The immunostimulatory effects of Chevimmun on the rainbow trout 
(Oncorhynchus mykiss)

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Abstract
The immunostimulatory effects of intraperitoneally injected Chevimmun, a fixed combination herbal product, was investigated in the rainbow trout. Administration of a 75% Chevimmun preparation resulted in significantly enhanced leucocyte migration (p<0.05), phagocytosis (p<0.001) and respiratory burst activity (p<0.001) in the peritoneal cavity. Antiprotease activity was also significantly elevated at 2 (p<0.001) and 7 days (p<0.05) post-injection.

Introduction
Chevimmun is a fixed combination product composed of Echinacea angustifolia, Eupatorium perfoliatum and Baptisia tinctoria. Although there is a large amount of information concerning the efficacy and mode of action of fixed combination herbal therapies in mammalian systems (Wagner and Jurcic, 1991; Bodinet and Freudenstein, 1999; Henneicke-Von Zepelin et al., 1999), this situation is not mirrored in the piscine model. Indeed, there is no published information concerning immunostimulation in the trout following administration of herbal therapies. The primary purpose of this study is to investigate localised and systemic immunostimulation in the rainbow trout (Oncorhynchus mykiss) in vivo, following intraperitoneal administration of Chevimmun.

Materials and Methods
Rainbow trout (Oncorhynchus mykiss) weighing 100-500g were obtained from Rothiemurchus Estate (Aviemore, UK) and stocked in 250l tanks, maintained at 12 ± 1°C, with a constant flow of aerated and dechlorinated mains water. Fish were fed ad libitum daily with commercial pellets (Ewos). An acclimation period of 2 weeks was observed prior to the experiments commencing.

Chevimmun, manufactured by Chevita GmbH, was supplied in 300ml aliquots by Aquaculture Vaccines Limited (Saffron Walden, UK). It is a fixed combination product composed of the following plant extracts: Echinacea angustifolia radix original tincture (200ml), Eupatorium perfoliatum original tincture (90ml) and Baptisia tinctoria radix original tincture (10ml). In order to achieve the final concentrations of Chevimmun as outlined below, it was diluted in sterile 0.15M PBS
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(pH 7.2) prior to administration. Concentration is therefore expressed in terms of percentage content of Chevimmun in the final preparation, with the amount injected varying in line with the body weight of the experimental animals.

To determine the effect of Chevimmun on total peritoneal leucocyte counts, two groups of 5 fish (100g) were anaesthetised with benzocaine (ethyl-4-aminobenzoate, BDH) solution (4mg ml⁻¹ of ethanol) diluted in water. Each fish was injected intraperitoneally with 250ml of either PBS (control) or a 75% Chevimmun preparation. At 1 day post-injection, peritoneal cells were isolated and processed as documented in Peddie et al. (2002). Counts were performed at under a light microscope at x400 magnification.

For the phagocytosis and NBT reduction experiments, two groups of 6 fish (300-500g) were injected with 1ml of either PBS or a 75% Chevimmun preparation. The experimental procedure for each assay was as outlined in Peddie et al. (2002). In the phagocytosis assay, leucocytes were allowed to adhere to microscope slides for 1h and subsequently incubated with yeast cells (1.2x10⁸ cells ml⁻¹ Leibovitz medium (L-15, Gibco)/PBS at a ratio of 1:1) for 1h at 18°C. Preparations were stained sequentially with Giemsa (BDH) and May-Grunwalds (BDH) and viewed at x1000 under oil immersion. In the NBT reduction assay, an equal volume of cell suspension was mixed with NBT (Sigma) (0.2%w:v in PBS) in vitro and incubated at 18°C for 30min. Blue stained adherent cells were counted in 10 fields of view at x400 magnification.

In order to determine the effects of Chevimmun on humoral parameters, two groups of 8 fish (300-500g) were injected intraperitoneally with either 1ml of PBS or 75% Chevimmun. Blood samples were taken on 0, 2, 4 and 7 days post-injection. Plasma was collected and stored at –20°C prior to use. The two humoral parameters investigated were lysozyme levels and antiprotease activity. The method used to detect lysozyme activity in trout plasma was based on the ability to lyse the Gram positive bacterium *Micrococcus luteus*. Quantification of antiprotease activity was based on the test-combination assay for trypsin (Thompson et al., 1994).

### Results

Chevimmun injected as a 75% preparation significantly increased the total number of leucocytes migrating into the peritoneal cavity at 1 day post-injection (p<0.05, by two-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PBS</th>
<th>Chevimmun</th>
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<tr>
<td>Total no. peritoneal leucocytes (x10⁶)</td>
<td>8.2 ± 0.95</td>
<td>16.3 ± 2.47</td>
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<tr>
<td>% Phagocytosis</td>
<td>75.4 ± 1.65</td>
<td>86.0 ± 1.40</td>
</tr>
<tr>
<td>Phagocytic index</td>
<td>2.5 ± 0.10</td>
<td>3.6 ± 0.13</td>
</tr>
<tr>
<td>No. of NBT +ve cells</td>
<td>0.9 ± 0.13</td>
<td>13.0 ± 1.25</td>
</tr>
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Table 1. The effect of Chevimmun on leucocyte migration, phagocytic activity and nitroblue tetrazolium (NBT) reduction in the peritoneal cavity of trout.
Phagocytic activity (measured as both % phagocytosis and phagocytic index) of leucocytes harvested from trout injected intraperitoneally with a 75% Chevimmun preparation was significantly higher than that recorded in the control group (p<0.001, by two sample T-tests) (Table 1). The total number of NBT positive cells in the peritoneal exudates of fish injected with the 75% Chevimmun preparation was over 12 times greater than in the control group (p<0.001, by two sample T-test) (Table 1).

With respect to the humoral parameters tested, there was no significant effect of Chevimmun on lysozyme activity (data not shown). However, there was a significant effect of treatment (p<0.001) and time (p<0.05) on antiprotease activity, as determined by two-way ANOVA (Fig. 1). An interaction (p<0.001) was also observed between these two parameters. Two sample t-tests at each time point revealed significantly higher antiprotease activity in the Chevimmun injected fish at 2 (p<0.001) and 7 (p<0.05) days post-injection.

Discussion

Administration of the 75% Chevimmun preparation resulted in an increase in the total number of leucocytes migrating into the peritoneal cavity at 1 day post-injection. Leucocyte migration to the site of injection is an important prelude to the inflammatory response and has been documented in fish following injection of a range of immunostimulants (MacArthur et al., 1984; Suzuki, 1986; Jørgensen et al., 1993; Peddie et al., 2002). Chevimmun injection significantly increased leucocyte phagocytic activity, a phenomenon previously described in mammalian systems exposed to fixed combination herbal immunostimulants (Wagner and Jurcic, 1991). Moreover, a 12-fold increase in the number of NBT positive cells was recorded in fish injected with Chevimmun. NBT reduction is associated with respiratory burst activity following the intra-cellular localisation of superoxide anions in leucocytes (Dalmo et al., 1997). With respect to systemic immune parameters, Chevimmun had a clear stimulatory effect on antiprotease activity at 2 and 7 days post-injection. This is an important finding given the role of proteinase inhibitors in the complement cascade (Dalmo et al., 1997) and their ability to counteract the extracellular digestion of host tissues by bacterial microorganisms (Bowden et al., 1997).

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References


