A COMPARISON OF SOME NON-SPECIFIC IMMUNE PARAMETERS DURING INFECTION WITH FLAVOBACTERIUM PSYCHROPHILUM

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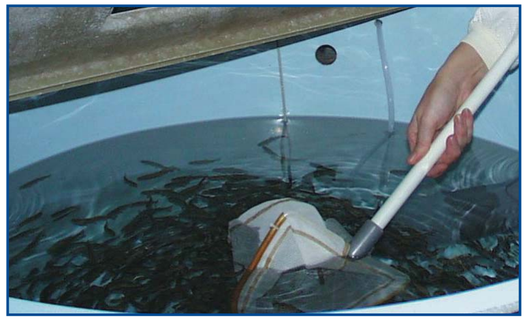
Introduction

Flavobacterium psychrophilum is a Gram negative bacterium responsible for causing systemic bacteraemia in salmonid fish (Bernardet *et al* 1988; Lehmann *et al* 1988; Lorenzen *et al* 1991), eels (*Anguilla anguilla*) and carp (*Cyprinus carpio*) (Lehmann *et al* 1991). In commercially reared juvenile rainbow trout (*Oncorhynchus mykiss*) the disease is known as Rainbow Trout Fry Syndrome (RTFS), Fry Mortality Syndrome (FMS) and Rainbow Trout Fry Anaemia (RTFA). Serious mortality can occur within the size range 0.5- 5.0 g (Branson 1995). The disease frequently develops before the onset of full immunocompetance consequently, non-specific defence mechanisms are important in resisting infection.

In this study, elevation and/or suppression of selected non-specific immune parameters during infection within *F. psychrophilum* was measured. The results for total serum protein levels, serum lysozyme activity, serum anti-proteases and alternative pathway complement activity are presented here.



Rainbow trout fry in holding tanks at the CEFAS Laboratory, Weymouth.

Experimental tank facility.

Materials and Methods

For intraperitoneal (IP) challenge studies fresh *F. psychrophilum* was isolated from the spleens of (5.0 g rainbow trout fry from an outbreak of RTFS at a South of England hatchery (accession no. UP295/97). Confirmatory identification was carried by ELISA (Rangdale 1995) and API Zym (BioMerieux) (Bernardet *et al* 1989). Three replicate groups of 150 3.0-4.0 g fry each received 100 µl of 5.5 x 10^{10} cells/ml in phosphate buffered saline (PBS); 150 control fry received 100 µl of PBS. Fish were monitored twice daily, mortality was recorded, swabs of ascitic fluid and spleens plated onto modified Anacker and Ordal media (Rangdale 1995) yellow pigmented bacteria were purified by re-streaking onto fresh media and identified as previously described. Caudal vein blood samples were pooled from 20 fish at 0, 1 and subsequently, every 5 days post injection. Blood samples were left to clot overnight at 4°C, sera were stored at -20°C.

The total serum protein content of sera was determined using the method of Lowry *et al* (1951). Serum lysozyme activity was measured using the method of Osserman and Lawler (1966) with *Micrococcus lysodeikticus* (Sigma). Anti-protease activity was determined using the method of Ellis (1990). Additionally, a semiquantative bioassay was devised to assess the activity of extracellular products (ECP) of *F. psychrophilum* (Plant 1997). The spontaneous haemolytic activity of the sera was assayed using 2 % unsensitised sheep red blood cells in complement fixation buffer (Oxoid).

Discussion

The results obtained in this study demonstrated that there were changes in the non-specific immune parameters of rainbow trout during infection with *F. psychrophilum*. The suppression of acute phase and total serum proteins can be attributed to release of exogenous proteases produced by the bacterium. These proteases, which have been partially characterised (Hofer, Rangdale and Richards 1997) are important virulence factors in the pathogenicity mechanisms of the bacterium. The increase in lysozyme activity in clinically infected fish is consistent with early activation of phagocytic cell populations. Increased lysozyme activity is considered to be an acute response to infection or stress as a classic barrier against disease. Complement acts both directly, via the formation of a membrane attack complex, and synergistically, by opsonisation of phagocytic cells. In fish exhibiting clinical disease, low levels of complement activity were indicative of successive complement consumption. However, complement appears to have a limited role in defence against RTFS. It is likely that the extracellular polysaccharide layer surrounding *F. psychrophilum* impedes the lytic action of complement proteins. Anti-protease activity was increased in infected fish but serum anti-proteases were ineffective at neutralising the activity of *F. psychrophilum*'s extracellular proteases (ECP). The resistance to ECP is indicative of an evolutionary pathogenic adaptation response aimed at resisting the host defence mechanisms.

In diseases such as RTFS, that cause early life stage mortality, innate immune defence mechanisms are particularly important. This study demonstrated the relevance of some of these mechanisms and indicated that enhancing non-specific immunity would lessen the impact of infection with *F. psychrophilum*.

