

**DNA VACCINATION DURING
LOW TEMPERATURE
AND PARTIAL STARVATION**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Intramuscular DNA vaccination against fish rhabdoviruses has been outstandingly successful when fish have been vaccinated under optimal conditions. Such conditions do not always apply to fish farms e.g. in winter or when fish are fed for maintenance. How DNA vaccines might work under these situations was examined in goldfish (*Carassius auratus L*), (Russell et al., 2000). To prevent the use of dangerous pathogens a plasmid which encodes the reporter betagalactosidase (b-gal) was used because 1) the limiting dose of this plasmid for antibody production by goldfish was known (Kanellos et al.,1999); and 2) the rate of destruction of the transfected b-gal positive myofibres is a measure of cytotoxic T cell activity in mice (Davis et al.,1997).

Goldfish, weighing 5g to 15 g, were kept at 15^oC for 1 month and then water temperatures were adjusted up to 24^oC or down to 9^oC and the fish were left to acclimatise for 1 month (Bennett et al., 1998) when they received 50 μ g plasmid by injection into the epaxial muscle behind the dorsal fin. Fish at 9^oC were then shifted down to 7^oC and then to 5^oC at intervals of 28 days. At 24^oC b-

gal-positive fibres appeared after 1 week and were destroyed by 2 weeks (Figure 1).

Antibody became detectable at 2 weeks and remained at a moderate level. At 15°C fibre destruction and antibody production occurred more slowly, at 4-8 weeks, and antibody became very high at 18 weeks. At ≤9°C, the production of antigen in muscle rose to a maximum between 4 to 18 weeks and antibody rose to a moderate level over this time (Figure 1).

These differing kinetics suggested that at 24 °C fish behaved like mice in that cytotoxic T cells caused the destruction of fibres as B cells started to produce antibody at 1 to 2 weeks after DNA injection. At ≤9°C antibody was made without the accompanying destruction of b-gal-positive fibres as if T but not B cells were less active at this temperatures, as suggested by hapten-carrier experiments with carp (Avtalion et al., 1976). The only example of cyprinid fish making antibody at 5°C is to bacterial flagellin (Azzolina et al., 1978) which is a T-independent antigen in mice. Fish lack IgG and so their sustained antibody production might require less T cell activity than mice.

When temperature is lowered fish eat less and so fish at 22 °C were put on a reduced diet of 0.33% bodyweight of goldfish food flakes (Tetra) each day compared to their usual intake of 3% for growth. The fish on 0.33% lost 20% of their weight in 6 weeks and then had to be killed under the terms of our Home Office license. The fish on 3% food gained 50% body weight. The fish on 1% food increased 14% bodyweight. Plasmid was injected at 2 weeks into the experiment. At 4 weeks fish on all 3 diets had antigen positive fibres and at 6 weeks, the end of the experiment, all fish had serum antibody (Figure 2).

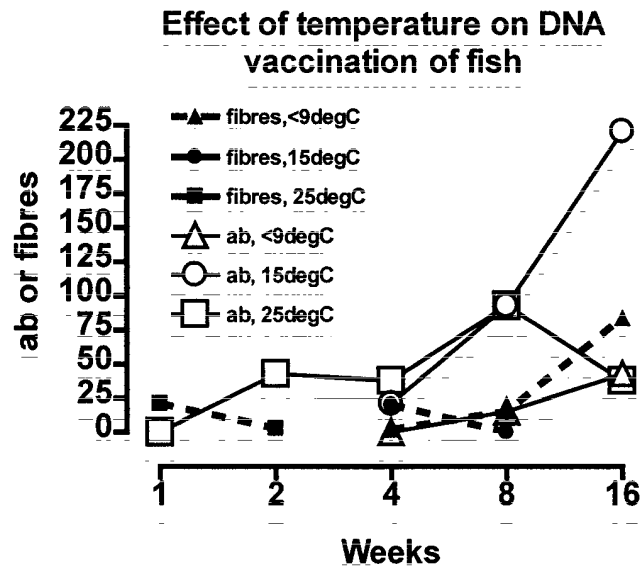


Figure 1.

The conclusions of this work were that intramuscular DNA vaccination works when fish are at low temperatures or on low food intake and so DNA might be a better means of immunising fish than with exogenous antigen. Future work could extend the range of temperatures and food intake and examine whether cytokines and dietary factors can improve the immune response under limiting conditions of food or temperature.

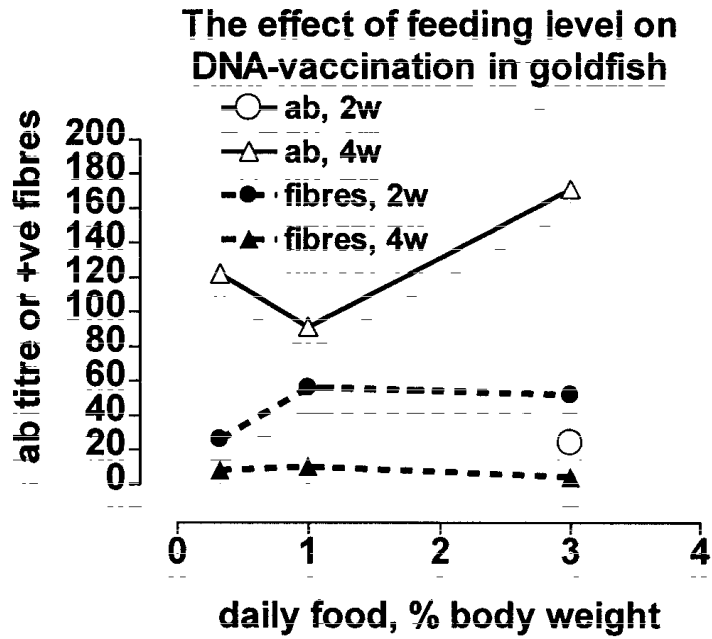


Figure 2

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Acknowledgements

This work was supported by a visiting fellowship to Prof. Ambali from the Wellcome Trust. We thank Mounir Negrou, Theo Kanellos and Andrew Mackie for assistance.

