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Changes in fatty acid concentrations in tissues of African catfish, *Clarias gariepinus* Burchell, as a consequence of dietary carnitine, fat and lysine supplementation

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A study was undertaken to examine the effect of different dietary carnitine (200 and 1000 mg/kg diet) and fat (90 and 190 g/kg diet) supplementation on growth and fatty acid concentrations of fish fed either with a low- (13 g/kg) or a high-lysine (21 g/kg) diet. African catfish (22·7 g/fish), *Clarias gariepinus* Burchell, juveniles were stocked (sixteen aquaria, twenty-five fish per aquarium) and fed for a maximum of 74 d. Dietary lysine had a clear effect on growth performance and feed conversion ratios, but dietary carnitine supplements had no effect. High-carnitine supplements increased total carnitine content ($P<0·0004$) and reduced tissue free carnitine: acyl-carnitine ratio ($P<0·05$) compared with low-carnitine supplements. High-fat supplements decreased liver carnitine concentrations. Clear effects on liver fatty acid concentrations were observed in high-carnitine-fed fish compared with low-carnitine-fed fish. The primary liver fatty acids affected were 18 : 2*n*-6 (linoleic acid), 20 : 5*n*-3 (eicosapentanoic acid) and 22 : 6*n*-3 (docosahexanoic acid). The whole-body fatty acid balance suggested that 20 : 5*n*-3 disappeared (apparently by β-oxidation) more readily than 18 : 2*n*-6 and/or 22 : 6*n*-3. From 774 mg 20 : 5*n*-3 eaten by high-lysine–high-fat–low-carnitine fish, 58 % was not assimilated into body tissues. High-carnitine-fed fish showed an increase in 20 : 5*n*-3 oxidation by 7 % compared with low-carnitine fish. Although dietary carnitine did not improve body growth, these results support the hypothesis that carnitine can enhance the mobilisation of long-chain fatty acids towards oxidation.

Carnitine: African catfish: Fatty acid balance: Lysine

Carnitine is synthesised in the liver from the amino acid lysine with methionine acting as a methyl donor (Broquist, 1997). It functions as a cofactor for the transport of fatty acids into the mitochondrial and thus facilitating the use of fatty acids for energy. Since total excretion rate of carnitine in mammals can reach 10·4 μmol/d (Brooks & McIntosh, 1975) and synthesis alone is not always sufficient (Cederblad & Lindstedt, 1976) fatty acid oxidation may become impaired if carnitine and/or its precursors are not supplemented in the diet. According to Van Kempen & Odle (1995) the capacity of fatty acid oxidation in neonatal pigs depends on dietary carnitine supplementation. Considering that carnitine supplements increase import of fatty acids into the mitochondria, it may be that carnitine can indirectly prevent protein from catabolism. Thus, animals fed diets with elevated carnitine and fat contents may have more protein energy available for growth. Early work of Bilinski & Jonas (1970) indeed showed that free fatty acid oxidation by trout muscle mitochondria was highly activated by the addition of carnitine. In the past decade evidence was provided both to support and to reject this hypothesis. Rabie & Szilagyi (1998) and Heo *et al.* (2000) also demonstrated that in broilers and piglets weight gain and fatty acid oxidation were improved by dietary carnitine. Gropp *et al.* (1994) showed that this effect, however, was more apparent when lysine and/or methionine were marginally present in quail. A number of studies in mammals have shown that lysine deficiency can diminish carnitine content of body tissue (Sachan & Mynatt, 1993; Krajcovicova-Kudlackova *et al.* 2000). Effects of dietary carnitine on growth and lipid oxidation was also reported in several aquaculture species, e.g. African catfish (*Clarias gariepinus* Burchell), Red Sea

Abbreviations: FAME, fatty acid methyl ester; FCR, feed conversion ratio; HSI, hepatosomatic index; PUFA, polyunsaturated fatty acid; SGR, specific growth rate.

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Table 1. Formulation of experimental diets

	Low-lysine				High-lysine			
	Low-fat		High-fat		Low-fat		High-fat	
	Low carnitine	High carnitine	Low carnitine	High carnitine	Low carnitine	High carnitine	Low carnitine	High carnitine
Ingredients (g/kg)*								
Premix†	25	25	25	25	25	25	25	25
Wheat meal	131	131	209	209	466	466	400	400
Corn gluten meal	180	180	308	308	–	–	–	–
Meat meal	–	–	–	–	90	90	132	132
Blood meal	–	–	–	–	17	17	–	–
Feather meal	100	100	0	0	100	100	100	100
Menhaden meal	150	150	150	150	150	150	150	150
Chalk	83	81	79	77	67	65	56	54
Capelin oil	55	55	120	120	55	55	106	106
Durabon binder	26	26	24	24	25	25	25	25
Gelatinized cornstarch	250	250	85	85	–	–	–	–
L-Lysine.HCl	–	–	–	–	5	5	6	6
Carniking‡	–	2	–	2	–	2	–	2
Crude protein	353	353	367	367	353·5	353·5	354·5	354·5
Crude fat	90	90	190	190	90	90	190	190
Ash	132	132	127	127	135·5	135·5	128	128
Carbohydrate§	372	372	257·5	257·5	374·5	374·5	275	275
Lysine	13·3	13·3	13·8	13·8	21·8	21·8	22·0	22·0
Carnitine	0·2	1·0	0·2	0·9	0·2	1·0	0·2	0·9

* All ingredients, with exception of Carniking, and formulations were provided by Provimi (Rotterdam, The Netherlands).
† Contained (per kg mixture): Vitamin A 774 mg, vitamin D 6·35 m, vitamin E 20 000 mg, $CuSO_4$ 501 mg, $ZnSO_4$ 15 000 mg, $MnSO_4$ 0·001 mg, $CoSO_4$ 500 mg, KI 500 mg, Na_2SeO_3 35 mg.
‡ Contained (g/kg): L-carnitine 500, silica 350, water 150 (Lonza Group Ltd, Basel, Switzerland).
§ Carbohydrate data were estimated from the measured values of DM, crude protein, crude fat and ash according to: carbohydrate=DM−crude protein−crude fat−ash (modified by Cloet and Heinsbroek, unpublished results from Brafield, 1985).

bream (*Pagrus major*), white prawn (*Penaeus indicus*) (Torreele *et al.* 1993; Chatzifotis *et al.* 1995; Jayaprakas & Sambhu, 1996 respectively). In contrast to these studies, extra carnitine did not alter growth or body lipid composition in tilapia, salmonids, ornamental cichlid fish (*Pelvicachromis pulcher*) and hybrid striped bass, *Morone chrysops* female × *M-saxatilis* male (Becker *et al.* 1999; Rodehutscord, 1995; Ji *et al.* 1996; Harpaz *et al.* 1999; Gaylord & Gatlin, 2000). These difference of results between species suggest that the effects of dietary carnitine supplements are associated with different factors such as age and feed composition. In addition, metabolic requirements of the species under study may be important for occurence of the effect.

Similar to the study of Gropp *et al.* (1994), we hypothesised that growth and fatty acid concentrations of fish tissues are positively related to dietary carnitine levels. Carnitine supplementation is expected to increase fatty acid oxidation and as a result protein:fat ratio in African catfish body will increase. In addition, one may expect that nutritional conditions, which give decreased carnitine synthesis, e.g. dietary lysine deficiency, will enhance the effect of dietary carnitine.

Since in the effects of carnitine on metabolism and concentration of long-chain fatty acids is most obvious, we decided to study C_{14}–C_{22}, as defined by Odle (1997), in tissues of the African catfish.

