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# Vaccination of European sea bass fry through bioencapsulation of *Artemia* nauplii

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European sea bass (*Dicentrarchus labrax*) fry vaccinated orally via bioencapsulation in *Artemia* nauplii or by bath method exhibited better performance than control fish in terms of growth, food conversion and resistance to stress. The comparable survival between vaccinated and non-vaccinated animals suggests that vaccination methods are not stressful. The present study shows that oral vaccination can be used to enhance growth in fish fry.

KEYWORDS: European sea bass, *Dicentrarchus labrax*, *Artemia*, Bioencapsulation, Oral vaccination, Growth, Food conversion, Resistance to stress

## INTRODUCTION

Intensification in aquaculture increases the chances of bacterial infections due to a more widespread use of antibiotics. The incidence of multiple-resistant bacterial strains seems to be increasing (Aoki and Kitao, 1985; Takashima *et al.*, 1985; Dixon, 1994). Vaccination can be used as a preventative strategy to circumvent such developments (Hjeltnes *et al.*, 1989).

Different ways of vaccination have proved their efficacy. Vaccination through injection is practised on older fish (Antipa and Amend, 1977; Cossarini-Dunier, 1985; Hastein and Refsti, 1986; Thorburn *et al.*, 1989) but is not feasible for early stages. The hyperosmotic infiltration method is very stressful (Croy and Amend, 1977). The spray technique does not offer a good and equal exposure of all animals to the vaccine, and methods such as dip and bath vaccination are less stressful to fish and can provide good protection (Egidius and Anderson, 1979; review: Smith, 1988). Maybe the most convenient way for vaccine delivery is oral administration, i.e. the vaccine is incorporated in the diet. Vigneulle and Baudin Laurencin (1991) and Campbell *et al.* (1993) indicate that vaccine antigens may be destroyed in the stomach before they reach areas such as the posterior gut where uptake and processing appear to be important. To safeguard vaccine antigenicity, different attempts of vaccine encapsulation have been tried (Juliano, 1985; Goosen *et al.*, 1989).

This work investigates the feasibility of oral vaccination in European sea bass (*Dicentrarchus labrax*) fry using the live prey *Artemia* as the carrier for the *Vibrio*

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*anguillarum* bacterin. An effective delivery method that ensures persistent antigen activity was documented earlier by Campbell *et al.* (1993). This oral vaccination is convenient as it uses *Artemia*, a commonly used live food in aquaculture. No extra labour is involved, making it a very cost-effective method. Furthermore, this method is non-stressful and mass vaccination of animals of different sizes can easily be performed. This oral vaccination is compared with the bath vaccination in terms of growth, food conversion, survival and resistance to stress. Lillehaug (1989) reported problems associated with prolonged bathing. The bath method used in this exercise is a slightly modified one.

## MATERIALS AND METHODS

### Fish

Sea bass larvae (50 days old) were purchased from a commercial hatchery (SEPIA International, Gravelines, France). Little mortality (< 10%) was caused by transportation, which might reflect the good quality of the fish. After 8 days of acclimation, the 2-month-old fish were distributed in 20 l aquaria equipped with a biofilter (Abelin *et al.*, 1989). The aquaria were filled with natural seawater (35‰ S) and the water temperature was maintained at 21 ± 1 °C with a submersible heater. The fish were weaned to a commercial formulated diet, (Lansy® W3, Artemia Systems SA, Baasrode, Belgium) over a period of 22 days (Fig. 1). Fish stocking density in the aquaria was 5 individuals $l^{-1}$ at the end of weaning. Dead fish were recorded daily and discarded. The fish were fed three times daily, and extra food siphoned out. The aquaria were cleaned and the water level restored after a partial change. Salinity, temperature and nitrogen levels were checked daily as routine operations.

### Incorporation of the vaccine in *Artemia*

*Artemia* cysts (Great Salt Lake, Utah, USA) were hatched and separated following the procedure outlined by Sorgeloos *et al.* (1986). Hatching duration was extended to 27 h to ensure that the nauplii had moulted into the instar II stage. One litre of the vaccine

