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A study on the use of canola oil in the feed of larval goldfish, *Carassius auratus* L.

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Abstract. Goldfish *Carassius auratus* L., larvae were raised on a diet of yeast, rice flour, vitamins and a lipid source consisting of 10% of either canola oil (CAN) or cod liver oil (COD) or a mixture of 5% of each (MIX). Survival of larvae raised on CAN or MIX diets was comparable to that of larvae raised on COD diets. In longer-term experiments, growth was superior and development more rapid in larvae receiving the COD diet.

Introduction

In the preparation of feeds for larval cyprinids, marine fish oils have frequently been used as the primary source of dietary lipid (e.g. Charlon, Durante, Escaffre & Bergot 1986; Dabrowski & Poczyczynski 1988). Marine fish oils, however, may be costly and are vulnerable to oxidation due to the presence of substantial quantities of highly unsaturated (n-3) fatty acids. Canola oil, on the other hand is comparatively inexpensive and stable. It contains about 9% 18:3(n-3) and 22% 18:2(n-6) in its fatty acid profile (Dosanjh, Higgs, Plotnikoff, Markert & Buckley 1988). When added to a diet at a level of 10%, this oil should satisfy the requirements of carps for the two families of essential fatty acids (reviewed by Dabrowski 1986). Canola oil has previously been successfully employed as a lipid supplement to the diet of salmonids (Dosanjh *et al.* 1988).

In this study, the efficacy of canola oil as a primary lipid source in the diet of a larval cyprinid, the goldfish, *Carassius auratus* L., was compared to that of a cod liver oil control. Parameters assessed were survival, weight gain and frequency of division of the swim bladder (Battle 1940), which was used as an index of development. In one experiment, fatty acid profiles of larval carcasses were also examined.

Materials and methods

Fish and incubations

Ovulated goldfish, *Carassius auratus* L., eggs were fertilized in a Tris-saline buffer and incubated in Petri dishes as described previously (Wiegand, Hataley, Kitchen & Buchanan 1989). A single female provided the eggs for each of experiments 1 and 2. Fertility was 93·0% and 95·0% in experiments 1 and 2, respectively, and the numbers of anatomically normal larvae hatching were 95·8% and 95·4% of the numbers of fertile eggs. Experiment 3 was

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performed on a pool of larvae from eggs donated by five females. Fertility of these eggs ranged from 96·8% to 99·5% and the numbers of anatomically normal larvae derived ranged from 79·6% to 96·7% of the numbers of fertile eggs ($\bar{X}$ = 88·8%).

Larvae were reared at 21° C ± 1·5 under 16L/8D photoperiod, in 850 ml of dechlorinated tap water in 1-l Berzelius beakers. Aeration was provided by an airstone immersed to a depth of 5 cm. Larvae were fed once per day, *ad libitum*. Excess food and faecal material were removed daily and about 50% of the water was changed at the same time.

The diets consisted of debittered brewer's yeast (43% by weight), brown rice four (43%), a commercial vitamin and mineral mix (4%) (New-Life Nutritional Products, Vancouver, Canada), and 10% of either canola oil (CAN) or cod liver oil (COD) or a mixture of 5% of each (MIX). Total crude lipid content of the diets was 11·5%. The dietary components were mixed manually and particle size ranged from about 10 to 500 μm. The largest particles (rice flour) were roughly rectangular in shape whereas smaller particles (rice flour or yeast) were more spherical. A variety of yeasts have been successfully employed in the culture of cyprinid larvae (e.g. Charlon *et al.* 1986; Dabrowski & Poczyczynski 1988) and rice flour has been used in the rearing of goldfish (Battle 1940).

Experiments

Experiment 1. Twenty-five larvae were placed in each of six beakers one day post hatch on 30 April 1991. Three groups each were fed the CAN and COD diets for 8 days at which time they were killed with tricaine methane sulphonate anaesthetic, counted and stored at −70°C. A sample of larvae was also taken at the initiation of the experiment.

Experiment 2. Forty larvae were placed in each of nine beakers 2 days post hatch on 8 May 1991 (day 0). The CAN, COD and MIX diets were fed to three groups of larvae each. Samples of nine larvae were taken from each beaker on days 8 and 14 and the remaining larvae were killed and stored on day 27. A sample was also taken and stored on day 0.

Experiment 3. Twenty-five larvae were placed in each of eight beakers, two days post hatch, on 12 May 1992. Four groups each were fed the CAN and COD diets. The larvae were killed on day 25; an initial sample was also taken on day 0.