Material and methods

Experimental animals and husbandry

This experiment was approved by the Dutch Experimental Animal and Welfare Committee according to the EC Directive applied to vertebrate animals.

African catfish (10 g) were obtained from a Dutch commercial catfish hatchery (Fleuren, Someren, The Netherlands). All fish were full siblings and had an identical nutritional history.

The experiment was conducted at the experimental facilities of the Fish Culture and Fisheries Group, Wageningen Institute of Animal Science (WIAS), Wageningen-UR. After arrival, the fish were equally distributed over four aquaria at a density of approximately 230 fish per 70 litre aquarium. The animals were allowed acclimatisation to the recirculation system conditions for 1 week. During this acclimatisation week, fish were fed on a low-carnitine–low-lysine–low-fat diet at a feeding rate of $24\,g/kg^{0{\cdot}8}$ per d.

After acclimatisation, fish of uniform size (22·7 g) were randomly assigned to each of sixteen aquaria, twenty-five fish per aquarium. The aquaria used were glass aquaria ($70 \times 35 \times 40$ cm) with a 90 litre capacity, filled to 70 litres, and a 25 % refreshment once per week. Each aquarium was connected to a recirculation system with capacity of $8{\cdot}25\,m^3$ water, and checked daily for dead fish. The flow

Table 2. Fatty acid concentrations of experimental diets (mg/g diet DM)

	Low-lysine		High-lysine	
	Low-fat Low–high-carnitine	High-fat Low–high-carnitine	Low-fat Low–high-carnitine	High-fat Low–high-carnitine
Fatty acids				
14 : 0	3·00	4·91	2·79	4·87
16 : 0	9·38	16·83	10·35	17·34
18 : 0	1·76	2·78	2·76	4·07
16 : 1*	3·00	4·95	2·35	5·08
18 : 1*n*-9	7·55	14·55	6·65	14·76
18 : 1*n*-7	1·21	2·07	1·12	2·21
20 : 1†	3·40	5·90	2·71	5·43
22 : 1‡	4·80	8·76	3·92	8·06
18 : 2*n*-6	4·81	8·87	4·90	4·49
18 : 3*n*-3	0·64	1·22	0·40	1·06
18 : 4*n*-3	1·23	2·11	0·90	1·91
20 : 5*n*-3	4·67	7·80	3·90	7·14
22 : 5*n*-3	0·54	0·88	0·30	0·82
22 : 6*n*-3	5·58	11·32	5·20	10·28
Σ SFA§	14·58	25·36	16·38	27·17
Σ MUFA‖	20·27	36·87	17·04	36·16
Σ *n*-6 PUFA¶	5·36	9·78	5·33	5·42
Σ *n*-3 PUFA**	13·14	24·24	10·95	22·07
Σ LCFA††	53·35	96·25	49·7	90·82
Σ *n*-3/Σ *n*-6	2·45	2·48	2·05	4·07

SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LCFA, long-chain fatty acid.
* Predominantly 16 : 1*n*-7.
† Includes 20 : 1*n*-11, 20 : 1*n*-9 and 20 : 1*n*-7.
‡ Includes 22 : *n*-13, 22 : 1*n*-11 and 22 : 1*n*-9.
§ Includes 15 : 0 and 17 : 0.
‖ Includes 14 : 1*n*-3, 15 : 1*n*-3 and 18 : 1*n*-5.
¶ Includes 16 : 3*n*-6 and 20 : 3*n*-6.
** Includes 16 : 3*n*-3, 18 : 2*n*-3 and 20 : 4*n*-3.
†† C_{14}–C_{22} as defined by Odle (1997).

rate (6 l/min), water temperature (27 ± 0·05°C), pH (7·03 ± 0·38), dissolved O_2 (>5 mg/l) and conductivity (6·07 ± 0·06 mS/cm) were checked daily. NH_4 (0·4 ± 0·1 mg/1), NO_3 (174 ± 29 mg/l) and NO_2 (0·2 ± 0·2 mg/l) were checked once per week. Fish were kept under a 12 h light–dark cycle. In case of mortality, fish were removed and daily feed amount was adjusted to the remaining number.

Diets, feeding and design

The eight experimental diets contained a lysine level of 13 or 21 g/kg, one of two levels of fat (90 and 190 g/kg), and one of two levels of carnitine (0·2 and 1·0 g/kg) in a 2 × 2 × 2 factorial design (Table 1). All diets were isonitrogeneous (350 g crude protein/kg) and close to isoenergetic (about 18 g crude protein/kJ gross energy). Low-carnitine and low-lysine diets were formulated using a combination of wheat meal and corn gluten as the primary protein source. Two levels of dietary lysine and carnitine were selected to represent the below-requirement level and the above-requirement level for catfish (Robinson *et al.* 1980; Torreele *et al.* 1993). Fat levels were set at the outer margins of the accepted inclusion range for African catfish (Uys, 1989).

Carniking® (Lonza Group Ltd, Basel, Switzerland) and L-lysine HCl ingredients were added to each diet by premixing the respective quantities in the premix prior to extrusion. The ingredients were mixed, extruded using co-rotating screw extruder (APV-Baker, Newcastle, UK) into 2·24 mm diameter × 5 mm length pellets and air-dried to 50 g moisture/kg. The floating diets were fed to fish slowly, in small amounts by hand, two times per day (09.00 and 17.00 hours) at a feeding level of 24 g/kg$^{0·8}$ per d, 7 d per week. The appetite of the fish was monitored carefully to minimise wastage of food. It was relatively easy to detect the point at which the catfish ceased active feeding from their behaviour since they stop agitating against the water surface and move away from the feeding area of the aquarium. Each feeding lasted about 4 min per aquarium. To obtain the same total feed intake, low-lysine groups were allowed more feeding days. Thus, the experimental sampling was designed in two periods (53 and 74 d).

Diets were analysed for proximate composition and fatty acid concentrations (Table 2) prior to the experiment. Treatments were tested in duplicate aquaria and fish were fed till a total feed intake of 120 g feed/fish was realised.

Sample collection, storage and analytical techniques

Two days before tissue sampling, twelve fish were taken from each treatment, killed by overdose of tricaine methane sulphonate (0·3 g/l; Crescent Research Chemicals, Phoenix, AZ, USA) and $NaHCO_3$ (0·4 g/l) and weighed individually to the nearest 0·1 g. Growth performance and feed conversion were measured from these animals in terms of percentage weight gain (specific growth rate, SGR), feed conversion ratio (FCR) and hepatosomatic index (HSI). Growth response parameters were calculated as follows: SGR $(\%/\mathrm{d}) = ((\ln W_f - \ln W_0)/t) \times 100$ where W_f is the weight of fish at time t ($t = 53$ or 74 d), W_0 is the weight of fish at time 0; FCR = total dry feed fed (g)/total wet weight gain (g); and HSI = wet weight liver (g)/wet weight fish (g) × 100. Proximate compositions and fatty acid concentration were measured from tissues (whole-body, liver and muscle) removed from fifteen fish from each aquarium deprived of food for 24 h prior to sampling. Tissues were pooled by aquarium, and immediately stored at −20°C. Frozen muscle samples were cut without thawing into pieces avoiding drip losses, minced using a meat mincer and pooled homogeneously. Frozen liver samples were homogenised using an Ultra Torax grinder (Janke & Kunkel GmbH, Staufen, Germany). After homogenisation, part of each sample was again frozen immediately and freeze-dried to eliminate water interference during certain analyses.