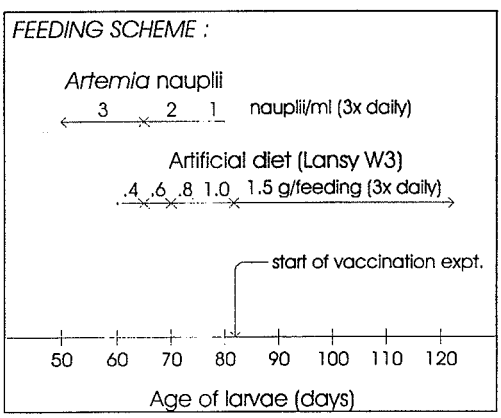


**FIG. 1.** Weaning scheme from live to artificial food.

suspension containing $10^9$ bacterial cells $ml^{-1}$ was added to a 9 l conical tank filled with filtered seawater and provided with continuous aeration. The water temperature was maintained at 28 ± 1 °C. The *Artemia* were added until reaching a density of 200 nauplii $ml^{-1}$. After 1.5 h incubation, the nauplii were harvested on a nylon sieve, washed and rediluted in 28 °C seawater before being distributed to the designated aquaria. A commercially available vaccine (Aquaculture Vaccines Ltd, Essex, UK) containing formalin-inactivated cultures of *Vibrio anguillarum* biotypes I and II was used.

### Vaccination of sea bass

The experiment consisted of four treatments with five replicates each. The treatments were assigned at random to the 20 aquaria. The 80-day-old weaned sea bass of 350 mg average weight were exposed to the vaccine, either by the bath method or by oral treatment. For the latter procedure, one group received one ration of the vaccine-loaded *Artemia* at a rate of 5 nauplii $ml^{-1}$, i.e. 1000 nauplii fish $^{-1}$ ('bioencapsulation'); in the second group, oral vaccination was repeated 2 weeks later ('booster'). The bath-vaccinated group was treated following a modified Egidius method where the vaccine dilution was 1:10 instead of 1:1000 and the exposure time 1 min instead of 1 h (Egidius and Anderson, 1979) ('bath'). The control group received untreated *Artemia* nauplii at the same density as the orally vaccinated group ('control').

### Data collection

Weekly sampling proceeded for 6 weeks post vaccination. Ten fish were collected from each replicate (i.e. 200 from 20 aquaria). Their total length and wet weight was measured. Fish were then dried at 60 °C for 24 h to determine dry weights. The food conversion ratio (FCR) was calculated as the amount of food distributed to the gain in weight over the experimental period. Fish survival (%) was computed based on the ratio of the number of live fish at the end of the experiment to that at the start. For a quick verification of the physiological state of the fish in the different treatments, a stress test was performed every 2 weeks. The test consisted of exposing the fish to a salinity shock. Ten fish from each aquarium were treated in a separate beaker following the protocol of Dhert *et al.* (1992). Since fish in the present experiment were older and were exposed to different experimental conditions, a preliminary test was performed to identify optimal salinities that ensured a good response in terms of onset of mortality, mortality rate and total mortality. As the fish grew older, their resistance to salinity increased, and every 2 weeks an adjustment of the test salinity was applied. The optimal salinity to run the stress test was 65‰ 2 weeks post vaccination, and was increased to 70‰ and 75‰ 4 and 6 weeks respectively post vaccination.

The sensitivity index for each treatment was computed as a mean of the summed cumulative mortalities from five replicates. Whenever applicable, type 1 ANOVA and Tukey's multiple range tests were used for data analysis.

## RESULTS

During the 4 weeks post vaccination, no significant difference could be detected between the different treatments in terms of total length, wet weight and dry weight. At 5 and 6 weeks, even though no significant difference was found between the different vaccination methods, the booster group exhibited significantly higher growth when compared with

**TABLE 1.** Total length (mm) of European sea bass during 6 weeks post vaccination. Values are means (standard errors in parentheses). Within weeks, means with the same superscript letter are not significantly different ($p < 0.05$)

| Treatment | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week |
|---|---|---|---|---|---|---|
| Control | 35.4[a] (0.4) | 39.0[a] (0.3) | 42.6[a] (0.2) | 45.4[a] (0.3) | 46.3[a] (0.7) | 49.1[a] (0.9) |
| Bath | 34.7[a] (0.4) | 38.7[a] (0.2) | 42.0[a] (0.5) | 46.0[a] (0.4) | 49.0[b] (0.5) | 52.2[b] (0.4) |
| Bioencapsulation | 35.2[a] (0.5) | 38.9[a] (0.4) | 42.1[a] (0.3) | 46.0[a] (0.4) | 48.9[b] (0.4) | 52.2[b] (0.3) |
| Booster | 35.0[a] (0.4) | 39.9[a] (0.5) | 43.6[a] (0.7) | 46.5[a] (0.6) | 49.7[b] (0.7) | 54.0[b] (0.6) |

