampled on day 16 showed evidence of infection in the kidney. At the end of the experiment on day 32, only four individuals (3.8%) were detected with bacteria in the kidney and they all were from fasted groups. We detected bacteria more frequently in mucus; in fed and fasted groups, on average, 18.5% (SD 23, $n = 3$) and 65.0% (SD 40) of the fish, respectively, were found to harbour bacteria in mucus. Given the small sample size (tank being the experimental unit; $n = 3$) and likely because of the large variation between tanks (0–44.4% in fed groups and 20.0–96.2% in fasted groups), these mean values were not statistically different.

In most tanks, cohabitants had died by day 9, that is, 7 days after introducing them into the tanks; a few cohabitants stayed alive a few more days, but all finally died. Mortality among test fish in fed and fasted groups, respectively, was 5% (2.5, 5.0 and 7.5% for replicates) and 12.5% (10.0, 12.5 and 15.0%), these values being statistically significantly different. All dead fish had a high level of infection. No fish died in control tanks during the experiment.

There was no difference in feed intake between the fed groups at day 16, but at the termination of the experiment, groups that had been fasted ate significantly more than the fed groups ($p < 0.05$). Whether or not the fish had been exposed to bacteria had no

effect on appetite (Fig. 1A). Coefficient of variation of feed intake was higher in fed groups (mean ± SD, $n = 3$, for control and infected groups, respectively, 0.64 ± 0.06 and 0.59 ± 0.11) than in fasted groups (control and infected groups, respectively, 0.41 ± 0.18 and 0.46 ± 0.19).