In brief, approximately 100 g sample was placed into the freeze-dryer (FTS system Inc., Stoneridge, NY, USA) and kept under a pressure of 26 Pa and a condenser temperature of −85°C. The shelf temperature was raised gradually during the freeze-drying process over 36 h from −20°C to room temperature. A small sample from each batch was further dried using the standard DM determination (ISO 6496 (International Organization for Standardization, 1983) to evaluate the water content of the freeze-dried material.

After freeze-drying, muscle samples were powdered using a foodstuff mincer (Retsch ZM 100 GmbH, Haan, Germany) and again homogenised. Because of high levels of fat in the liver samples, it was not possible to homogenise them using the foodstuff mincer. Instead, the freeze-dried liver was homogenised using a coffee grinder.

DM was determined by drying the samples for 4 h at 103°C (ISO 6496 (International Organization for Standardization, 1983)) and ash content was measured by ashing the samples for 4 h at 550°C (ISO 5984 (International Organization for Standardization, 1978)). Crude protein was measured by Kjeldahl according to ISO 5983 (International Organization for Standardization, 1978) procedures and calculated as N content multiplied by 6·25. Crude fat was determined by Soxhlet extraction (EEG 18.1.84 no. 15/29–30). Gross energy was measured by bomb calorimetry (IKA-C-7000; Fa. IKA-Analysentechnik, Weitersheim, Germany), and fatty acids were measured in duplicate from freeze-dried sample by high resolution GC according to a modified procedure of Lepage & Roy (1984). DM matter, ash, and protein analyses were done from fresh sample of muscle and liver tissues in triplicate and in duplicate respectively. Fat and energy analyses were done from freeze-dried material in duplicate and triplicate respectively. Carnitine determination was performed from freeze-dried material by radiometric detection of free- and acyl-carnitine following the procedure of Christiansen & Bremer (1988).

Fatty acid analysis

Fatty acid methyl ester (FAME) analysis from liver and muscle tissues was performed using a high resolution GC method, employing capillary columns and flame ionisation detection, fitted with an automatic split type injection 1 μl:20 μl (type SSL 71; Fison Instruments, Milano, Italy). A small amount (0·2 g) sample was saponified and transesterified with methanolic KOH and the methyl esters

extract into hexane according to a modified direct transesterification technique as described by Lepage & Roy (1984). Tricosanoic acid methyl ester (23:0; Sigma T9900, Sigma, St Louis, MO, USA) was used as internal standard because it is a stable fatty acid, not present in fish tissue and therefore can be easily identified during chromatography. Injection of 1 μl was performed in high resolution Mega 2 Series (Fison Instruments), fitted with a 30 m × 0·25 mm i.d. column silicate type and 0·25 μm DB-WAX film 122-7032; J&W Scientific, Folsom, USA). Detector type was the flame ionisation detector and the carrier gas was He with a flow rate of 2 ml/min. The oven was programmed to start at 80°C, rising 5°C/min until 140°C and then 2°C/min until 200°C. Thereafter, it rose 1°C/min to the final temperature of 250°C. The injection and detector temperatures were 200°C and 250°C respectively. Peak identification and quantification was done by using the relative retention times between each FAME from diet or tissue and the reference standards for the most common FAME (Sigma kit no. 189-19; Sigma). These include 14 : 0, 14 : 1*n*-5, 15 : 0, 15 : 1*n*-5, 16 : 0, 16 : 1*n*-7, 17 : 0, 17 : 1*n*-7, 18 : 0, 18 : 1*n*-9, 18 : 2*n*-6, 18 : 3*n*-3, 18 : 3*n*-6, 20 : 0, 20 : 1*n*-9, 20 : 2*n*-6, 20 : 3*n*-3, 20 : 3*n*-6, 20 : 4*n*-6, 20 : 5*n*-3, 21 : 0, 22 : 0, 22 : 1*n*-9, 22 : 2*n*-6, 22 : 6*n*-3, 23 : 0, 24 : 0 and 24 : 1*n*-9. The results are expressed as FAME and only main FAME are presented. Integration was done by using Chrom-card for Windows, version 1.17 (Fisons Instruments).

Fatty acid balance was calculated assuming no faecal losses of the fatty acids. Therefore, the difference between fatty acid intake and its accumulation in fish body equals their disappearance (apparent oxidation) (Cunnane & Yang, 1995).

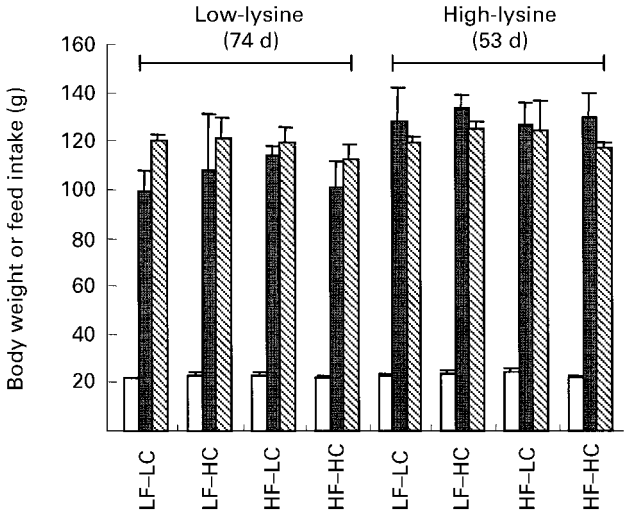

Fig. 1. Effect of feeding different experimental diets on body weight and feed intake of African catfish (*Clarias gariepinus* Burchell). For details of diets and procedures, see Tables 1 and 2. LF, low-fat; HF, high-fat; LC, low-carnitine; HC, high-carnitine. □, Initial weight; ■, final weight; ▨, feed intake. Values are means for two aquaria per treatment with twelve fish per treatment, with standard deviations shown by vertical bars.

Statistical analysis

Results are reported as mean values and standard deviations per treatment unless otherwise stated. Normality was tested using Shapiro-Wilk test. Homogeneity was checked using the absolute residuals according to Levene's test. Non-homogeneous data were arcsine transformed prior to further