Analyses

Dry weights of individual larvae were determined by drying to a constant weight at 63°C on pre-weighed pieces of aluminium foil. A six-place, Mettler 'Micro Gram-atic' balance was used for this purpose.

In experiment 2, the fatty acid profiles were determined for both eviscerated, day 27 larval carcasses and day 0 larvae immediately upon thawing. Extraction of larval lipids and the preparation and capillary gas chromatographic analyses of fatty acid methyl esters were performed as described previously (Wiegand, Kitchen & Hataley 1991). Samples of the feeds were processed the same way. To estimate fatty acid mass in the larval bodies, a known mass of tritridecanoin was added to the extracts as an internal standard.

At termination of experiments 2 and 3, all larvae were examined for separation of the swim bladder into two chambers (Battle 1940).

Statistical analysis

Analysis of variance was performed on dry weight data after logarithmic transformation. If *ANOVA* revealed significance, group means were compared with Duncan's New Multiple Range Test. Significance was accepted at $P < 0{\cdot}01$. Frequencies of division of the swim bladder and of mortality were compared using Model II, 2×2 contingency tables, and χ^2 test of independence (Sokal & Rohlf 1969).

Results

Experiment 1. Survival of larvae to termination of day 8 was identical for fish fed the CAN and COD diets, 94·7% ± 2·3 (SD). Dry weights were determined for 10 larvae from each beaker and 10 larvae from the initial samples. There were no significant differences in dry weights among the three groups of larvae fed either diet, or between the CAN- and COD-fed fish. Dry weights of both the CAN (215 μg ± 8 (SE), $n = 30$) and COD (220 μg ± 9, $n = 30$) larvae were significantly higher than those of the larvae sampled at initiation of the experiment (127 μg ± 7) ($P < 0{\cdot}01$).

Experiment 2. Total mortality, expressed as a percentage of larvae initially placed in the beakers averaged 6·7% ± 2·9 (SD), 11·7% ± 2·9, and 10·0% ± 4·3 for the CAN, COD and MIX larvae, respectively.

Dry weights were determined for up to nine larvae for the day 8 and 14 samples from each beaker and up to 8 larvae from day 27. Several larvae were damaged in handling reducing the numbers in some samples. At no sampling time were there any significant differences in dry weights among the three groups of larvae receiving a particular diet. Data for the three groups fed each diet at each time were thus pooled for further analysis. On day 14, dry weights of larvae fed each of the diets were significantly greater than those of corresponding larvae on days 0 and 7 (Fig. 1). Similarly, on day 27, dry weights were significantly higher than on the three previous sample days. In addition, on day 27, dry weights of COD larvae were significantly higher than those of larvae receiving the other two diets.

Division of the air bladder was visible in 47 of 52 COD larvae sampled on day 27. This proportion was significantly greater than in the CAN (29 of 58) or MIX (34 of 54) larvae. The latter two groups were not significantly different from each other.

The fatty acid profiles of day 27 larval carcasses reflected those on the respective diets (Table 1). Larvae that received the CAN diet had comparatively high proportions of monoenes and (n-6) polyunsaturates, especially 18:1(n-9) and 18:2(n-6), in their carcass lipids. Larvae that received the COD diet had high proportions of saturates and (n-3) polyunsaturates, especially 16:0 and 22:6(n-3). Fatty acid proportions in the MIX larvae were typically intermediate between those in the other two groups.

For all three groups of fish, proportions of 16:0, 18:0, 20:4(n-6) and 22:6(n-3) in carcass lipids exceeded the proportions in the respective diets. Alternatively, proportions of 18:1(n-9), 18:2(n-6) and 18:3(n-3) were consistently lower in the carcass lipids than in the dietary lipids. The MIX and COD larval carcasses also had lower proportions of 20:5(n-3) than did the respective diets.

The total fatty acid mass of the initial controls was determined to be 22·9 μg per larva. The fatty acid masses of the eviscerated day 27 larval carcasses were 74·2 μg per larva for both the CAN and MIX larvae and 114 μg per larva for the COD larvae. The average coefficient of variation for fatty acid mass determination was 6·0%.

