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The Effect of Bacterial Additives on the Production Rate and Dietary Value of Rotifers as Food for Japanese Flounder, *Paralichthys olivaceus*

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ABSTRACT

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Two food additives containing live lactic bacteria were given to S-type rotifers fed on live algae and baker's yeast. One of them improved the production rate of rotifers (59 rotifers ml^{-1} day^{-1}) in comparison with the control group fed without any additive (48 rotifers ml^{-1} day^{-1}). The second additive did not improve the production rate (42 rotifers ml^{-1} day^{-1}), but it improved the dietary value of the rotifers. Indeed, at day 18, the mean length of Japanese flounder fed on these rotifers (7.5 mm) was significantly higher than that of the control group (7.3 mm). The total amount of aerobic bacteria in rotifers was also affected by this additive. Three thousand bacteria per rotifer were recorded in the production tanks. Bacterial growth was particularly high during the enrichment phase. After 17 h of enrichment with an emulsion of cuttlefish liver oil, 99 000 bacteria were counted per control rotifer. The second bacterial additive limited the bacteria to 54 000 per rotifer after 17 h of enrichment. This may be a reason why this additive improved the dietary value of the rotifers.

INTRODUCTION

The bacteria associated with rotifers were studied by Nicolas and Joubert (1986), who found that most belonged to the genus *Pseudomonas*. Some of these bacteria were shown to be detrimental to larval turbot, whereas food additives containing selected strains of live bacteria improved the production rate of L-type rotifers and the growth rate of turbot larvae (Gatesoupe, 1989).

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Two new experiments were performed (1) to study the effect of bacterial additives on S-type rotifers and their associated flora and (2) to evaluate the dietary value of such rotifers for the culture of Japanese flounder, *Paralichthys olivaceus*.

MATERIAL AND METHODS

Experiment 1

S-type rotifers were cultured in cylindrical 35-cm diameter 30-l tanks. The tanks had a flat bottoms and were filled up to 27 l with seawater. The temperature was kept between 25 and 27° C. At the beginning of the experiment, each tank was stocked with 1.8 million rotifers. Once a day, 9 l were drawn from each tank, and replaced by 9 l of algae. The harvest of rotifers was discarded only when the mean number of the rotifers fed with the best diet was more than 225/ml (i.e. on days 5, 7, 9, 10, 12, 13 and 14). On the other days, the harvested rotifers were carefully collected on a 70-μm net kept in seawater. They were then transferred back into the culture tanks.

The control rotifers were fed once a day with marine chlorella, *Nannochloropsis oculata*, at a rate of 9 l/tank, i.e. about 3 million cells per ml of culture medium. Baker's yeast was given twice a day (326 mg dry weight per million rotifers, each day). The experimental rotifers were fed with the same diet plus one portion of bacterial additive per day (8 mg/l). Two additives were tested: (1) Adjulact 1000, a spray-dried powder containing live lactic bacteria (*Streptococcus thermophilus* and *Lactobacillus helveticus*) cultured on lactoserum and other additives (1 million bacteria/mg) and (2) Acosil, a spray-dried extract from sprouting cereal grains, fermented with selected strains of lactic bacteria. This latter coarse-grained extract was passed through a 40-μm net bag so that only small particles and soluble substances were introduced into the culture medium.

The two experimental diets were compared to the control diet without bacterial additives. Each diet was tested in five replicates. The rotifers were counted daily. The production of rotifers in each tank was computed as their final number, minus their initial number, plus the total harvest of the rotifers which were discarded. This production was divided by the culture volume and the duration of the experiment. From day 6 to day 14, tanks were sampled at random for bacterial counts, so that each tank was sampled once during the experiment. Under sterile conditions, the medium containing rotifers was filtered through a 70-μm net and diluted 1000 or 10 000 times with seawater. The total aerobic bacteria were counted on Petrifilm SM plates (3M Co.). The number of bacteria per rotifer was obtained by difference between the number in the filtered medium and the total number in a sample which was not filtered but crushed in a glass homogenizer. After one-way analysis of variance, the data were compared in a Student–Newman–Keuls test (Sokal and Rohlf, 1969, pp. 239–246).