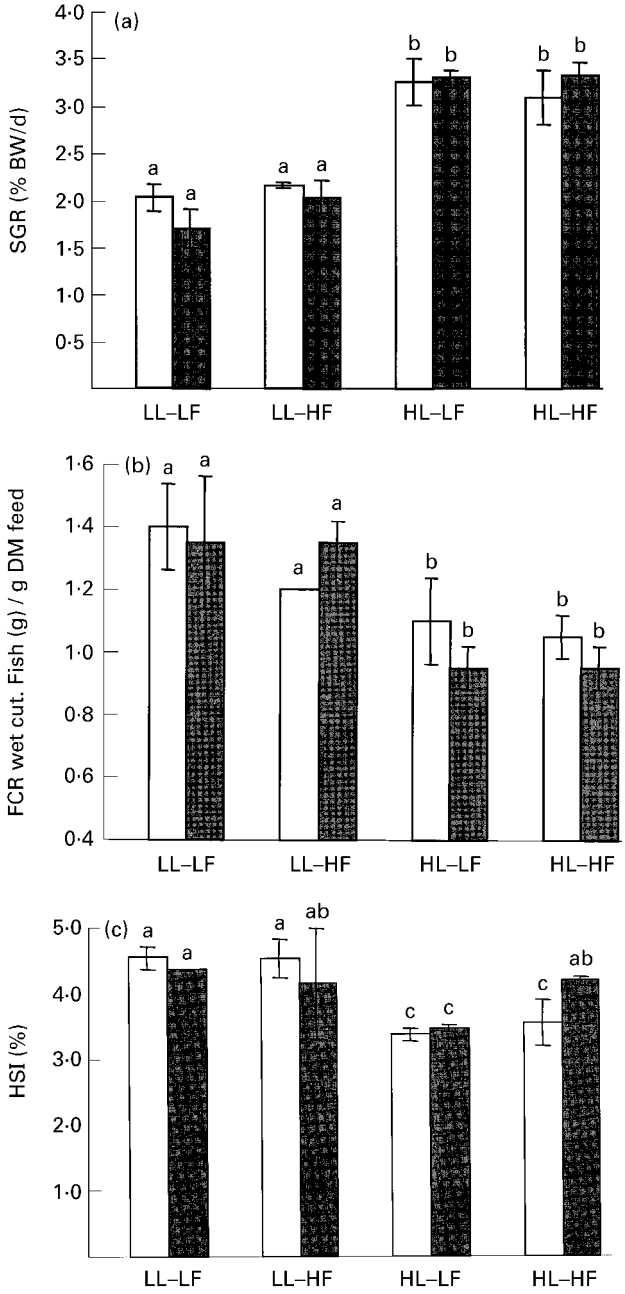

Fig. 2. Effect of feeding different experimental diets on (a) specific growth rate (SGR), (b) feed conversion ratio (FCR) and (c) hepato-somatic index (HSI) of African catfish (*Clarias gariepinus* Burchell). For details of diets and procedures, see Tables 1 and 2 and p. 626. LL, low-lysine; HL, high-lysine; LF, low-fat; HF, high-fat; BW, body weight. □, Low-carnitine; ■, high-carnitine. Values are means for two aquaria per treatment with twelve fish per treatment, with standard deviations shown by vertical bars. [a,b,c]Mean values with unlike superscript letters were significantly different (ANOVA, $P<0·05$).

statistical analysis. Proximate analysis, FAME analysis and growth data were subjected to the three-way ANOVA according to the model:

$$Y_{ijkl} = \mu + Car_i + Lys_j + Fat_k + (Lys \times Car)_{ji} + (Lys \times Fat)_{jk} + (Car \times Fat)_{ik} + (Car \times Lys \times Fat)_{ijk} + e_{ijkl},$$

where Y_{ijkl} corresponds to the growth performance, tissue proximate composition, carnitine content or fatty acid concentrations, Car_i relates to the dietary carnitine effect, Lys_j to the dietary lysine effect, Fat_k to the dietary fat effect, $(Lys \times Car)_{ji}$ to the lysine–carnitine interaction effect, $(Lys \times Fat)_{jk}$ to the lysine–fat effect; $(Car \times Fat)_{ik}$ to the carnitine–fat effect; $(Car \times Lys \times Fat)_{ijk}$ to the carnitine–lysine–fat interaction, and e_{ijkl} represents the error term.

Differences between means were reported as significant if $P<0{\cdot}05$. All statistical analyses were performed using SAS program (Version 6, 1990; Statistical Analysis Systems Inc., Cary, NC, USA).

Results

Growth and proximate composition

The average cumulative mortality during the experiment was <5 %. Fish grew from a mean initial weight of 22·7 g to final weights of 105·5 g for low-lysine groups (74 d) and to 129·5 g for high-lysine groups (53 d) (Fig. 1). This gain coincides with a SGR of 2·3 % body weight/d for low-lysine groups and 3·3 % body weight/d for high-lysine group (Fig. 2). Differences in SGR were also reflected in FCR, which averaged 1·0 g/g in high-lysine groups and 1·5 g/g in low-lysine groups (Fig. 2). Dietary carnitine supplements showed no significant effect on the HSI (Fig. 2, $P>0{\cdot}05$). High-lysine diets highly improved SGR and decreased FCR ($P<0{\cdot}001$). Fish receiving low-lysine diets showed higher HSI than fish fed the high-lysine diets ($P<0{\cdot}01$). Dietary fat supplements showed no apparent effect on growth rates and no interaction whatsoever was observed.

Tissue composition of catfish at the end of the growth experiment varied in crude fat and crude protein contents (Fig. 4). Protein:fat ratio was reduced in all tissues studied as a consequence of high dietary fat supplements ($P<0{\cdot}05$). Dietary low-lysine supplements also increased body fat contents compared with dietary high-lysine supplements. Dietary carnitine supplements significantly affected whole-body and liver fat content only in low-lysine–low-fat groups.

Tissue carnitine composition

Overall, carnitine content in tissues were positively related to dietary carnitine level ($P<0{\cdot}001$, Fig. 3). High-carnitine fed fish showed about a 3·5-fold increase in carnitine content over the low-carnitine groups ($P<0{\cdot}001$). Liver contents of both free- and acyl-carnitine esters were approximately forty times lower than the carnitine contents of the muscle tissue and fifteen times lower than the contents of the whole body. Fish fed low-lysine diets showed lower liver free-carnitine concentration than high-lysine animals ($P<0{\cdot}01$). High-fat supplements also reduced liver carnitine. A lysine *v.* carnitine interaction was observed ($P<0{\cdot}01$), but a carnitine *v.* fat interaction was not observed.

Fatty acid concentration

Effects of dietary carnitine, fat and lysine supplements on fatty acid concentration of the liver and the muscle tissues are shown in Tables 3 and 4 respectively, and the statistical significance is represented in Table 5. Muscle total fatty acids concentration ranged from 271 to 335 mg/kg (Table 3). Muscle *n*-3 and *n*-6 long-chain polyunsaturated fatty acids (PUFA) concentrations were slightly affected after 74 d by high-carnitine intake ($P<0{\cdot}1$). The principal fatty acid affected was docosahexanoic acid (22:6*n*-3). The effect of carnitine on muscle fatty acid concentrations were observed only when fish were offered the low-lysine–low-fat diets.

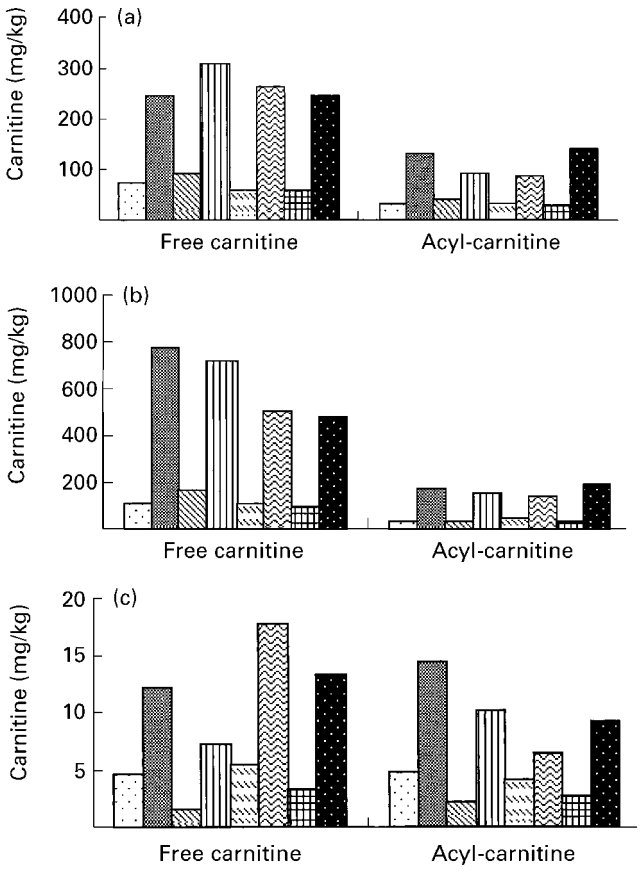

Fig. 3. Effect of dietary carnitine supplements on free- and acyl-carnitine content in African catfish (*Clarias gariepinus* Burchell) (a) Whole-body; (b) muscle tissue; (c) liver. For details of diets and procedures, see Tables 1 and 2 and p. 626. ⊡, Low-lysine–low-fat–low-carnitine; ▩, low-lysine–low-fat–high-carnitine; ▨, low-lysine–high-fat–low-carnitine; ◫, low-lysine–high-fat–high-carnitine; ▧, high-lysine–low-fat–low-carnitine; ▩, high-lysine–low-fat–high-carnitine; ⊞, high-lysine–high-fat–low-carnitine; ■, high-lysine–high-fat–high-carnitine. Values are means for two aquaria per treatment with fifteen fish per aquarium.