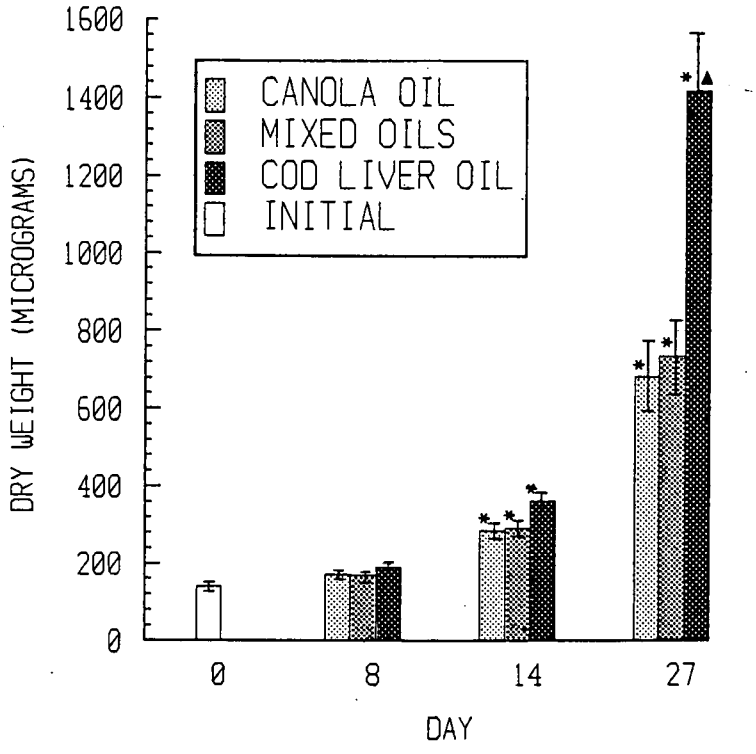

Figure 1. Dry weights (± SEM) of larvae from initial sample day 0 (n = 9) and larvae fed diets containing the three different lipid sources experiment 2 (n = 22–26). Asterisks – Significantly ($P < 0{\cdot}01$) greater than previous samples of larvae fed the same diet and day 0 larvae. Triangle-Significantly ($P < 0{\cdot}01$) greater than other groups the same day.

Experiment 3 Survival of larvae fed the CAN diet averaged 88·0% ± 0·0. There were no significant differences in dry weight among the four CAN groups and data were therefore pooled for further analysis. Mean dry weight of the CAN larvae on day 25 was 523 μg ± 44 (n = 32). This was significantly higher than the dry weight of the initial controls, 138 μg ± 5 (n = 10). Division of the swim bladder was observed in six of 88 CAN larvae.

Survival and growth of the four groups of COD larvae were quite variable. In one group, designated COD–LOW, the survival rate of 60% was significantly lower than that of the remaining COD groups, designated COD–R, which was 86·7% ± 6·1 (SD). None of the COD-LOW larvae had divided swim bladders and their dry weight, 340 μg ± 38 (n = 8), was significantly lower than that of the COD–R larvae, 708 ± 64 (n = 24). Division of the swim bladder was observed in 11 of 65 COD–R larvae on day 25. Dry weights of both COD–LOW and COD–R larvae were significantly higher than those of the initial controls. No reason for the poor performance of the COD-LOW group was apparent.

The differences in both dry weights and frequencies of swim bladder division of the COD–R and CAN larvae approached significance, $0{\cdot}01 < P < 0{\cdot}05$.

Discussion

This study demonstrates that canola oil can be an adequate lipid source for the feed of larval

goldfish, at least in the short term of 1 to 2 weeks post hatch. Over the longer term of 3 to 4 weeks, survival can be expected to remain high in larvae fed canola oil but superior growth and more rapid development can be expected with a marine fish oil. These latter trends were obscured somewhat in experiment 3, presumably due to the more heterogeneous nature of the larval population than that in experiment 2.

As with other studies of fish nutrition, carcass fatty acid profiles in experiment 2 reflected those of the diets. It should be noted, however, that four fatty acids, 16:0, 18:0, 20:4(n-6) and 22:6(n-3), were found in higher proportions in the larval carcass lipids than in the respective feed lipid. These same fatty acids are incorporated into body tissues during embryonic

Table 1. Fatty acid compositions[a] of the feeds and larval bodies in experiment 2

