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Essential fatty acids in the diet of silver perch (*Bidyanus bidyanus*): effect of linolenic and linoleic acid on growth and survival

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Abstract

The development of more cost-effective feeds for silver perch, depends on a better understanding of the nutrient requirements of this species. The dietary requirements of silver perch for linoleic acid (LA) and α-linolenic acid (ALA) were examined in a 4×4 factorial experiment, with diets containing incrementally increasing proportions of LA (10%, 20%, 30%, 40% of total fatty acids (TFA)) and ALA (0%, 10%, 20%, 30% of TFA) and only trace amounts of the highly unsaturated fatty acids (HUFA). Three additional treatments were included in the study: a reference diet (FF) that contained significant amounts of HUFA, a defatted reference diet (DF), and a defatted reference diet with its lipid composition reconstituted to mimic fatty acid composition of the FF diet (FF-DF). The lipid content was kept constant across all diets (100 g kg^{-1}), except for the DF diet (22 g kg^{-1}). After feeding the experimental diets to silver perch fingerlings (mean initial weight.1.8 ± 0.12 g) for 57 days, weight gain was highest in fish fed the diets containing HUFA: FF diet (12.0 ± 1.13 g) and FF-DF diet (10.7 ± 0.67 g). Fish fed the DF diet had the lowest weight gain (7.0 ± 0.96 g). The growth rate of silver perch fed the LA/ALA series of diets increased with increasing dietary LA content to a maximum when the LA content was about 27% of TFA, after which the growth rate declined with further inclusion of LA. Silver perch did not appear to respond to dietary ALA. Feed conversion ratio (FCR) did not vary significantly with changes to dietary LA or ALA content, but was significantly better with the diets containing HUFA. Survival was >80% in all treatments and did not vary significantly with treatment. These results demonstrate that silver perch require the dietary total lipid content to be greater than 22 g kg^{-1}. In the absence of HUFA, and with a total lipid

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content of 100 g kg^{-1}, juvenile silver perch require a dietary LA content of 27% of TFA (equivalent to 17 g kg^{-1} of diet) for optimum growth. There appears to be no nutritional benefit in formulating silver perch diet to contain a specific level of ALA when the diet contains some LA (>8% of TFA or >5.3 g kg^{-1} of diet). However, the enhanced weight gain of the fish fed the FF and FF-DF diets demonstrates that silver perch have a requirement for some or all of the HUFA present in fish oils.
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1. Introduction

The essential fatty acids (EFA) for fish are broadly recognised to comprise polyunsaturated fatty acids (PUFA) with carbon chain lengths of 18 and HUFA with carbon chain lengths of 20 and 22, of both the *n*-3 and *n*-6 series. These fatty acids cannot be synthesised by the fish de novo, though the 18-carbon PUFA can be converted by some species to longer-chain, more highly unsaturated fatty acids of the same series (Sargent et al., 1989). Hence, these fatty acids must be provided in the diet to meet the fishes′ requirements. Fish use EFA for normal growth and for cellular structure and function, including the maintenance of membranes and eicosanoid metabolism (Henderson and Tocher, 1987; Shepherd and Bromage, 1988). A dietary deficiency of EFA is manifested as poor growth, increased water content of the muscle, high liver lipid content, poor feed efficiency, shock syndrome, fin erosion, mitochondrial swelling and a decrease in haemoglobin (Stickney and Andrews, 1972; Castell et al., 1972a,b; Watanabe et al., 1974).

A major difference appears to exist between the EFA requirements of freshwater fish and those from a marine habitat. In general, freshwater fish require either linoleic acid (LA, 18:2*n*-6) or linolenic acid (ALA, 18:3*n*-3) or both of these fatty acids, while marine fish require a dietary source of HUFA, mainly eicosapentaenoic acid (EPA, 20:5*n*-3) and/or docosahexaenoic acid (DHA, 22:6*n*-3) (Sargent et al., 1989). Even within the freshwater fish species, there are large variations in EFA requirements, with some species requiring EPA and DHA whereas others, including Nile tilapia and Zilli's tilapia, appearing to not need these HUFAs (Sargent et al., 1989). In contrast to the earlier findings with tilapia, recent studies by Chou and Shiau (1999) and Chou et al. (2001) have demonstrated that hybrid tilapia, in addition to their requirement for LA, have a requirement for EPA and/or DHA.