Table 3. Fatty acid concentrations of muscle total lipids (mg/g tissue DM)*
(Mean values and standard deviations of two aquaria per treatment with fifteen fish per aquarium)

	Low-lysine								High-lysine							
	Low-fat				High-fat				Low-fat				High-fat			
	Low-carnitine		High-carnitine		Low-carnitine		High-carnitine		Low-carnitine		High-carnitine		Low-carnitine		High-carnitine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fatty acids																
14 : 0	10·20	0·40	10·58	0·09	13·44	0·85	12·11	1·01	11·72	0·71	11·47	0·07	13·88	0·47	12·90	0·21
16 : 0	87·95	5·81	82·72	1·86	82·48	0·22	77·84	3·16	71·26	3·59	70·16	0·82	69·50	0·54	69·66	0·16
18 : 0	18·62	1·50	19·40	0·16	20·81	0·46	20·29	1·59	19·15	1·21	19·92	0·07	20·06	0·34	19·00	0·26
16 : 1†	15·46	0·82	17·70	0·26	17·29	0·62	16·17	0·88	16·04	0·96	14·81	0·13	16·39	0·64	16·15	0·23
18 : 1*n*-9	62·02	1·99	62·52	4·26	65·55	0·30	61·82	7·27	61·26	4·37	59·31	0·04	58·88	0·63	59·43	0·00
18 : 1*n*-7	5·94	0·16	6·49	0·03	6·39	0·07	6·08	0·45	6·36	0·02	6·35	0·04	7·04	0·15	6·68	0·21
20 : 1‡	10·96	0·30	11·79	0·02	14·50	0·38	13·20	0·86	12·33	0·08	12·31	0·29	14·41	0·47	13·66	0·40
22 : 1§	6·99	0·67	7·40	0·14	10·60	0·19	9·19	0·50	8·19	0·27	8·39	0·03	12·04	0·40	10·84	0·68
18 : 2*n*-6	18·43	0·47	20·66	0·16	33·01	1·80	28·83	2·84	18·80	0·58	18·58	0·15	18·81	0·33	17·65	0·10
20 : 2*n*-6	0·99	0·04	0·94	0·03	1·22	0·04	1·18	0·12	0·81	0·05	0·82	0·02	0·91	0·04	0·88	0·04
20 : 4*n*-6	1·03	0·03	1·21	0·04	1·38	0·06	1·33	0·01	1·03	0·06	1·07	0·04	1·27	0·00	1·21	0·02
18 : 3*n*-3	1·84	0·12	2·12	0·01	3·50	0·30	2·98	0·28	2·59	0·00	2·54	0·01	3·35	0·09	3·04	0·15
18 : 4*n*-3	2·17	0·44	2·69	0·01	4·05	0·38	3·29	0·29	2·93	0·04	2·80	0·01	4·36	0·13	3·71	0·29
20 : 5*n*-3	7·21	0·91	8·65	0·24	13·28	1·12	10·73	0·86	9·29	0·08	9·39	0·10	14·20	0·04	12·06	0·20
22 : 5*n*-3	2·33	0·11	2·87	0·03	3·66	0·15	3·16	0·25	2·87	0·10	3·01	0·01	3·63	0·21	3·41	0·09
22 : 6*n*-3	19·23	0·78	24·53	0·47	34·19	1·93	32·00	1·93	22·24	0·27	22·17	0·37	30·88	1·40	29·32	1·53
Σ SFA‖	118·43	4·74	114·39	2·11	119·08	1·17	112·26	5·83	104·22	5·58	103·70	0·70	106·21	1·40	104·05	0·88
Σ MUFA¶	102·52	2·63	107·14	3·89	116·15	1·50	108·12	10·07	105·59	5·72	102·64	0·48	110·74	1·09	108·57	1·24
Σ *n*-6 PUFA**	22·79	0·28	25·33	0·14	38·32	2·03	33·86	3·30	22·78	0·87	22·37	0·12	22·77	0·35	21·53	0·00
Σ *n*-3 PUFA††	34·69	0·66	42·95	0·24	61·72	4·07	54·95	3·80	42·28	0·54	42·34	0·52	59·54	1·84	54·5	2·42
Σ LCFA‡‡	278·43	2·66	289·81	5·99	335·27	7·73	309·19	21·13	274·87	44·33	271·05	11·75	299·26	3·65	288·65	2·94
Σ *n*-3/Σ *n*-6	1·52	0·01	1·70	0·02	1·61	0·02	1·62	0·05	1·86	0·05	1·89	0·01	2·62	0·04	2·53	0·11

SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LCFA, long-chain fatty acid.
* For details of diets and procedures, see Tables 1 and 2 and p. 626.
† Predominantly 16 : 1*n*-7.
‡ Includes 20 : 1*n*-11, 20 : 1*n*-9 and 20 : 1*n*-7.
§ Includes 22 : *n*-13, 22 : 1*n*-11 and 22 : 1*n*-9.
‖ Includes 15 : 0 and 17 : 0.
¶ Includes 14 : 1*n*-3, 15 : 1*n*-3 and 18 : 1*n*-5.
** Includes 16 : 3*n*-6 and 20 : 3*n*-6.
†† Includes 16 : 3*n*-3, 18 : 2*n*-3 and 20 : 4*n*-3.
‡‡ C_{14}–C_{22} as defined by Odle (1997).

Table 4. Fatty acid concentrations of liver total lipids (mg/g tissue DM)*
(Mean values and standard deviations of two aquaria per treatment with fifteen fish per aquarium)