**TABLE 2.** Wet weight (mg) of European sea bass during 6 weeks post vaccination. Values are means (standard errors in parentheses). Within weeks, means with the same superscript letter are not significantly different ($p < 0.05$)

| Treatment | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week |
|---|---|---|---|---|---|---|
| Control | 422.5[a] (12.0) | 524.8[a] (14.0) | 681.1[a] (13.7) | 890.1[a] (10.0) | 925.1[a] (43.3) | 1100.8[a] (52.2) |
| Bath | 410.9[a] (5.6) | 517.0[a] (13.3) | 669.5[a] (25.8) | 936.7[a] (22.3) | 1109.6[ab] (44.4) | 1323.7[ab] (65.6) |
| Bioencapsulation | 414.0[a] (14.2) | 518.0[a] (19.2) | 667.7[a] (18.6) | 954.8[a] (37.3) | 1136.6[b] (45.4) | 1347.4[ab] (45.3) |
| Booster | 419.7[a] (14.2) | 567.8[a] (23.1) | 726.3[a] (17.4) | 972.9[a] (47.0) | 1151.3[b] (47.5) | 1473.5[b] (81.6) |

the control (Tables 1, 2 and 3). Although there was no significant difference in terms of FCR between the different vaccination methods, only the bioencapsulation as well as the booster treatment exhibited better performance than the control, with the booster treatment again reflecting the best FCR (Table 4).

Vaccination methods do not seem to affect fish survival (Table 4). As of the sixth week post vaccination, the sea bass fry subjected to a high-salinity stress test exhibited a different pattern of response in terms of rate and total mortality (Fig. 2), and also in terms of sensitivity index (SI); although all vaccinated fish exhibited smaller SI values than the unvaccinated group, the lowest SI value was obtained with the booster-treated fish (Table 5).

## DISCUSSION

The present study confirms the earlier finding of Smith (1987) that vaccinated fish acquire better FCR than unvaccinated ones. In our study, fish responded positively in terms of growth and FCR 5 weeks post vaccination.

**TABLE 3.** Dry weight (mg) of European sea bass during 6 weeks post vaccination. Values are means (standard errors in parentheses). Within weeks, means with the same superscript letter are not significantly different ($p < 0.05$)

| Treatment | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week |
|---|---|---|---|---|---|---|
| Control | 104.0$^{a}$ (2.8) | 133.8$^{a}$ (3.5) | 181.4$^{a}$ (4.3) | 225.5$^{a}$ (2.9) | 244.7$^{a}$ (12.1) | 299.1$^{a}$ (17.0) |
| Bath | 101.5$^{a}$ (2.2) | 130.7$^{a}$ (3.4) | 172.4$^{a}$ (7.2) | 235.5$^{a}$ (6.7) | 294.7$^{ab}$ (13.2) | 360.1$^{ab}$ (21.6) |
| Bioencapsulation | 102.2$^{a}$ (3.8) | 135.2$^{a}$ (4.9) | 172.8$^{a}$ (4.2) | 240.9$^{a}$ (10.5) | 302.8$^{b}$ (13.1) | 369.6$^{ab}$ (13.2) |
| Booster | 103.7$^{a}$ (3.1) | 144.7$^{a}$ (5.7) | 186.4$^{a}$ (7.1) | 245.8$^{a}$ (14.1) | 304.4$^{b}$ (13.5) | 407.4$^{b}$ (19.1) |

**TABLE 4.** Food conversion ratios and survival of European sea bass during 6 weeks post treatment. Values are means (standard errors in parentheses). Within columns, means with the same superscript letter are not significantly different ($p < 0.05$)

| Treatment | Food conversion ratio | Survival (%) |
|---|---|---|
| Control | 1.90$^{a}$ (0.13) | 93.9$^{a}$ (3.9) |
| Bath | 1.48$^{ab}$ (0.10) | 95.1$^{a}$ (2.3) |
| Bioencapsulation | 1.44$^{b}$ (0.06) | 97.8$^{a}$ (0.6) |
| Booster | 1.29$^{b}$ (0.09) | 95.9$^{a}$ (2.6) |

Oral vaccination through bioencapsulation in the water flea was reported to improve survival in juvenile Ayu (*Plecoglossus altivelis*) (Kawai *et al.*, 1989). The present study failed to clearly demonstrate such an effect. This is maybe attributed to the good quality of the sea bass used, which was demonstrated by the negligible mortality during transport and the high survival rate throughout the experiment,

Resistance to stress can be used as an indicator of fish or shrimp quality (Tackaert *et al.*, 1989; Dhert *et al.*, 1992). This test has been used here to check if resistance to stress could be correlated with immunity success. The present study is unfortunately not conclusive, i.e. vaccinated sea bass exhibited a significantly lower sensitivity index, but they were larger than the controls on the sixth week, and could consequently resist better.