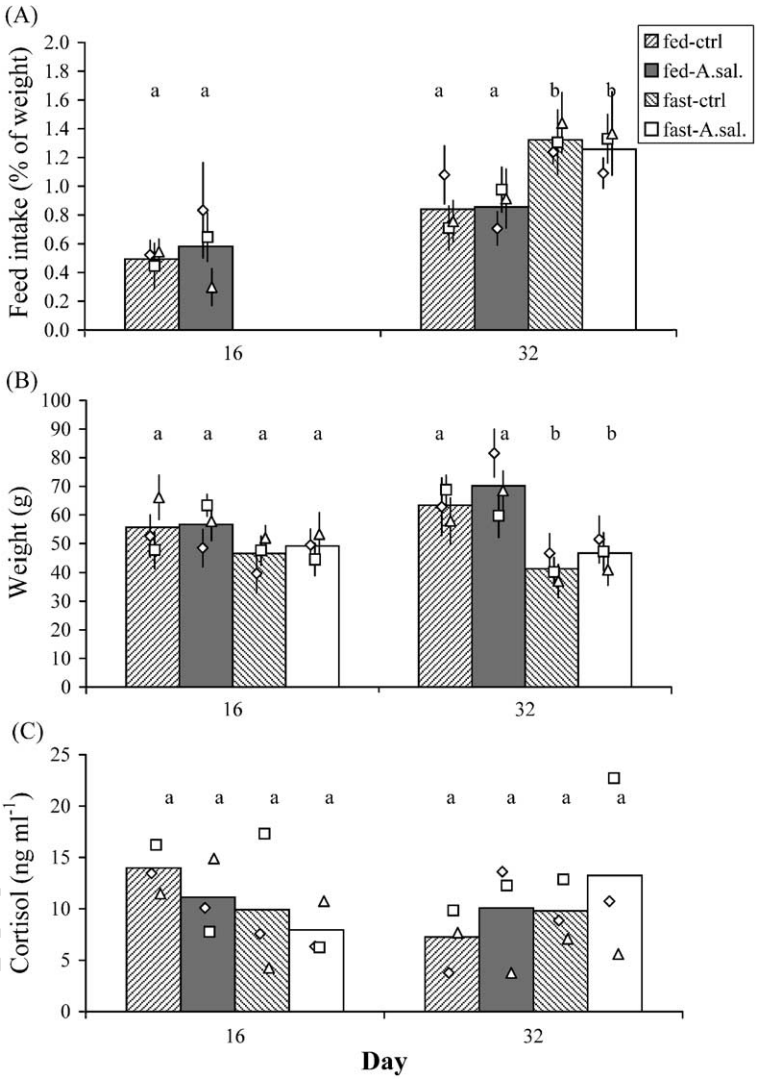

Fig. 1. (A) Feed intake, (B) weight and (C) plasma cortisol of juvenile chinook salmon in fed and fasted groups (ctrl = control groups, A.sal = groups infected with *A. salmonicida*). Bars represent treatment means on days 16 and 32, on top of which individual tank means ($n = 3$) within each treatment are depicted; error bars show S.E. within each tank ($n = 10$) in (A) and (B), pooled samples were used for cortisol (C). Feed intake is based on X-radiography, fasted fish were fed only at the termination of the experiment. Within each day, values that are denoted by different letters are significantly different from each other.

On day 16, there were no statistical differences in size of fish among any of the treatments. At the end of the experiment 16 days later, fasted groups were significantly smaller than fed groups (Fig. 1B). Again, no effect of the exposure to the pathogen was observed.

Plasma cortisol concentrations were low on both sampling days (treatment means between 7.0 and 14.0 ng ml^{-1}) in all groups and no statistically significant differences were found between the treatments (Fig. 1C).

4. Discussion

We only induced low levels of furunculosis in test fish with only a few fish dying although we anticipated high rates of mortality (60–70%) within about 2–3 weeks after exposure to infected cohabitants. We do not know the reason why mortality and infection rates were so low, especially because our fish should have become infected also through the digestive tract as they ate (Enger, 1997). In other experiments with this pathogen also with seemingly well-acclimated animals, high mortality rates have been reported (e.g. Néji and de la Noüe, 1998; Nordmo et al., 1998). Our preliminary experiments suggested (data not shown) that high mortalities should have occurred. In addition, in experiments in our laboratory with chinook salmon fingerlings exposed to the same strain of bacteria by the same route and in similar temperature, mortalities of 60–80% occurred consistently (Ögüt and Reno, unpublished data).

Only a few of our test fish had infection in the kidney, but many individuals had *A. salmonicida* in or on the skin mucus particularly in the fasted groups (20–96%). It is difficult to say why the variation between tanks was so large, but in this type of experiment, one is dealing with not just one but rather two living organisms, both of which induces variability in the system and the variation may be multiplied. Cipriano et al. (1992) reported values similar to ours from groups of lake trout (*Salvelinus namaycush*) and Atlantic salmon undergoing epizootic of furunculosis. They sampled both mucus and kidney and found that 56% of lake trout had *A. salmonicida* on mucus, while only 6% of the same individuals had it in the kidney. In Atlantic salmon, the respective values were 37% and 4%.

While a decrease of about 25% in feed intake in rainbow trout (*Oncorhynchus mykiss*) after *A. salmonicida* infection has been reported (Néji and de la Noüe, 1998), we found that exposing the fish to the bacteria did not decrease feed intake when compared to the control fish, at least not at the group level. As there were no differences in feed intake between infected and control groups, no differences were observed in fish size between these groups. We could not demonstrate reduction in individual level of feed intake in infected fish. We have previously demonstrated an exponential negative relationship between infection levels of *R. salmoninarum* in juvenile chinook salmon and feed intake and that only relatively high concentrations of bacteria really decrease feed intake (Pirhonen et al., 2000). Similarly, Damsgård et al. (1998a) have found that Atlantic salmon infected with infectious pancreatic necrosis virus must exhibit relatively high virus titres before any changes in appetite or growth could be detected. If that is the case with furunculosis, then the relatively low infection levels might explain why no differences were observed in feed intake between infected and control fish.

