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The Propagation of Cod *Gadus morhua* L.

PROPERTIES OF A NEW ARTIFICIAL DIET FOR FISH LARVAE, INCLUDING COD *Gadus morhua* L.

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ABSTRACT

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A dry feed produced from cod roe has been shown to support growth of cod larvae, although not beyond metamorphosis. Cod larvae respond to the feed by producing trypsin. Since cod roe is very rich in polyunsaturated fatty acids, which are essential for fish larvae, it is extremely sensitive to oxidation which leads to formation of toxic levels of hydroperoxides.

INTRODUCTION

During the last few years we have been developing a new starter diet for salmonids using industrial cod roe as the major raw material. Salmon fry grow up to three times faster on the new diet than on a commercial starter feed (Raa, 1982; Raa and Molvik, 1983). More important, however, is the fact that the salmon fry started to grow on our diet a few weeks earlier than on commercial ones, indicating that it embodies some principle which induces the on-set of digestive functions and growth of the larvae.

It has been suggested that the inability of dead feed to support growth of cod larvae might be due to lack of certain signal substances (Flüchter, 1982; Huse et al., 1982). Accordingly, we decided to examine whether the growth inducing principle in the salmonid feed was active also with cod larvae.

MATERIALS AND METHODS

Protein of cod roe was determined by standard Kjeldahl procedure, multiplying N-content by 6.25.

The protein content of fish larvae was calculated on a basis of amounts of free amino acids in a hydrolysate (6 N HCl, 110°C, 24 h) of the wet larvae, using an automatic amino acid analyser (JEOL, JLC-6AH).

Carbohydrate was determined by the anthrone reaction (Spiro, 1966) in dried samples hydrolysed in 98 - 100% formic acid for 24 h at 110°C.

Fatty acid composition was determined by gas chromatography after saponification and methylation of extracted lipid, according to the following procedure:

- 20 g roe, 20 ml chloroform and 40 ml methanol were mixed in a Warring Blender for 60 sec, followed by 20 ml chloroform and 20 ml water, each blended for 30 sec after addition. The chloroform phase was collected on a Buchnerfunnel and the solvent removed by evaporation in a rotavapor.
- 50 µl of the extracted lipid was saponified in 2 ml of 5% NaOH in methanol/water (1:1 v/v) by boiling for 45 min.
- The saponified lipid was neutralized by adding 0.5 ml of 10 M HCl, and then methylated by boiling in 10% BCl_3 in methanol for 30 min. The methylated fatty acids were collected by phase separation into hexane, and the solvent was subsequently evaporated under a stream of N_2.
- The fatty acid composition was finally determined by chromatography on a 6 Ft - 2 mm glass column packed with GP 10% SP 2330 on 100 - 120 mesh Chromosorb W-AW (Supelco Inc.).

Linear temperature programming from 160 to 200°C with 2 min and 16 min hold at 200°C was used. Both the detector and injector temperature was 250°C.

The trypsin(-ogen) content in the fish larvae was estimated by radioimmunoassay (Hjelmeland et al., 1984; Hjelmeland et al., in prep.).

The artificial diet was prepared from immature cod roe by boiling the fresh roe in water for 30 min before mixing the denatured roe particles with 0.5 g vitamins[1)] and a mixture of antioxidants in a vegetable oil. The moist particles were freeze dried and packed under N_2 in sealed aluminium bags.

The feeding trials were performed in small experimental plexiglass cylinders (3 l) supplied continuously with filtered (0.45 µm) seawater at 4 - 8°C. On day 5 after hatching, 200 - 300 cod larvae were transferred to these tanks and fed by hand every hour from 8.00 h until 23.00 h. Larvae (10) were sampled at regular intervals, weighed and subjected to amino acid analysis.

RESULTS AND DISCUSSION

Being rich in protein (Table 1) and long chain polyunsaturated fatty acids (Table 2), cod roe should constitute a good basis for a formulated diet for fish larvae (Cowey et al., 1976; Takeuchi and Watanabe, 1976; Leger et al., 1979; Kanazawa et al., 1982; Millikin, 1982; Watanabe, 1982).

1)
Vitamin premix contained in mg/0.5 g: 7.8 thiamine, 22.5 riboflavin, 17.0 pantothenic acid, 17.0 nicotinic acid, 7.8 pyridoxine, 1.1 biotin, 1.7 folic acid, 0.01 cobalamin, 225.0 ascorbic acid, 115.0 cholin, 35.0 inositol, 5.5 menadione sodium bisulphite, 50.0 para-aminobenzoic acid and 45.0 α-tocopherol.

TABLE 1

Chemical composition of cod roe.

	Dry weight (%)
Protein	75 - 80
Lipid	8 - 10
Carbohydrate	4 - 5

TABLE 2

The major fatty acids in cod roe.

Abbreviation	Name	%
14:0	myristic acid	2.1
16:0	palmitic acid	16.5
16:1, n-7	palmitoleic	5.4
18:1, n-9	oleic acid	14.5
20:1, n-9	gadoleic acid	4.3
20:5, n-3	5, 8, 11, 14, 17-eicosapentaenoic acid	14.4
22:6, n-6	4, 7, 10, 13, 16, 19-docosahexaenoic acid	23.3

It has, moreover, a thin chorion layer (Davenport et al., 1981) which upon proper processing of the feed will represent no significant mechanical barrier for digestive enzymes. But, due to the high content of the long chain polyunsaturated fatty acids (C-20:5,n-3 and C-22:6,n-3), the roe is very vulnerable to oxidation. Unless proper precautions are taken to protect these fatty acids, toxic levels of hydroperoxides are formed. Experiments with salmon and char fry have demonstrated that the nutritional quality of some diets based on cod roe deteriorated quickly as a result of hydroperoxide formation (Fig. 1). Retarded growth has been observed at a peroxide