Experiment 2

The experimental rotifers were produced in 500-l tanks with the same shape as those used in the previous experiment. They were fed daily with chlorella (3 million cells/ml), baker's yeast (326 mg dry matter per million rotifers) and Acosil (8 mg/l). The control rotifers were produced in 10-t tanks with the same food concentration of chlorella and yeast as in the experimental tanks. The rearing temperature range was 25-28°C. The day before feeding to fish larvae, the rotifers were concentrated and put into 30-l tanks (200–300 rotifers/ml) with chlorella (10 million cells/ml) and cuttlefish liver oil emulsion for 17 h. The emulsion was made with a commercial premix (Riken-Bitamin Co., Tokyo) which contained cuttlefish liver oil (95.78%), BHT (0.02%) and several emulsifiers (2.7% sorbitan fatty acid ester, 1.4% polyglycerol fatty ester, 0.1% lecithin). The premix was thoroughly blended with tapwater (one volume of premix for 20 volumes of water), then poured into the rotifer tanks (50 mg premix/l). During this enrichment, the experimental rotifers were also fed with Acosil (8 mg/l). Rotifers were sampled for bacterial counts after enrichment. The counting technique was the same as in the previous experiment. Four replicates were compared in a Student's *t* test.

Japanese flounder were reared in cylindrical 100-l tanks. The water temperature was kept at 18°C, with continuous water renewal. The water at the outlet, which was not recirculated, passed through a 70-μm net, so as to retain the rotifers in the larval tanks. The initial stocking density was 22.5 larvae/l. The larvae were fed with enriched rotifers twice a day from day 4 to day 17. The experimental rotifers were distributed to six replicate groups of flounder, while the control rotifers were given to fish in five other tanks. On day 18, at the end of the experiment, the survival rates of flounder were compared in a Student's *t* test. After one-way analysis of variance, the mean lengths of fish in the experimental groups were set against those of the controls in an a priori comparison test (Sokal and Rohlf, 1969, pp. 226–235).

RESULTS

The results of the two experiments are summarized in Table 1.

Experiment 1

The daily mean concentrations of the rotifers are shown in Fig. 1. The mean daily production rates were 48, 42 and 59 rotifers ml^{-1} day^{-1} with the control diet, Acosil and Adjulact respectively. The mean production obtained with Adjulact was higher than that of the two other treatments. The production rates of the Acosil and control treatments were not significantly different.

There were no significant differences between treatments in the numbers of bacteria in the rotifers and in the media. The means were 0.5 million bacteria/ml of medium and 3000 bacteria per rotifer.

TABLE 1

Summary of the results obtained in the two experiments. Superscripts a and b indicate significant differences

	Control	Acosil	Adjulact
Experiment 1			
Production rate (rotifer ml^{-1} day^{-1})	48^b	42^b	59^a
Total bacteria (10^6 ml^{-1})	0.3	0.7	0.4
Bacteria in rotifers (10^3 $rotifer^{-1}$)	3	5	2
Experiment 2			
Bacteria after 17 h of enrichment			
Total (10^6 ml^{-1})	2.5	3.9	-
In rotifers (10^3 $rotifer^{-1}$)	99^a	54^b	-
Flounder on day 18 after hatching			
Survival rate (%)	48	47	-
Mean length (mm)	7.29^b	7.53^a	-