Silver perch is an omnivorous, freshwater species of fish. The quantitative requirements of this species for EFA have not yet been determined. Anderson and Arthington (1989) found that they accumulated dietary fatty acids in both the depot fat and the phospholipids. When the fish were transferred to a fat-free diet the rate of loss of LA, ALA, EPA and DHA was slow and there appeared to be a preferential incorporation of the HUFAs, arachidonic acid (AA, 20:4*n*-6) and DHA, into the phospholipid. These results suggest a requirement for both *n*-3 and *n*-6 fatty acids. Anderson and Arthington (1992) also found that silver perch have some ability to chain elongate and desaturate dietary LA and ALA to their longer chain analogues. Our hypothesis was that silver perch would be similar to tilapia, with a requirement for both LA and ALA but that it would not require dietary HUFA for optimal growth. In this study we have determined the requirements of silver perch for LA

and ALA, essentially in the absence of HUFA. Additionally, through the inclusion of a reference diet, we have also been able to clarify whether silver perch have a requirement for a dietary source of HUFAs.

2. Materials and methods

2.1. Experimental design and diet formulation

The study comprised a 57-day growth assay with 19 dietary treatments with four replicates, randomly allocated to 76 tanks each stocked with 8 fish (mean initial weight 1.8 ± 0.12 g). The diets contained the same amount of protein (450 g kg^{-1} dry matter basis) and, with the exception of one reference diet, all the diets had the same total lipid content (100 g kg^{-1} dry matter basis). Sixteen of the diets comprised a 4×4 factorial design with incrementally increasing content of LA (10%, 20%, 30%, 40% of total fatty acids (TFA)) and ALA (0%, 10%, 20%, 30% of TFA) and with only trace amounts of HUFA. The remaining three treatments consisted of reference diets: (i) a full-fat version of the Allan et al. (2000) 95LC2 diet (FF), that contained marine lipid and associated HUFA (Table 1); (ii) a defatted

Table 1
Composition of base diets used in silver perch essential fatty acid requirement study

Ingredient (g kg^{-1}, as used basis)	Full fat 95LC2	Defatted 95LC2[a]
Fish meal (69% CP)[b]	50.0	49.9
Meat meal (53% CP)[c]	368.8	374.4
Corn gluten meal (60% CP)	51.9	52.5
Canola meal (30% CP)	50.0	54.6
Peanut meal (39% CP)	50.0	54.6
Field peas (dehulled, 24% CP)	103.9	113.4
Lupins (dehulled, 41% CP))	73.6	76.3
Mill run (21% CP)	202.0	205.0
Cod liver oil	32.1	0.0
DL-methionine	2.7	2.9
Vitamin Premix[d]	7.5	8.2
Mineral Premix[e]	7.5	8.2
Nutrient (g kg^{-1} on dry-matter basis)		
Ash	172	176
Crude protein	433	463
Total lipid	101	24
Gross energy (MJ kg^{-1})	18.5	17.6

[a] Fish meal, meat meal, corn gluten meal, lupins and mill run were all defatted before use, following methods described. CP = crude protein content, as used prior to defatting.
[b] Fish meal derived from an Australian jack mackerel, *Trachurus declivis.*
[c] Meat meal from rendered ovine material.
[d] As described by Allan et al. (2000).
[e] As described by Allan et al. (2000).

reference (DF) equivalent to 922 g kg^{-1} of defatted 95LC2 (Table 1) with 78 g kg^{-1} of diatomaceous earth as an inert filler; and (iii) a lipid reconstituted reference diet (FF-DF) identical to DF except that the diatomaceous earth was replaced with a lipid mixture so that its lipid content and fatty acid profile matched that of the FF diet (Table 2 and 3). The 16 test diets were formulated to specific fatty acid contents (Table 2 and 3), based on the approach used by Glencross and Smith (1999, 2001). The diets were prepared on a dry-matter basis to contain 922 g kg^{-1} of the defatted version of 95LC2 and 78 g kg^{-1} of individual lipid mixtures that contained the appropriate ratio of LA and ALA unique to each diet.

2.2. Diet preparation

Diets were formulated using low-fat ingredients so that the wide range of inclusion levels of LA and ALA could be achieved with minimal HUFA content, yet maintaining the total lipid content at a previously established, satisfactory level (Allan et al., 2000). Individual ingredients (meat meal, fish meal, lupins, mill run, corn gluten meal) were defatted using a crude extraction process involving *n*-hexane and ethanol.

In a preliminary step, 1 kg batches of Australian fish meal (Triabunna) (Gibsons, Glenorchy, Tasmania, Australia) or meat meal (Fletcher International, Dubbo, NSW, Australia) were placed in glass beakers, saturated with *n*-hexane (2 l), mixed thoroughly and allowed to settle. The solvent was then decanted and ingredients were air-dried. Subsequently, smaller batches of the pre-extracted fish meal and meat meal were extracted twice with n-hexane and twice with ethanol. In this process, the material (200 g) was vigorously mixed with solvent (400 ml) for 2 min in a high-speed blender (Waring, Model 32 BL 80). The resulting mixture was passed through a filter cloth (40 μm) to remove the excess solvent. The fish meal (and the meat meal) was air-dried and the batches combined and remixed between each extraction step. Lipid was removed from dehulled lupin meal (*L. angustifolius*, var. Gungurru), corn gluten meal and mill run using one hexane and one ethanol extraction step in the high-speed blender.