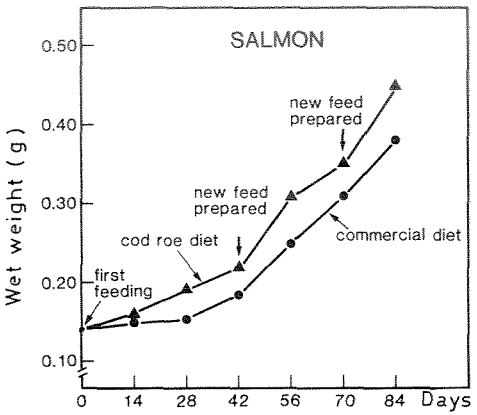

Fig. 1. Growth of salmon fry fed on commercial starter feed and on dry cod roe diet not properly protected from lipid oxidation.

value of 6 mmol per kg lipid in the cod roe diet (Gregersen, 1982). If, however, the peroxide formation was completely arrested, a feed stored for a long time (months) supported growth equally as well as the newly prepared one.

Hydroperoxides are very toxic molecules which are able to kill fish and other animals if present in the diet in consentrations above a certain level (Chow, 1979). At sub-lethal concentrations the animal is able to detoxify the hydroperoxides continuously. This metabolic detoxification is, however, energy-consuming for the larvae. This is even more critical for the small cod larvae than for much larger fry of the salmonids, which have greater resources to draw upon. Moreover at low temperatures, energy production may be insufficient to detoxify hydroperoxides. Any starter feed, and in particular one for cod larvae, should therefore be completely free from hydroperoxides.

Feeding trials in 1982 have shown that cod larvae ingested our new starter diet and grew significantly well on it, but the feed could not support growth of the larvae beyond meta-

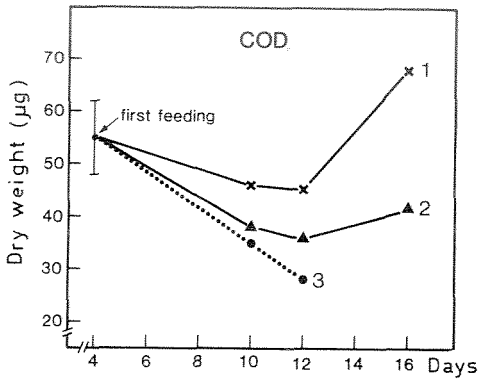

Fig. 2. Growth of cod larvae fed on dry cod roe diet (1) and standard diet (2) and starving larvae (3). After Huse et al. (1982).

morphosis (Huse et al., 1982). Our diet was also clearly better than a "standard diet" composed of hen's egg, peptone, cod liver oil and fish protein autolysate enriched with vitamins and minerals. These preliminary feeding trials thus supported the suggestion that the cod roe diet might well contain some growth inducing principle.

The repeated feeding trials during the 1983 season confirmed the first trial in 1982 (Fig. 2). The cod larvae grew on the diet, as demonstrated by direct weight gain recordings and measurements of protein content (Fig. 3). It has also been demonstrated that the cod larvae started producing trypsin when ingesting the new diet (Fig. 4), thus further supporting the suggestion that the diet indeed contains an inducer of digestive function and growth in newly hatched larvae. We have still not succeeded, however, in getting the larvae beyond metamorphosis.

Cod larvae are continuous feeders and must, therefore, have constant access to feed particles. Otherwise there is a risk of subjecting the larvae to starvation. This makes very difficult demands on any artificial diet; the particles should remain loosely suspended in the water column, intact, and not disintegrate too quickly. Until now we have not been able to

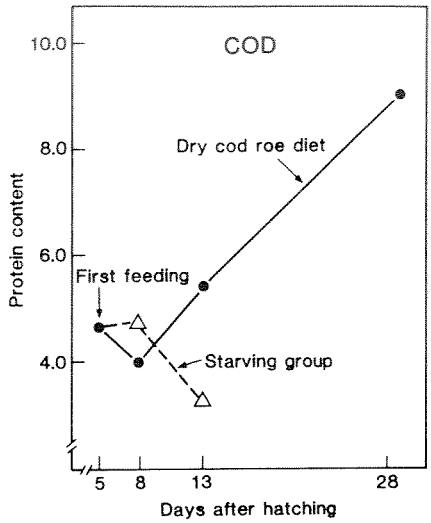

Fig. 3. Relative protein content of feeding and starving cod larvae.

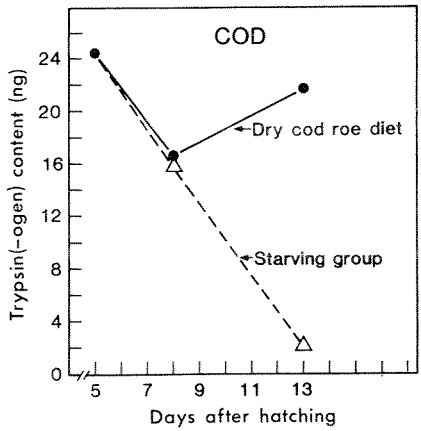

Fig. 4. Trypsin(-ogen) content of feeding and starving cod larvae.

construct a dry or moist pellet which will remain in the water column like a planktonic organism.

In conclusion, we have not yet come to a final breakthrough in our attempts to develop an artificial starter diet for cod. We do believe, however, that the dry roe feed constitutes a useful basic experimental formula from which more optimal diets can be developed. There is certainly scope for improvement in the chemical composition relating to osmoregulatory functions and feeding behaviour of the larvae, and by constructing a food particle of the right buoyancy and density which will not alter the specific gravity of the larvae.

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