	Low-lysine								High-lysine							
	Low-fat				High-fat				Low-fat				High-fat			
	Low-carnitine		High-carnitine		Low-carnitine		High-carnitine		Low-carnitine		High-carnitine		Low-carnitine		High-carnitine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fatty acids																
14 : 0	6·27	0·14	8·30	0·69	7·56	0·06	7·02	0·29	8·19	0·59	8·77	0·18	11·21	1·21	9·32	0·65
16 : 0	118·75	0·30	150·38	1·98	115·98	10·15	114·61	0·9	129·96	10·52	155·97	0·46	170·32	5·32	156·58	19·00
18 : 0	36·60	0·56	56·22	7·49	43·76	8·02	34·18	0·52	53·84	10·28	74·78	3·74	101·99	7·02	90·89	2·40
16 : 1†	24·08	1·28	36·11	2·17	16·99	1·79	21·89	2·00	25·23	2·51	27·76	0·15	25·37	2·47	24·59	4·26
18 : 1*n*-9	108·44	6·96	140·46	2·47	93·00	15·31	101·62	0·78	123·13	16·97	131·52	12·80	116·83	10·98	124·37	27·99
18 : 1*n*-7	6·64	0·26	8·32	1·16	6·26	0·80	7·08	0·23	6·44	0·02	6·82	0·05	7·64	0·71	6·87	0·96
20 : 1‡	11·11	0·47	14·52	0·29	12·71	1·89	12·02	0·26	13·43	0·94	16·06	0·51	21·99	0·44	18·54	0·58
22 : 1§	1·81	0·12	1·82	0·17	2·97	0·42	2·33	0·42	2·29	0·04	3·09	0·19	7·19	0·33	5·01	0·22
18 : 2*n*-6	5·17	0·93	12·55	1·12	7·48	1·22	10·43	3·49	8·56	0·01	10·07	0·47	14·67	1·72	10·86	0·02
20 : 2*n*-6	0·90	0·10	1·64	0·11	1·26	0·21	1·55	0·29	1·12	0·03	1·40	0·05	1·92	0·05	1·63	0·01
20 : 4*n*-6	tr	–	1·14	0·04	tr	–	tr	–	1·14	0·12	1·19	0·01	1·60	0·08	1·08	0·39
22 : 2*n*-6	1·11	0·19	0·99	0·25	1·01	0·19	1·14	0·02	0·68	0·01	0·80	0·12	0·93	0·19	1·15	0·45
18 : 3*n*-3	tr	–	tr	–	tr	–	tr	–	tr	–	tr	–	2·01	0·36	1·22	0·09
20 : 5*n*-3	0·24	0·03	2·71	0·12	0·53	0·02	1·47	0·83	1·95	0·07	2·52	0·46	9·33	1·62	4·59	0·04
22 : 5*n*-3	0·34	0·03	2·05	0·09	0·78	0·27	1·30	0·51	1·37	0·04	1·90	0·31	7·36	0·20	4·23	0·52
22 : 6*n*-3	0·69	0·47	15·17	0·41	2·46	1·12	8·08	3·95	10·65	0·03	14·15	1·79	39·50	1·09	27·51	3·92
Σ SFA‖	162·68	0·97	216·32	4·73	168·91	18·88	157·23	1·71	193·65	21·42	241·44	3·50	286·68	11·02	259·02	22·09
Σ MUFA¶	152·76	8·34	202·19	6·31	133·05	20·22	146·34	0·79	171·62	20·36	186·55	13·05	181·22	15·09	181·09	33·53
Σ *n*-6 PUFA**	8·23	1·49	19·50	1·21	11·05	1·54	15·71	4·60	13·87	0·25	15·93	0·47	21·30	2·30	17·17	0·87
Σ *n*-3 PUFA††	2·58	0·26	23·18	0·05	4·32	2·24	13·37	5·88	16·66	0·19	21·85	2·71	63·30	1·72	41·06	4·56
Σ LCFA‡‡	326·25	9·80	461·19	3·14	317·33	44·75	332·65	9·30	395·80	43·15	465·77	14·83	552·50	8·54	498·34	52·51
Σ *n*-3/Σ *n*-6	0·32	0·09	1·19	0·07	0·38	0·15	0·93	0·13	1·2	0·01	1·37	0·21	2·99	0·24	2·40	0·39

tr, trace (<0·5 mg/g); SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LCFA, long-chain fatty acid.
* For details of diets and procedures, see Tables 1 and 2 and p. 626.
† Predominantly 16 : 1*n*-7.
‡ Includes 20 : 1*n*-11, 20 : 1*n*-9, and 20 : 1*n*-7.
§ Includes 22 : *n*-13, 22 : 1*n*-11 and 22 : 1*n*-9.
‖ Includes 15 : 0 and 17 : 0.
¶ Includes 14 : 1*n*-3, 15 : 1*n*-3 and 18 : 1*n*-5.
** Includes 16 : 3*n*-6 and 20 : 3*n*-6.
†† Includes 16 : 3*n*-6 and 18 : 2*n*-3.
‡‡ C_{14}–C_{22} as defined by Odle (1997).

Carnitine tended to decrease the concentration of the saturated fatty acids, mainly 16:0, when fish were fed low-lysine diets. Concentrations of *n*-3 and *n*-6, however, were elevated by the intake of high-fat diets ($P<0·05$). Within the low-lysine groups, high-fat supplements caused an increase of *n*-3 and *n*-6 PUFA deposition respectively, from 38·8 (13·7 %) to 58·3 mg/kg (18·1 %) and from 24·1 (8·5 %) to 36·1 mg/kg (11·2 %) over the feeding trial.

In contrast to muscle, liver of fish fed high-carnitine diets showed an increase in fatty acid concentration, regardless of dietary lysine and fat level. The fatty acids 18 : 2*n*-6, 20 : 5*n*-3, 22 : 6*n*-3 were the primary fatty acids affected (Table 4). Dietary carnitine supplements severely raised *n*-3 PUFA liver concentration from 2·6 (0·8 %) to 23·2 mg/g (5 %) when fish were fed 90 g fat and from 2·3 (0·7 %) to 13·4 mg/g (4 %) when fed 190 g fat ($P<0·005$), and interactions were also observed (Table 5). The elevation of *n*-3 PUFA concentrations were associated with a 20-fold increase of 22 : 6*n*-3 and 10-fold increase of 20 : 5*n*-3 concentrations ($P<0·005$). Similarly, high-carnitine intake increased 18 : 2*n*-6 concentration 2-fold ($P<0·05$). High-carnitine level coupled with low-fat level increased deposition of the saturated and monounsaturated fatty acids.

Whole-body fatty acid balance

Effects of dietary treatments on linoleic acid (18 : 2*n*-6), eicosapentanoic acid (20 : 5*n*-3) and docosahexanoic acid (22 : 6*n*-3) balance in the whole body of the juvenile African catfish are presented in Table 6. The whole-body fatty acid balance showed that dietary carnitine and fat supplements clearly affected accumulation and disappearance of 20 : 5*n*-3, 18 : 2*n*-6 and 22 : 6*n*-3. Fish fed high-lysine supplements accumulated less 18 : 2*n*-6 in their body than the low-lysine animals. In conclusion, dietary carnitine and fat supplements significantly influenced the whole-body concentrations of linoleic acid and interactions between lysine and fat supplements were observed.

The disappearance of 20 : 5*n*-3 was by far the highest among the PUFA, ranging from 52·5 to 65·3 %. Dietary fat and carnitine supplements also affected accumulation of 20 : 5*n*-3 in the whole body of the African catfish. Within the low-lysine–low-fat groups, 533·4 and 535 mg 20 : 5*n*-3 were consumed respectively by the fish fed the low- and high-carnitine diets, 202 (37·9 %) and 197·5 mg (36·9 %) accumulated, and 331·3 (62·1 %) and 337·5 mg (63·1 %) disappeared. Carnitine supplements slightly increased the whole-body disappearance of 22 : 6*n*-3. Within the low-lysine–low-fat groups, 640·3 and 636·8 mg 22 : 6*n*-3 were consumed respectively by fish fed low- and high-carnitine diets, 581 (90·9 %) and 569·7 mg (89·5 %) accumulated, and 58·5 (9·1 %) and 67·1 mg (10·5 %) disappeared. In general, fish fed 190 g fat/kg diet showed reduced accumulation of *n*-6 and *n*-3 long-chain PUFA compared with fish fed 90 g fat/kg diet.