The proximate and fatty acid composition of the ingredients were determined before formulation of two batch diets: full-fat and defatted versions of a previously tested silver perch diet 95LC2 (Allan et al., 2000) (Table 1). In formulating the defatted 95LC2 diet, the inclusion rate of the ingredients was adjusted from that of the full-fat 95LC2 diet to compensate for the change in nutrient composition brought about by defatting, and to ensure that the relative proportion of protein from each ingredient was consistent among diets. Prior to formulation of the experimental diets, the proximate and fatty acid composition of the two versions of 95LC2 were determined.

All ingredients for the full-fat and defatted versions of 95LC2 were thoroughly dry mixed before being ground in a hammermill (Raymond Laboratory Mill, Transfield Technologies Pty, Rydalmere, Australia) so that all of the mixture would pass through a 710-ìm screen. Each diet batch was dry mixed again (Hobart Mixer, Troy Pty, Ltd. Ohio, USA) to ensure a homogeneous dispersion of ingredients. In the preparation of test diets (Table 2), 1.2 kg (dry basis) lots were withdrawn from the bulk mix of defatted 95LC2 and warmed in a convection oven prior to the addition of wet ingredients. Premixed oils (93.6 g) were warmed in a water bath to about 40 °C and thoroughly mixed before being

Table 2
Ingredient composition g kg^{-1} (dry matter) of experimental diets

Ingredient	Diet designation																		
	FF	FF-DF	DF	10/0	20/0	30/0	40/0	10/10	20/10	30/10	40/10	10/20	20/20	30/20	40/20	10/30	20/30	30/30	40/30
95LC2 FF[a]	1000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
95LC2 DF[b]	–	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0	922.0
Diatom. Earth[c]	–	–	78.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cod liver oil	–	24.8	–	0.6	–	–	–	0.6	–	–	–	0.6	–	–	–	1.0	1.0	–	–
Coconut oil	–	20.0	–	50.1	50.1	47.1	16.1	50.1	47.8	36.3	24.7	50.1	36.6	23.9	10.8	40.2	15.6	–	–
ALA	–	1.6	–	–	–	–	–	9.3	–	–	–	18.2	–	–	–	27.9	6.4	6.1	7.3
Oleic acid	–	–	–	27.3	5.6	–	–	18.0	–	–	–	9.1	–	–	–	8.0	–	–	–
Linseed oil	–	–	–	–	–	–	–	–	18.2	17.7	18.0	–	36.3	36.3	36.2	–	40.1	40.1	40.0
Canola oil	–	–	–	–	–	6.4	3.9	–	–	2.9	–	–	–	–	–	–	14.9	19.8	0.6
Safflower oil	–	0.8	–	–	11.2	24.0	35.3	–	8.0	21.1	34.9	–	4.5	17.8	31.0	–	–	12.0	30.1
Olive oil	–	24.3	–	–	11.1	0.5	22.7	–	4.0	–	0.4	–	0.6	–	–	0.9	–	–	–
MCT[d]	–	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fish oil TAG[e]	–	5.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

FF = Full fat reference diet, FF-DF = Reconstituted defatted reference diet, DF = Defatted reference diet. 10/0 to 40/30 are designations of test diets according to their linoleic and linolenic acid content (LA/ALA), expressed as % of total fatty acids.

[a] Full-fat 95LC2 diet (Table 1).

[b] Defatted 95LCF2 diet (Table 1).

[c] Diatomaceous earth.

[d] Medium chain triacylglycerols.

[e] Triacylglycerols.

added drop wise to the defatted 95LC2. These ingredients were mixed (Model A707a, Kenwood MGF Australia, Elizabeth, SA, Australia) for about 10 min before distilled water was added (700–800 ml) and mixing continued for a further 5 min. Reference diets were prepared in a similar manner. All diets were cold pelleted through a meat mincer fitted with a die-plate with 1.5-mm diameter holes (Barnco Australia, Leichardt, NSW, Australia). The strands of feed were dried in a convection oven at < 35 °C for approximately 5 h until moisture content was < 10%, before being broken up into ~ 5 mm long pellets, and analysed to determine the fatty acid composition (Table 3). During the course of the experiment, all treatments diets were stored in a nitrogen atmosphere to prevent possible oxidation of EFA.