These low levels of infection, mainly observed as bacteria associated with mucus, did not induce a clinically evident stress response in our fish, as indicated by low levels of plasma cortisol. Our results confirm previous findings (Milne et al., 1979; White and Fletcher, 1986; Reddy and Leatherland, 1995; Varnavsky et al., 1995) that fasting does not affect plasma cortisol concentrations.

5. Conclusion

The results of this experiment as well as those of Pirhonen et al. (2000) indicate that after fasting or food restriction, even infected fish may eat abundantly when offered a ration. These observations may be relevant for application of medicated diet, as it is likely that fasting of a population of sick fish before administration of medicated ration could increase the probability that sick individuals would also eat. Our results showed a decrease in coefficient of variation in feed intake after fasting, indicating a smaller variation in feed intake between individuals in infected groups. Fasting might be advantageous before administration of medicated ration for infected fish to ensure more even distribution of feed amongst the fish.

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References

Barton, B.A., Schreck, C.B., Fowler, L.G., 1988. Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile chinook salmon. Prog. Fish-Cult. 50, 16–22.

Cipriano, R.C., Ford, L.A., Teska, J.D., Hale, L.E., 1992. Detection of *Aeromonas salmonicida* in the mucus of salmonid fishes. J. Aquat. Anim. Health 4, 114–118.

Damsgård, B., Mortensen, A., Sommer, A.-I., 1998a. Effects of infectious pancreatic necrosis virus (IPNV) on appetite and growth in Atlantic salmon, *Salmo salar* L. Aquaculture 163, 185–193.

Damsgård, B., Mortensen, A., Sommer, A.-I., Ugelstad, I., Eliassen, R., 1998b. Appetite and health status in Atlantic salmon. The Feeding Behaviour of Fish in Culture, Second Workshop of the COST 827 Action on Voluntary Food Intake in Fish, 20–22 August 1998, Umeå, Sweden, p. 23.

Enger, Ø., 1997. Survival and inactivation of *Aeromonas salmonicida* outside the host—a most superficial way of life. In: Bernoth, E.-M., Ellis, A.E., Midtlyng, P.J., Olivier, G., Smith, P. (Eds.), Furunculosis, Multidisciplinary Fish Disease Research. Academic Press, San Diego, pp. 159–177.

Enger, Ø., Thorsen, B.K., 1992. Possible ecological implications of the high cell surface hydrophobicity of the fish pathogen *Aeromonas salmonicida*. Can. J. Microbiol. 38, 1048–1052.

Exton, M.S., 1997. Infection-induced anorexia: active host defence strategy. Appetite 29, 369–383.

Farbridge, K.J., Leatherland, J.F., 1992. Temporal changes in plasma thyroid hormone, growth hormone and fatty

acid concentrations, and hepatic 5′-monodeiodinase activity, lipid and protein content during chronic fasting and re-feeding in rainbow trout. Fish. Physiol. Biochem. 10, 245–257.

Jobling, M., Christiansen, J.S., Jørgensen, E.H., Arnesen, A.M., 1993. The application of X-radiography in feeding and growth studies with fish: a summary of experiments conducted on Arctic charr. Rev. Fish. Sci. 1, 223–237.

Klesius, P., Lim, C., Shoemaker, C., 1999. Effect of feed deprivation on innate resistance and antibody response to *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. Bull. Eur. Assoc. Fish Pathol. 19, 156–158.

Manning, M.J., 1998. Immune defence system. In: Black, K.D., Pickering, A.D. (Eds.), Biology of Farmed Fish. Sheffield Academic Press, Sheffield, England, pp. 180–221.

Midtlyng, P.J., Lillehaug, A., 1998. Growth of Atlantic salmon *Salmo salar* after intraperitoneal administration of vaccines containing adjuvants. Dis. Aquat. Org. 32, 91–97.

Milne, R.S., Leatherland, J.F., Holub, B.J., 1979. Changes in plasma thyroxine, triiodothyronine and cortisol associated with starvation in rainbow trout (*Salmo gairdneri*). Environ. Biol. Fish 4, 185–190.