Discussion

African catfish

The African catfish is a scaleless fish with an eel-like body shape and a slightly depressed head. African catfish are air-breathers, omnivores, and normally found in turbid waters. It is farmed mainly in Africa and Europe (Huisman & Richter, 1987), although it is now also receiving attention in India, China and some East European countries, and also has recently been introduced into Brazil (Hecht *et al.* 1996). It was chosen as a model species because it is a fish easy to farm (robust, rapid growth, closing live-cycle) and because of its well-documented nature of growth and body

Table 5. Three-way ANOVA of individual fatty acid concentration of liver total lipids†

	Statistical significance of effect (three-way ANOVA)‡					
	Carnitine	Lysine	Fat	Carnitine × lysine	Lysine × fat	Carnitine × fat
Fatty acids						
14 : 0	NS	*	*	*	*	*
16 : 0	**	*	NS	NS	*	*
18 : 0	NS	*	*	NS	*	*
16 : 1§	*	NS	*	*	*	*
18 : 1*n*-9	*	*	*	NS	NS	NS
18 : 1*n*-7	NS	NS	NS	*	*	NS
20 : 1	NS	*	*	NS	*	*
22 : 1	*	*	*	NS	*	*
18 : 2*n*-6	*	*	*	*	*	*
18 : 3*n*-6	NS	*	NS	NS	*	NS
20 : 2*n*-6	*	*	*	*	*	*
20 : 4*n*-6	*	*	NS	*	*	*
22 : 2*n*-6	NS	NS	NS	NS	NS	NS
20 : 5*n*-3	NS	**	**	NS	***	***
22 : 5*n*-3	NS	**	**	*	***	***
22 : 6*n*-3	*	**	*	*	***	***

*$P<0·05$, **$P<0·01$, ***$P<0·005$.
† For details of diets and procedures, see Tables 1 and 2 and p. 626.
‡ A three-way interaction was not observed.
§ Predominantly 16 : 1*n*-7.

Table 6. Effects of dietary treatments on linoleic acid (18 : 2*n*-6), eicosapentanoic acid (20 : 5*n*-3) and docosahexanoic acid (22 : 6*n*-3) balance in the whole-body (wet weight basis) of the African catfish (*Clarias gariepinus* Burchell)*† juvenile

(Mean values for two aquaria per treatment with fifteen fish per aquaria)

	Low-lysine				High-lysine			
	Low-fat		High-fat		Low-fat		High-fat	
	Low carnitine	High carnitine	Low carnitine	High carnitine	Low carnitine	High carnitine	Low carnitine	High carnitine
Duration of feeding trial (d)	74	74	74	74	53	53	53	53
18 : 2*n*-6								
Intake (mg)	525·4	547·8	999·9	924·2	535·2	534·9	492·9	463·4
Body content (mg)								
Initial	7·3	7·8	7·6	7·3	7·7	7·8	8·2	7·5
Final	492·0	504·9	883·8	772·0	419·4	424·7	466·8	416·7
Accumulation (mg)	484·7	497·2	876·1	764·6	411·7	416·8	458·5	409·2
% 18 : 2*n*-6 intake	92·2	90·8	87·6	82·7	76·9	77·9	93·0	88·3
Disappearance (mg)§	40·7	50·7	123·8	159·5	123·4	118·1	34·3	54·2
% Intake	7·8	9·2	12·4	17·3	23·1	22·1	7·0	11·7
20 :5*n*-3								
Intake (mg)	533·4	535·0	894·3	810·8	453·2	462·2	857·4	773·9
Body content (mg)								
Initial	7·3	7·8	7·7	7·4	7·8	7·9	8·3	7·6
Final	209·4	205·4	354·6	288·5	209·6	227·5	370·1	278·8
Accumulation (mg)	202·0	197·5	346·9	281·1	201·8	219·6	361·8	271·3
% 20 : 5*n*-3 intake	37·9	36·9	38·8	34·7	44·5	47·5	42·2	35·1
Disappearance (mg)§	331·3	337·5	547·3	529·8	251·4	242·6	495·6	502·6
% Intake	62·1	63·1	61·2	65·3	55·5	52·5	57·8	64·9
22 : 6*n*-3								
Intake (mg)	640·3	636·8	1296·6	1177·9	604·3	616·3	1243·3	1106·1
Body content (mg)								
Initial	23·4	25·0	24·5	23·6	24·8	25·1	26·5	24·1
Final	605·2	594·7	1012·2	885·3	558·2	573·2	909·8	786·6
Accumulation (mg)	581·8	569·7	987·7	861·6	533·5	548·0	883·3	762·5
% 22 : 6*n*-3 intake	90·9	89·5	76·2	73·1	88·3	88·9	71·0	68·9
Disappearance (mg)§	58·5	67·1	308·9	316·3	70·8	68·3	360·0	343·6
% Intake	9·1	10·5	23·8	26·9	11·7	11·1	29·0	31·1

* Body weight 100–130 g.
† For details of diets and procedures, see Tables 1 and 2 and p. 626.
§ Excludes excretion.

composition, which enables a better evaluation of the results.

Performance

Since all treatment groups consumed the same amount of feed, differences in SGR in some groups were reflected in differences in FCR, which varied between 1·0 and 1·6 g/g. Low-lysine–high-fat–low-carnitine and high-lysine–high-fat–high-carnitine groups grew 2·3 and 3·3 % body weight/d respectively. In those situations, the total feed allowance was consumed in different periods, i.e. 74 *v.* 53 d.

Extra lysine supplements to low-lysine diets improved SGR and decreased FCR ($P<0·05$). Since juvenile catfish have a high potential for growth and high food intake, it seems that amino acid imbalance may have a larger impact on growth depression and feed conversion efficiencies (Conceição *et al.* 1998*a*) than in adult fish. Fish receiving low-lysine diets showed higher HSI than fish fed the high-lysine diets ($P<0·005$). These animals had increased fat and decreased protein deposition in the liver (Fig. 4). In addition, Conceição *et al.* (1998*b*) showed that amino acid imbalances between the dietary and the fish amino acid profiles lead to an increase in lipid deposition. Several other studies have also shown supplementation of extra lysine to diets increased protein and reduced fat in tissue of catfish (Munsiri & Lovell, 1993; Li & Robinson, 1998). It has been proposed that fish fed lower dietary fat:carbohydrate ratio tend to produce higher HSI (Higgs *et al.* 1992) and lower growth (Erfanullah, 1998).

The present study showed that dietary carnitine supplementation in low-carnitine diets slightly affected