2.3. Experimental management

Prior to stocking, silver perch (*Bidyanus bidyanus*) were held in 10,000-l tanks and fed a standard silver perch diet SP35 (Allan et al., 2000). Fingerlings were sedated in a bath of ethyl *p*-aminobenzoate (30 mg l^{-1}) then caught at random, weighed individually and systematically stocked into 76 aerated tanks (8 fish $tank^{-1}$). A sample of the fish used to stock the experiment was frozen and stored for later analysis. Fish that died during the experiment were replaced with weighed, fin clipped fish and these were later excluded from all estimates of fish weight gain and feed conversion ratio (FCR).

Experimental tanks (fibreglass, 70 l, rectangular, base 360 × 670 mm, depth 300 mm) were supplied with pre-heated, continuously flowing water (600 ml min^{-1}). All water was filtered of particulate material, and passed through a 2-m^3 biological filter and an ultra violet conditioning unit (Australian Ultra Violet Products, Seven Hills, NSW, Australia) before recirculation to the laboratory. Approximately 30% of the circulating water was continuously discharged as effluent. Automatically controlled fluorescent lighting provided a 12 h light:12 h dark photoperiod. All tanks were siphoned twice weekly to remove accumulated faeces. Replacement fish were held in a polyethylene tank (200 l) in the same laboratory being supplied with single pass water from the same system.

Water quality was monitored weekly by sampling from 20 tanks on a rotating basis, using a model 611 Intelligent Water Quality Analyser (Yeo-Kal Electronics, Brookvale, NSW, Australia). During the experiment, water temperature (range 25.1–27.1 °C), dissolved oxygen (6.4–8.1 mg l^{-1}), pH (between 7.9 and 8.9) and salinity (1.0–6.5 g kg^{-1}) was measured using this equipment. Nitrite and ammonia (< 40 μg l^{-1} NO_2-N and < 100 μg l^{-1} total ammonia-N, respectively) were measured using the indophenol blue colorimetric method (Parsons et al., 1984). Un-ionised ammonia concentrations, measured as ammonia nitrogen (NH_3-N) were calculated weekly from measurements of total ammonia nitrogen (total ammonia-N) (Allan et al., 1990).

Experimental diets were first fed to the fish 24 h after stocking into tanks. Fish were fed a weighed ration of their respective diets twice daily (40% at 08:15 h and 60% at 15:00 h), starting at 3% biomass day^{-1}, and thereafter the feed rates were adjusted daily to meet satiation, following a scoring protocol designed to track the satiation feed intake of each tank. Any uneaten food was collected from the tanks approximately 20 min after feeding, dried and weighed. Fish were fed for a period of 57 days after which they were weighed and killed with an overdose of anaesthetic (ethyl-*p*-aminobenzoate). Weight gain (g),

Table 3
Mean (±SEM) of total lipid (g kg^{-1} dry matter)[1] and fatty acid composition (% by weight of total fatty acids)[2] of experimental diets

Nutrient	Diet designation[a]																		
	FF	FF–DF	DF	10/0	20/0	30/0	40/0	10/10	20/10	30/10	40/10	10/20	20/20	30/20	40/20	10/30	20/30	30/30	40/30
Total lipid	100	101	22	99	97	99	100	100	100	100	100	100	100	100	100	100	100	100	100
	(1.1)	(0.5)	(0.9)	(1.5)	(0.9)	(0.6)	(0.7)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)
Sum SFA	36.4	32.1	27.1	45.9	50.5	48.3	25.1	48.1	48.9	39.4	30.8	47.4	38.8	29.5	20.2	38.3	22.5	11.7	12.3
	(0.07)	(0.01)	(0.05)	(0.09)	(0.03)	(0.08)	(0.08)	(0.07)	(0.07)	(0.05)	(0.03)	(0.09)	(0.06)	(0.03)	(0.01)	(0.12)	(0.02)	(0.01)	(0.02)
Sum MUFA	37.7	43.9	37.8	45.4	30.6	21.4	36.4	34.6	21.3	19.1	20.2	23.0	20.5	21.0	21.6	21.4	29.1	32.1	22.0
	(0.07)	(0.04)	(0.05)	(0.08)	(0.03)	(0.05)	(0.05)	(0.05)	(0.03)	(0.02)	(0.01)	(0.03)	(0.03)	(0.03)	(0.02)	(0.03)	(0.03)	(0.03)	(0.01)
LA	12.0	12.6	31.9	8.0	18.1	29.0	37.2	9.8	18.5	30.5	38.2	10.0	18.9	28.3	37.4	10.1	18.8	27.9	38.0
	(0.02)	(0.07)	(0.06)	(0.02)	(0.02)	(0.06)	(0.04)	(0.02)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.02)	(0.02)	(0.11)	(0.04)	(0.01)	(0.02)
ALA	1.3	1.7	2.2	0.6	0.7	1.2	1.2	7.3	11.2	10.8	10.7	19.3	21.7	21.2	32.1	32.1	32.1	32.1	32.1
	(0.01)	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.02)	(0.03)	(0.04)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
AA	0.5	0.4	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
	(0.01	(0.01)																	
EPA	4.9	3.7	0.3	0.1	tr	tr	tr	0.1	tr	0.1	tr	0.1	tr	tr	tr	0.1	0.1	tr	tr
	(0.02)	(0.01)	(0.01)	(0.01)				(0.01)		(0.01)		(0.01)				(0.01)	(0.01)		
DHA	4.6	3.7	tr	tr	tr	tr	0.1	tr	tr	tr	tr	0.1	tr	tr	tr	0.2	0.1	tr	tr
	(0.03)	(0.02)					(0.01)					(0.01)				(0.01)	(0.01)		
Sum n-6	12.5	13.0	31.9	8.0	18.1	29.0	37.2	9.8	18.5	30.5	38.2	10.0	18.9	28.3	37.4	10.1	18.8	27.9	38.0
	(0.02)	(0.02)	(0.06)	(0.02)	(0.02)	(0.06)	(0.04)	(0.02)	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.02)	(0.02)	(0.11)	(0.04)	(0.01)	(0.02)
Sum n-3	13.1	11.0	3.3	0.7	0.8	1.2	1.3	7.5	11.3	11.0	10.8	19.6	21.8	21.3	20.8	30.2	29.5	28.3	27.7
	(0.06)	(0.05)	(0.04)	(0.02)	(0.03)	(0.02)	(0.05)	(0.01)	(0.03)	(0.04)	(0.03)	(0.03)	(0.02)	(0.03)	(0.01)	(0.02)	(0.03)	(0.02)	(0.02)