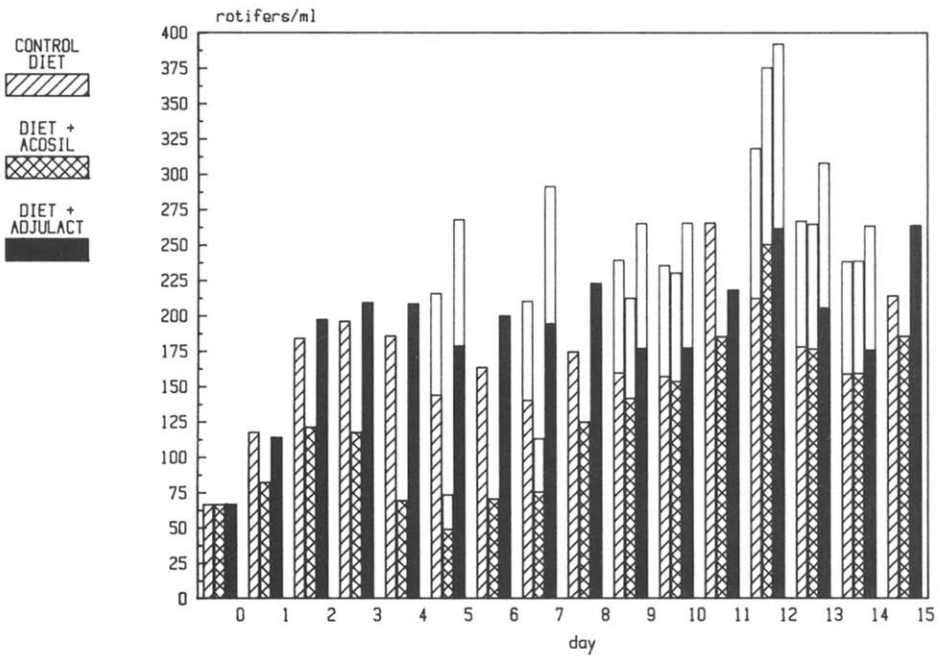

Fig. 1. The effect of the bacterial additives on the daily mean concentrations of rotifers. The white areas in bars correspond to the discarded rotifers.

Experiment 2

After 17 h of enrichment, the number of bacteria in the rotifers fed with Acosil (54 000 bacteria per rotifer) was significantly less than that observed in the control rotifers (99 000 bacteria per rotifer). There was no significant difference in the number of bacteria in the enrichment media (3 million bacteria/ml).

The survival rates of flounder at day 18, with a mean of 47%, were not significantly affected by the treatments. The mean length of the flounder fed with Acosil rotifers (7.53 ± 0.10 mm) was significantly higher than that of the flounder fed with the control rotifers (7.29 ± 0.09 mm).

DISCUSSION AND CONCLUSION

Adjulact increased significantly the production rate of S-type rotifers fed on chlorella plus yeast, as was observed previously on L-type rotifers fed with yeast, vitamins and fish oil emulsion (Gatesoupe, 1989). A similar effect on the production rate of rotifers was not observed with Acosil.

In the first experiment, the amount of bacteria was low and similar levels were found in the routine production of rotifers. In the second experiment, the bacterial concentration in enriched rotifers was very high. Most bacteria in rotifers were thus produced during the enrichment phase. This throws more light on the beneficial effect of the addition of antibiotics to the enrichment medium of rotifers on the survival and growth rate of turbot (Gatesoupe, 1989). The rotifers fed and enriched with Acosil had a bacterial concentration significantly lower than the control. This may be a reason why the flounder fed with these rotifers had a higher growth rate. As Acosil had no effect on the production rate of rotifers, it can be inferred that there was no indirect effect. Japanese flounder seem very resistant to the bacterial environment, since no prophylactic care had been applied during their larval rearing. Consequently, the use of bacterial additives and a strict attention to the bacterial environment during the enrichment of rotifers are important, not only for turbot but also for much more resistant species.

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REFERENCES

Gatesoupe, F.J., 1989. Further advances in the nutritional and antibacterial treatments of rotifers as food for turbot larvae, *Scophthalmus maximus* L. In: N. de Pauw, E. Jaspers, H. Ackerfors and N. Wilkins (Editors), Aquaculture — a Biotechnology in Progress. European Aquaculture Society, Bredene, Belgium (in press).

Nicolas, J.L. and Joubert, M.N., 1986. Bactéries associées aux productions de *Brachionus plicatilis*. In: GERBAM, 2nd Int. Coll. Mar. Bacteriol. 1-5 Oct. 1984, Brest, France. IFREMER, Actes Colloq., 3: 451-457.

Sokal R.R. and Rohlf, F.J., 1969. Biometry. Freeman, San Francisco, CA, 776 pp.