	Feed[c]			Larval bodies[c]			
Fatty acid[b]	CAN	MIX	COD	INIT	CAN	MIX	COD
14:0	0·1	2·2	4·3	0·9	1·1	1·4	1·9
16:0	6·1	10·7	14·7	22·4	12·1	16·0	16·5
16:1(n-9)	0·04	0·1	0·1	1·3	2·0	1·6	1·0
16:1(n-7)	0·4	3·0	5·3	2·7	1·9	3·2	4·0
18:0	2·7	3·1	3·5	7·2	6·1	7·9	7·6
18:1(n-11)	–	d	0·3	1·2	–	d	0·6
18:1(n-9)	53·3	38·6	22·8	10·8	33·2	21·5	14·9
18:1(n-7)	2·7	3·0	3·2	3·5	3·1	3·1	3·2
18:2(n-6)	21·6	15·5	9·0	2·2	10·3	6·8	4·7
18:3(n-6)	–	0·1	0·3	0·2	2·4	0·8	0·6
18:3(n-3)	8·0	5·2	2·4	0·2	1·8	1·4	0·8
18:4(n-3)	0·03	0·8	1·6	0·1	0·8	0·3	0·5
20:0	0·6	0·4	0·3	0·2	0·3	0·2	0·2
20:1(n-11)	–	0·5	1·0	0·2	–	0·2	0·5
20:1(n-9)	1·5	2·0	2·5	1·5	1·3	1·2	1·4
20:3(n-6)	–	0·05	0·1	1·6	1·9	1·2	0·7
20:4(n-6)	–	0·4	0·8	5·4	3·3	2·8	2·2
20:4(n-3)	–	0·2	0·5	0·2	0·3	0·2	0·4
20:5(n-3)	–	4·2	9·0	3·2	0·5	2·9	5·6
22:1(n-11)	–	0·9	1·8	–	–	0·1	0·3
22:1(n-9)	0·6	0·5	0·3	–	0·2	0·1	0·1
22:4(n-6)	–	0·06	0·1	0·7	0·3	0·2	0·2
22:5(n-6)	–	0·1	0·2	1·4	1·4	0·9	0·9
22:5(n-3)	–	0·6	1·4	2·0	0·7	1·3	1·9
22:6(n-3)	–	3·0	6·7	23·5	6·9	16·6	20·0
Σ saturates	9·7	17·0	23·6	31.6	20·5	26·5	27·3
Σ monoenes	58·6	48·8	37·9	21·8	42·5	31·8	26·8
Σ (n-6)	21·7	16·3	10·6	11·9	19·9	12·9	9·5
Σ (n-3)	8·0	14·0	21·7	29·2	11·0	22·8	29·4
Σ unknowns	2·0	3·8	6·2	5·6	6·1	6·0	7·0

[a] Values are mean weight percentage for triplicate analyses. Coefficients of variation for individual fatty acids were generally less than 3%.

[b] Only fatty acids comprising ≥ 0·5% in any one sample are listed. Other fatty acids identified were 12:0, 15:0, 16:1(n-5), 17:0, 18:1(n-5), 20:1(n-7), 20:2(n-6), 20:3(n-3).

[c] Feeds or bodies of day 27 larvae that received feeds containing canola oil (CAN), a mixture of canola and cod liver oils (MIX) or cod liver oil (COD). INIT — larvae sampled at initiation of feeding.

[d] Not resolved from 18:1(n-9).

development in higher proportions than those present in the original yolk (Wiegand *et al* 1991). Similarly, 18:1(n-9), 18:2(n-6) and, in the case of larvae fed MIX or COD diets, 20:5(n-3), were found in lower proportions in larval carcass lipids than in feed lipid. These latter fatty acids are also preferentially depleted during embryonic and early larval development in goldfish (Wiegand *et al.* 1991). Although some aspects of the fate and functions of individual fatty acids in fish are known (Henderson &Tocher 1987), these consistent trends indicate that the subject is worthy of more study.

One possible problem with canola oil as a primary lipid source in larval diets is the ability of the larvae to produce adequate amounts of longer chain (n-3) polyunsaturates. Canola oil contains neither 22:6(n-3) nor 20:5(n-3), of which both are found in marine oils, and the latter is found in freshwater ecosystems (Henderson & Tocher 1987). Estimation of the mass of 22:6(n-3) on the basis of data from the total fatty acid mass determination and from Table 1 yielded masses of 5·4 μg and 5·1 μg 22:6(n-3) per larva from the day 0 larvae and day 27 eviscerated carcasses of the CAN larvae respectively. This suggests that either larvae have some capacity to synthesize 22:6(n-3) or that there is almost no turn over of this fatty acid derived from the yolk. Longer studies are required to resolve this question. It is also noteworthy that the proportions of 22:6(n-3) in the CAN larval carcasses were similar to those in some wild freshwater fish samples (Henderson & Tocher 1987) but that those of 20:5(n-3) were much lower. It remains to be determined whether or not this is a significant deficiency. A MIX-type diet may thus be the best alternative.

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