SFA=saturated fatty acids; MUFA=monounsaturated fatty acids.

[a] See Table 2.

[1] Mean fat content determined from triplicate analyses (Folch et al., 1957).

[2] The sum of SFA, MUFA, n-6 and n-3 groupings doesn't always equal 100%, due to the presence of minor unidentified fatty acids.

apparent feed intake (AFI) (g), FCR and % survival were measured. Fish carcasses from the initial and final samples were freeze-dried and homogenised for lipid and fatty acid analysis, the results of which will be reported separately. All measured indices were reported as mean ± SEM.

2.4. Biochemical analyses

Dry matter was determined by weighing before and after drying at 105 °C for 16 h and cooling in a vacuum desiccator; ash by heating a weighed and dried sample at 550 °C for 16 h before cooling in a desiccator and re-weighing (AOAC International, 1999). Crude protein was determined by the Kjeldahl method. Gross energy was determined by isothermal bomb calorimetry using a Leco AC200 Bomb Calorimeter (Leco, St. Joseph, MI, USA). Total lipid was determined gravimetrically following a chloroform-methanol (2:1) extraction using the method of Folch et al. (1957). Fatty acids were analysed as the methyl esters (Lepage and Roy, 1986) using a flame ionisation, capillary gas chromatograph (Hewlett Packard 5890 series II, Agilent Technologies, USA) fitted with a fused silica capillary column (DB 225, 30 m × 0.25 mm, ID × 0.25 μm, J and W Scientific,

Table 4
ANOVA table with orthogonal a priori contrasts and fatty acid mean and interactive effects for weight gain, apparent feed intake (AFI, g tank^{-1}) and feed conversion ratio (FCR) of silver perch fed reference diets and diets with varying inclusions of LA and ALA comprising a 4 × 4 factorial array

Contrast	*Df*	MS	*F*	Significance
Weight gain (g)				
DF vs. Rest of data	1	13.92	5.17	< 0.05
FF + FF-DF vs. LA/ALA series	1	54.18	20.1	< 0.01
FF vs. FF-DF	1	3.54	1.31	ns
LA main	3	7.82	2.90	< 0.05
ALA main	3	0.83	0.31	ns
LA × ALA interaction	9	1.91	0.71	ns
AFI				
DF vs. Rest of data	1	735	1.04	ns
FF + FF-DF vs. LA/ALA series	1	4691	6.64	< 0.05
FF vs. FF-DF	1	27	0.04	ns
LA main	3	2227	3.15	ns
ALA main	3	766	1.09	ns
LA × ALA interaction	9	549	0.78	ns
FCR				
DF vs. Rest of data	1	1.92	67.1	< 0.01
FF + FF-DF vs. LA/ALA series	1	0.51	17.7	< 0.01
FF vs. FF-DF	1	0.08	2.79	ns
LA main	3	0.06	1.94	ns
ALA main	3	0.07	2.29	ns
LA × ALA interaction	9	0.04	1.24	ns

[1]Natural values are reported but significance of differences were obtained from arcsine-transformed data.