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Results

Clinical disease signs of RTFS were reproduced in experimentally challenged fish from day 5 post injection, with the first mortality occurring on day 6. In total 8 % of laboratory challenged fish died within 30 days. This approximately corresponded with the concurrent mortality at the infected farm.

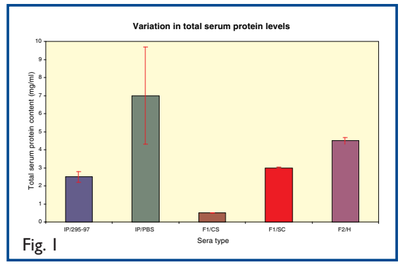

Fig. 1

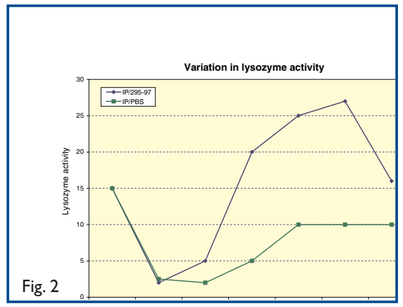

Fig. 2

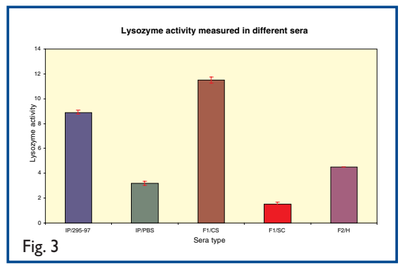

Fig. 3

Total Serum Protein

Levels of serum proteins were consistently lower in experimentally and farm-infected fry than in sham-(PBS) challenged and healthy fish. In sham-challenged fry an initial peak in serum protein in response to injection was recorded. No peak was observed in fry injected with the bacterium, and, subsequently a rapid decrease was measured. Low levels were sustained throughout the trial.
Figure 1 shows the total serum protein levels recorded on day 6 post injection (corresponding with the first mortality), and from farm-infected and non-infected fry. Significant differences (students t-test)($p<0.01$) were recorded between clinically sick and non-diseased fish.

Serum Lysozyme Response

Figure 2 shows lysozyme activity recorded in sera from *F. psychrophilum* and sham- injected fry throughout the first 7 days of the trial. Statistically significant variation (students t-test: $p<0.01$) in activity was observed during this initial phase, but these differences were not maintained throughout. Comparisons with clinically-infected, sub-clinically-infected and healthy sera (**Figure 3**) show clear elevation in activity in fry with RTFS.

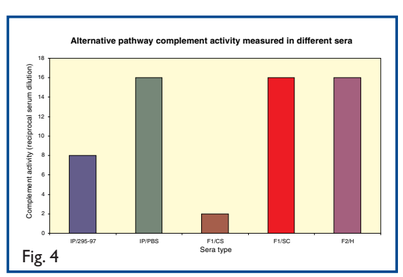

Fig. 4

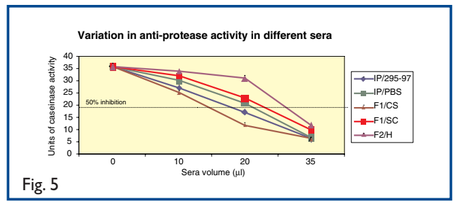

Fig. 5

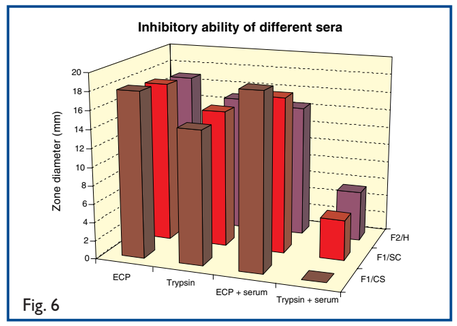

Fig. 6

Anti-protease Activity

Caseinase activity was considerably increased in sera from fry showing clinical disease signs from both laboratory trials and the fish farm compared with sham- infected, sub-clinically infected and healthy fish (**Figure 5**). However, *F. psychrophilum* ECP activity (estimated activity 1525.5 units of trypsin) was not notably inhibited by sera from any source (**Figure 6**).

Alternative Pathway Complement Activity

Figure 4 shows spontaneous haemolytic activity of complement as measured by lysis of unsensitised sheep red blood cells on day 6 post challenge. In fry showing clinical RTFS signs complement activity was decreased compared with sham-infected, sub-clinically-infected and healthy fry.

Note. Sub-clinically infected fish were defined as those where recovery of *F. psychrophilum* was possible from internal lesions, but that exhibited no behavioural or external signs of disease.

KEY	
IP/295-97	INTRAPERITONEAL ROUTE /ISOLATE NO.
IP/PBS	INTRAPERITONEAL ROUTE /PBS.
F1/CS	INFECTED FARM/ FISH WITH CLINICAL SIGNS
F1/SC	INFECTED FARM/ SUB-CLINICAL INFECTION
F2/H	DISEASE FREE

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