Johnson *et al.* (1982) related immunity development to the fish body weight. Different values are reported concerning the minimum weight for vaccination. Horne *et al.* (1984) set this limit at 5 g. Anggawati-Satyabudhy *et al.* (1989) successfully vaccinated 3.2 g rainbow trout (*Oncorhynchus mykiss*). In this study, sea bass were vaccinated at 350 mg wet weight at 21 ± 1 °C, and the response to the vaccine could be detected when the fish reached 1.1 g wet weight.

The variables which mostly affect antigen uptake during immersion are adequate fish

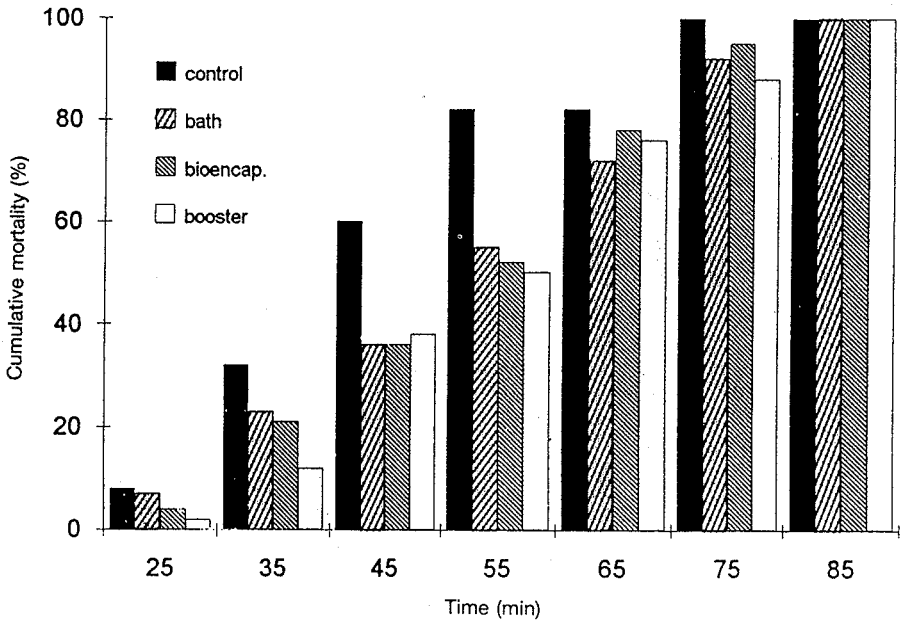


**FIG. 2.** Cumulative mortalities per treatment for sea bass subjected to a 75‰ salinity stress test 6 weeks post vaccination.

**TABLE 5.** Sensitivity indices (SI) of post-treated European sea bass. Values are means (standard errors in parentheses). Within weeks, means with the same superscript letter are not significantly different ($p < 0.05$)

| | Sensitivity Index | | |
|---|---|---|---|
| Treatment | 2nd week | 4th week | 6th week |
| Control | $82.4^{b}$ (0.87) | $83.6^{b}$ (0.81) | $90.0^{a}$ (1.52) |
| Bath | $85.2^{a}$ (1.11) | $86.4^{a}$ (0.40) | $72.2^{b}$ (1.07) |
| Bioencapsulation | $81.4^{b}$ (0.68) | $82.2^{c}$ (0.80) | $73.0^{b}$ (1.55) |
| Booster | $84.6^{a}$ (0.68) | $86.2^{a}$ (0.49) | $67.6^{b}$ (5.05) |

size and exposure to sufficient antigen over time (Tatner, 1987). The results which we obtained with the modified bath method seem to indicate that the above conditions were met.

## CONCLUSIONS

1. The vaccination methods applied do not harm sea bass fry.
2. Although no definitive scientific explanation can be provided, this study shows that oral vaccination can be used to enhance growth and FCR in sea bass fry.

## ACKNOWLEDGEMENT

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