Folsom, CA, USA) and with hydrogen as the carrier gas. The injector and detector temperatures were set at 250 °C, and the column oven temperature was programmed to increase from 170 °C to 220 °C at 1 °C min^{-1}. Identification and quantification were by comparison with internal standards (tridecanoic acid (13:0) and heneicosanoic acid (21:0) in conjunction with fatty acid mixed standards (Nu-Check-Prep, Elysian, MN, USA).

2.5. Statistical analyses

The effect of diet on fish weight gain, AFI, FCR and survival was tested initially using a single-factor ANOVA to examine all treatment contrasts. Subsequently, the interactive effects of fatty acid type and proportion in the diet were examined as a within 4×4 factorial design (SAS Procedure GLM, SAS Institute, Cary, NC). Orthogonal comparisons (a priori) were applied to the means of treatment groupings (SAS Procedure GLM) as follows, (i) diet DF (total lipid 22 g kg^{-1}) versus all other treatments (total lipid 100 g kg^{-1}); (ii) within treatments with total lipid of 100 g kg^{-1}, diets FF and FF-DF (moderate HUFA) verses LA/ALA series (low HUFA); (iii) FF diet verses FF-DF diet, to examine the effect of the defatting process; (iv) LA main effect; (v) ALA main effect; and (vi) LA × ALA interaction. Orthogonal comparisons were made even if the overall analysis of variance failed to reach significance (Sokal and

Table 5
Mean weight gain, apparent feed intake (AFI), feed conversion ration (FCR) and survival (%) of silver perch fed reference diets and diets with varying inclusions of LA and ALA comprising a 4×4 factorial array

Contrast	*n*	Weight gain (g)	AFI (g $tank^{-1}$)	FCR	Survival (%)
DF	4	7.00[b]	150	2.72[b]	96.9
Rest of data	72	8.92[a]	137	2.01[a]	94.8
FF+ FF-DF	8	11.37[a]	159[a]	1.78[a]	98.4
LOA/LNA series	64	8.61[b]	134[b]	2.04[b]	94.3
FF	4	12.04	161	1.68	100.0
FF-DF	4	10.71	157	1.88	96.9
LOA 10	16	7.89[a]	119	2.01	93.0
LOA 20	16	8.63[ab]	136	2.11	92.2
LOA 30	16	9.55[a]	148	1.99	96.9
LOA 40	16	8.38[ab]	132	2.07	95.3
LNA 0	16	8.48	135	2.07	96.9
LNA 10	16	8.51	130	2.03	93.0
LNA 20	16	8.95	142	2.12	93.0
LNA 30	16	8.51	126	1.96	94.5
Residual SE		1.641	26.6	0.169	8.48

Within contrasts and biological responses, means with different superscript letters are significantly different ($P < 0.05$).

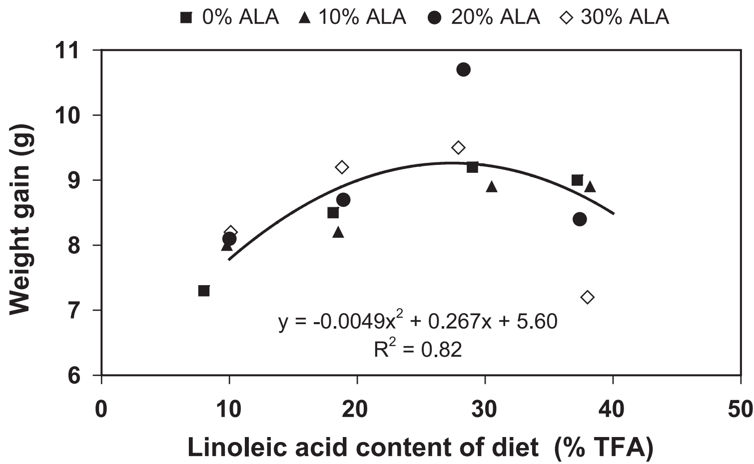

Fig. 1. Mean weight gain ($n = 4$) of silver perch in response to dietary linoleic acid content (% of total fatty acids, % TFA) in diets with different α-linolenic acid (ALA) content (% TFA).

Rohlf, 1981). Percentage survival data were analysed both before and after arcsine transformation.

3. Results

3.1. Weight gain, AFI, FCR and survival

For the a priori comparisons, feeding fish with the DF diet, which contained 22 g kg^{-1} of total lipid, resulted in significantly less weight gain (7.00 g) and poorer FCR (2.72) than