Murray, J., Murray, A., 1977a. Suppression of infection by famine and its activation by refeeding—a paradox? Perspect. Biol. Med. 20, 471–483.

Murray, M.J., Murray, A.B., 1977b. Starvation suppression and refeeding activation of infection: an ecological necessity? Lancet 1, 123–125.

Murray, M.J., Murray, A.B., 1979. Anorexia of infection as a mechanism of host defence. Am. J. Clin. Nutr. 32, 593–596.

Murray, J., Murray, A., Murray, N., 1978. Anorexia: sentinel of host defence? Perspect. Biol. Med. 22, 134–142.

Murray, M.J., Murray, N.J., Murray, A.B., Murray, M.B., 1995. Infections during severe primary undernutrition and subsequent refeeding: paradoxical findings. Aust. N. Z. J. Med. 25, 507–511.

Nakamura, K., Aoike, A., Hosokawa, T., Rokutan, K., Koyama, K., Nishi, Y., Yoshida, A., Kawai, K., 1990. Effect of food-restriction stress on immune response in mice. J. Neuroimmunol. 30, 23–29.

Néji, H., de la Noüe, J., 1998. Effect of animal and vegetal protein diets on feed intake and apparent digestibility of nutrient in rainbow trout (*Oncorhynchus mykiss*) infected by *Aeromonas salmonicida*, with and without chronic hypoxia stress. Can. J. Fish Aquat. Sci. 55, 2019–2027.

Nordmo, R., Ramstad, A., Holth Riseth, J.M., 1998. Induction of experimental furunculosis in heterogeneous test populations of Atlantic salmon (*Salmo salar* L.) by use of a cohabitation method. Aquaculture 162, 11–21.

Pirhonen, J., Schreck, C.B., Gannam, A., 2000. Appetite of chinook salmon (*Oncorhynchus tshawytscha*) naturally infected with bacterial kidney disease. Aquaculture 189, 1–10.

Redding, J.M., Schreck, C.B., Birks, E.K., Ewing, R.D., 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 56, 146–155.

Reddy, P.K., Leatherland, J.F., 1995. Influence of the combination of time of feeding and ration level on the diurnal hormone rhythms in rainbow trout. Fish Physiol. Biochem. 14, 25–36.

Schreck, C.B., 1996. Immunomodulation: endogenous factors. In: Iwama, G., Nakanishi, T. (Eds.), The Fish Immune System: Organism, Pathogen, and Environment. Fish Physiology, vol. 15. Academic Press, San Diego, pp. 311–337.

Varnavsky, V.S., Sakamoto, T., Hirano, T., 1995. Effects of premature seawater transfer and fasting on plasma growth hormone levels of yearling coho salmon (*Oncorhynchus kisutch*) parr. Aquaculture 135, 141–145.

White, A., Fletcher, T.C., 1986. Serum cortisol, glucose and lipids in plaice (*Pleuronectes platessa* L.) exposed to starvation and aquarium stress. Comp. Biochem. Physiol. 84A, 649–653.

Wing, E.J., Young, J.B., 1980. Acute starvation protects mice against *Listeria monocytogenes*. Infect. Immun. 28, 771–776.

Wing, E.J., Barczynski, L.K., Boehmer, S.M., 1983. Effect of acute nutritional deprivation on immune function in mice. Immunology 48, 543–550.

Wing, E.J., Barczynski, L.K., Sherbondy, J.M., 1986. Effect of acute nutritional deprivation on macrophage colony-stimulating factor and macrophage progenitor cells in mice. Infect. Immun. 54, 245–249.

Wise, D., Johnson, M., 1998. Effect of feeding frequency and Romet-medicated feed in survival, antibody response, and weight gain in fingerling channel catfish *Ictalurus punctatus* after natural exposure to *Edwardsiella ictaluri*. J. World Aquacult. Soc. 29, 165–175.

Wolf, K., 1989. Fish Viruses and Fish Virus Diseases. Cornell Univ. Press, Ithaca, NY, USA.