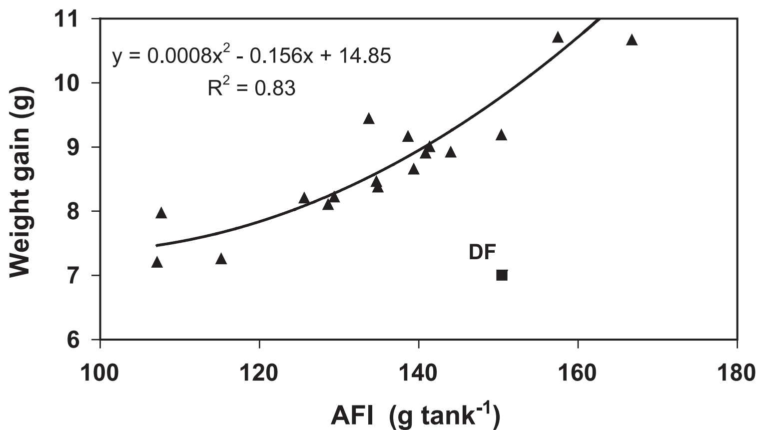

Fig. 2. Mean weight gain of silver perch from all treatments relative to apparent feed intake (AFI, g tank^{-1}). Data indicated with ■ DF refers to DF treatment, ▲ refers to all other data.

with higher lipid (100 g kg^{-1}) (weight gain, 8.82 g; FCR, 2.01) and AFI of these treatments were not significantly different (Tables 4 and 5). There were no significant differences in weight gain, AFI and FCR between fish fed diet FF and the reconstituted reference diet (FF-DF) (Table 5). There was a significant difference between the combined FF and FF-DF treatments and the rest of the 100 g kg^{-1} total lipid treatments (LA/ALA series), respectively, for weight gain (11.37 g vs. 8.61 g), AFI (159 g $tank^{-1}$ vs. 134 g $tank^{-1}$) and FCR (1.78 vs. 2.04) (Table 5).

For the within factorial comparison of fatty acid type and dietary fatty acid content, there was no interaction ($P>0.05$) between the main effects for weight gain, AFI and FCR (Table 4). Weight gain increased with increasing proportion of LA in the diet up to 27.1% of TFA, while altering the amount of ALA in the diet had no significant ($P>0.05$) effect on any of the measured production traits (Table 5, Fig. 1).

Excluding the data for the DF diet, weight gain of fish increased curvilinearly with increasing feed intake (Fig. 2). Survival was high in all treatments (95 ± 4.3%), with no significant differences among treatments (Table 5).

4. Discussion

The survival of fish in this study was high (>80%), and the growth rates on the reference diets were similar to those obtained in other studies with healthy fish of the same size under the same temperature conditions (Allan et al., 2001). Weight gain was generally quite closely related to AFI ($R^2 = 0.83$) with the exception of fish fed diet DF, where weight gain was significantly depressed with respect to AFI.

Weight gain and FCR of silver perch was best with diet FF (12.04 g, 1.68, respectively), but not significantly greater that that obtained with the reconstituted reference diet, FF-DF (10.71 g, 1.88, respectively). This was not unexpected since the formulation and gross chemical composition of the two diets were very similar, the latter differing only in the pre-extraction of lipids from the ingredients and the quantitative replacement in the formulation with an oil mixture of defined composition. This result demonstrates that the lipid extraction process itself did not significantly affect the nutritional value of the diet, thereby establishing the suitability of the defatted 95LC2 diet formulation as a basis for the formulation of the LA/ALA series of diets.

Weight gain and FCR of fish fed the DF diet were significantly poorer ($P < 0.05$, $P < 0.01$, respectively) than that of the fish diets containing 100 g kg^{-1} of total lipid, demonstrating that their dietary lipid requirement exceeds 22 g kg^{-1}. Although the dietary total lipid requirement of silver perch fingerlings appears not to have been expressly determined, satisfactory growth rates have been observed with diets containing between 54 and 92 g kg^{-1} in previous studies (Allan et al., 2000, 2001; Stone et al., 2000). The total lipid content of diets in this study (100 g kg^{-1}) was marginally higher than used in previous studies but this did not appear to have had a deleterious effect.

All diets in the LA/ALA series contained the same total lipid content but had very low levels of HUFA (< 0.1% of TFA). Increasing the concentration of LA in the diets, brought about a curvilinear improvement in fish weight gain with the asymptotic response occurring with LA at 27.1 % of TFA (Fig. 1), which equates to about 18 g kg^{-1} of diet. In contrast,

increasing the concentration of ALA in the diets from 0.6% of TFA (or 0.4 g kg^{-1} of diet) to 32% of TFA (or 21 g kg^{-1} of diet) did not result in a change in growth rate or FCR. However, the requirement for ALA in the total absence of any other PUFA or HUFA has not been tested in this study. LA was present in all the diets of the LA/ALA series (minimum content 8% of TFA, or approximately 5.3 g kg^{-1} of diet), but there were only traces of other PUFA and HUFA present. As fish are unable to convert LA to ALA, and as there was no interaction between dietary LA and LNA in growth response, AFI or FCR, it is unlikely that the LA content of the diets would have affected the observed response to ALA.

There was a marked reduction in weight gain at the highest inclusion level of LA (40% of TFA, or 23 g kg^{-1} diet), particularly when the ALA content was also at the maximum inclusion level (30% of TFA), (Fig. 1). This observation is consistent with the findings of Takeuchi and Watanabe (1979) who reported that excess amounts of dietary ALA (80% of TFA) and total *n*-3 PUFAs (40% of TFA) resulted in poor weight gain and low feed efficiency in rainbow trout. These results suggest that silver perch have a requirement for LA with the optimum at about 27% of TFA, but that the requirement for ALA is considerably lower. This result is consistent with the findings of Takeuchi et al. (1983) with Nile tilapia.

Recent studies with hybrid tilapia (Chou and Shiau, 1999; Chou et al., 2001) have demonstrated a requirement for dietary LA and EPA/DHA but not ALA. This has also been demonstrated in other freshwater species: ayu (Kanazawa et al., 1982) and channel catfish (Satoh et al., 1989). Though the FF and FF-DF diets and the LA/ALA series of diets all contained the same amount of total lipid, the FF and FF-DF diets contained much higher levels of the *n*-3 series HUFAs. The EPA and DHA content of the FF diet was 4.9% and 4.6% of TFA (or about 2.9 and 2.7 g kg^{-1} of diet), respectively, and FF-DF diet contained 3.7% of TFA for both EPA and DHA (or 2.0 g kg^{-1} diet). In contrast, there was less than 0.1% of TFA (or 0.06 g kg^{-1} of diet) of either EPA or DHA in each of the LA/ALA series of diets, and only traces of AA. In addition, the LA and ALA content of the FF and FF-DF diets were each about 12% of the TFA (or about 7 g kg^{-1} of the diet), which was well below the level that resulted in the greatest weight gain in the LA/ALA series of diets. The weight gain of fish fed these two treatments was significantly greater, and FCR significantly better than for any of the diets comprising the LA/ALA series, demonstrating a requirement for EPA and DHA, and possibly AA. In the treatments with low levels of LA and high levels of ALA, the balance of fatty acids would tend to favour the conversion of ALA to EPA and DHA via the $\Delta 6$ desaturase pathway (Sargent et al., 1989). However, in contrast to the diets containing EPA and DHA, the performance of these diets was poor. This would suggest that though silver perch may be the capacity to chain elongate and desaturate ALA to EPA and DHA (Anderson and Arthington, 1992), the rate at which this happens is insufficient to meet the fish’s requirements for rapid growth.

A strong relationship between the growth of the fish and AFI was seen in this study, with the exception of the fish fed the DF diet. The apparent feed intake was greater, and FCR values were significantly better for the FF and FF-DF diets in comparison to the LA/ALA series of diets, which remained relatively constant. These results demonstrate that there was a nutritional deficiency in the LA/ALA series of diets. They also show that the fish fed the LA/ALA series did not increase their feed intake to overcome the deficiency but suggest

that the fish were consuming as much feed as their physical capacity would allow, at each of the two daily feeding sessions.

This study has shown that silver perch, when fed diets containing 100 g kg^{-1} of total lipid which contains little or no HUFA, have a requirement for LA of the order of 27% of TFA (or about 18 g kg^{-1} of diet) and a much lower requirement for ALA. The study has also demonstrated the importance of the HUFA present in cod liver oil for improving growth in silver perch. The presence of AA in the diets containing HUFA, albeit at low levels (0.5% of TFA), should not be overlooked. Given the lack of response of silver perch to the *n*-3 series fatty acid, ALA, and positive response to LA, it is possible that the response seen with the inclusion of cod liver oil was due to the presence of AA in these diets (Castell et al., 1994). Further research is warranted to identify the key fatty acids(s) and to determine the optimal amount of HUFA in the diet. As LA is one of the *n*-6 series of fatty acids and cannot be converted to the *n*-3 series, it is highly likely that the requirement for LA would not alter appreciably with the addition of EPA and DHA to the diet. However, it could alter if AA is included in the diet, but the apparent inability of silver perch to meet EPA/DHA requirements from ALA would suggest that conversion of LA to AA would be also at a rate insufficient to meet requirements. Hence, in studies to determine the optimum dietary contents of AA, EPA and DHA, it would be appropriate for the LA content to be fixed at 27% of TFA and the ALA content set at some arbitrary, though relatively low level.

There has been much interest in reducing the amount of fish meal and other ingredients of marine origin in silver perch diets (Allan et al., 2000; Stone et al., 2000). However, it is clear from these studies that for optimal growth, diets for silver perch must be formulated to contain at least some of the HUFA present in fish oils, which implies including in the diet formulation an ingredient of marine origin that has a significant lipid content. However, the optimal levels of EPA, DHA and AA in the diet for silver perch remain to be established.

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