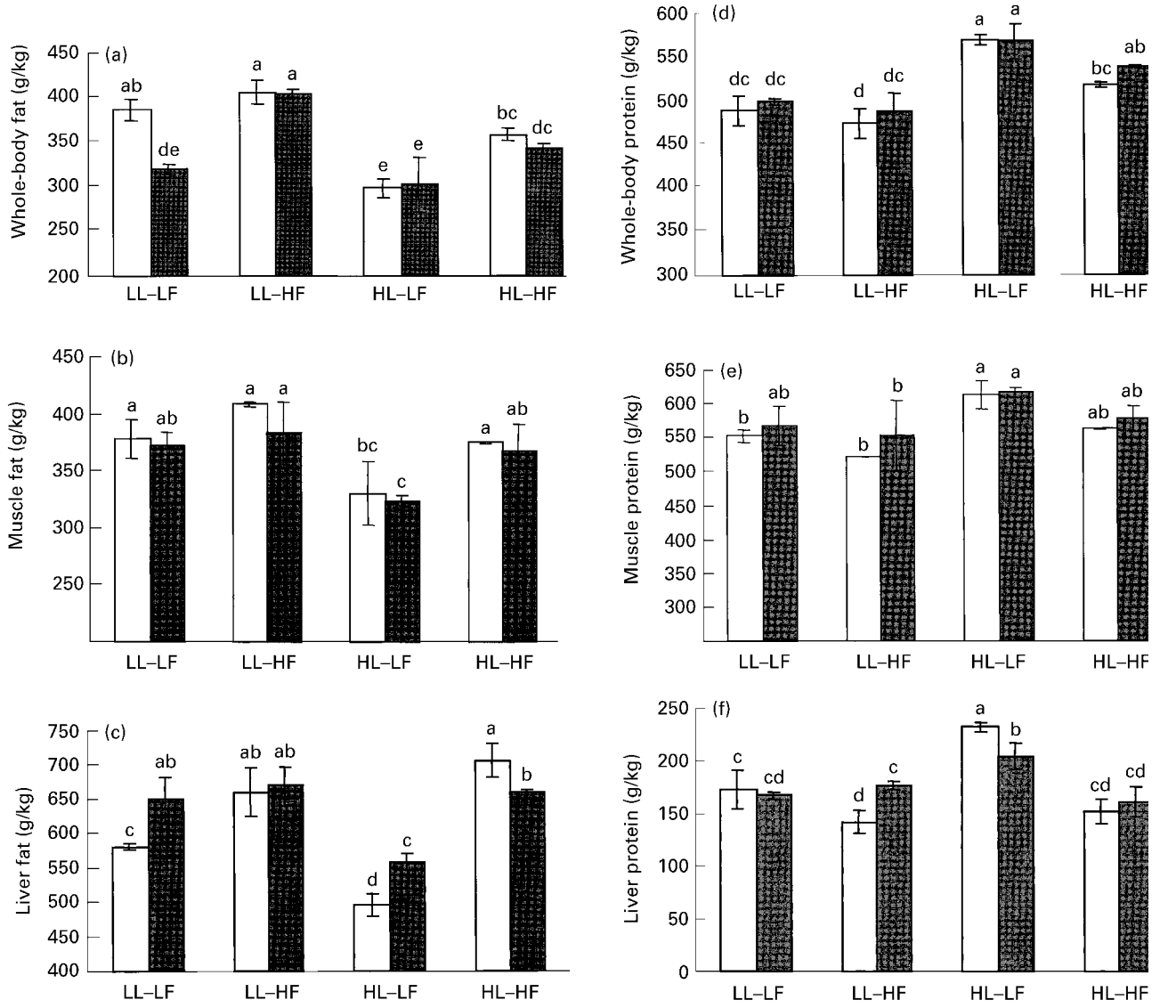

Fig. 4. Effect of feeding different experimental diets on (a, b, c) fat and (d, e, f) protein content in whole-body, muscle and liver tissues of African catfish (*Clarias gariepinus* Burchell). For details of diets and procedures, see Tables 1 and 2 and p. 626. LL, low-lysine; HL, high-lysine; LF, low-fat; HF, high-fat. □, Low-carnitine; ■, high-carnitine. Values are means for two aquaria per treatment with twelve fish per treatment, with standard deviations shown by vertical bars. [a,b,c,d,e]Mean values with unlike superscript letters were significantly different (ANOVA, $P<0·05$).

SGR and FCR in African catfish. Our results did not support those of Torreele *et al.* (1993), who clearly found faster and more efficient growth with increased levels of dietary carnitine. Torreele *et al.* (1993) fed commercial diets with higher crude protein levels compared with our present study. In addition, Torreele *et al.* (1993) studied growing animals from 5 to about 30 g, while in the present experiment, the initial fish size was already 23 g.

Growth rates (per unit of body mass) decrease as fish increase in size therefore a dietary carnitine deficiency may be more easily expressed at small fish size. Our results are in agreement with several other studies (Rodehutscord, 1995; Chatzifotis *et al.* 1996; Harpaz *et al.* 1999), that observed some effect of carnitine on growth and body composition. The absence of a clear effect of carnitine supplementation on weight gain of fish fed low-lysine diets suggests that low-lysine level in our present study did not impair the carnitine biosynthesis capacity. It may also be that lysine deficiency overrules any effect of carnitine.

Tissue carnitine content

Dietary lysine and methionine are required for the biosynthesis of carnitine (Broquist, 1997). Dietary lysine deficiency is therefore associated with reduced total body carnitine. It has been reported that endogenous carnitine synthesis alone may not be sufficient to meet the energy demands in fast growing juvenile animals (Rebouche & Seim, 1998; Bamji, 1984). Carnitine homeostasis in animals is maintained by a combination of absorption of carnitine from the diet, a modest rate of biosynthesis, and an efficient reabsorption of carnitine. Thus, fish may become carnitine-deficient if carnitine and/or its precursors are not present in the diet.

In the present study, high-carnitine groups accumulated significantly more free- and acyl-carnitine than low-carnitine groups. This suggests that dietary carnitine was absorbed and entered in the intermediary metabolism. Carnitine was stored mainly in the muscle tissue. Muscle free-carnitine was lower ($P<0{\cdot}01$) in fish fed high-lysine diets. No differences in acyl-carnitine level were observed. Three alternatives are suggested as explanation. First, high-lysine levels may lead to some extent to a decrease in carnitine biosynthesis. Davis *et al.* (1993) also showed that rats fed high-lysine diet had lower plasma carnitine concentration than did controls. Second, this depression may be caused by increased excretion in kidney. Third, being a carnitine storage site implies that muscle carnitine may be transported to other body tissues during high metabolic demands.

In contrast to muscle, liver free-carnitine content was substantially increased and acyl-carnitine decreased when fish were fed high-lysine diets compared with the low-lysine groups. High-fat–high-carnitine groups showed lower free carnitine:acyl-carnitine ratio compared with low-fat–low-carnitine group. This decline was caused by an increase in the carnitine ester content, thus indicating an increase in carnitine mobilisation towards lipid oxidation.

Fatty acid concentrations

Overall, the decrease of muscle fatty acid concentrations corresponds well with the decrease in muscle total fat content (Fig. 4). It is feasible to speculate that this reduction was due to a decrease in muscle C_{14}–C_{22} fatty acid concentrations. Few changes in muscle fatty acid concentrations were observed in high-carnitine fish compared with low-carnitine animals.

High-carnitine supplements have severely increased PUFA concentration, in particular 22 : 6*n*-3 and 20 : 5*n*-3, in the livers of fish fed low-lysine diets. Recent evidence suggests that 22 : 6*n*-3 and 20 : 5*n*-3 appear to be synthesised in liver mitochondria by the recently elucidated fatty acid desaturases for which carnitine is an essential co-factor (Infante & Huszagh, 2000; Wynn & Ratledge, 2000). Our current results showed that feeding extra carnitine to low-lysine–low-fat-fed fish may overrule the repression of PUFA biosynthesis and/or fatty acid desaturation and elongation. Muscle fat content was not significantly affected by high-carnitine supplements. High-carnitine lowered whole-body fat content and increased the protein:fat ratio only in low-lysine–low-fat groups (Fig. 4, $P<0{\cdot}05$). The latter findings and the fact that the great majority of lipids in catfish are deposited in intraperitoneal fat indicates that the increase in liver fatty acid concentrations is derived from intraperitoneal tissue. However, we did not measure intraperitoneal fat in the present study.

Whole-body fatty acid balance

To the best of our knowledge, this is the first study to report the effect of carnitine on fatty acid balance in fish. Without tracers, the quantitative method can be utilised to determine the partitioning of dietary fatty acids between accumulation, and disappearance (or apparent oxidation) in response to altered nutrient and energy demands. The results showed that fatty acid disappearance was stimulated significantly by high-carnitine and high-fat supplements in the diet. Fatty acid oxidation was assumed to be equal to fatty acid disappearance. We assumed however complete digestion of fatty acids. This may have resulted in overestimation of lipid oxidation rate. In addition, the fatty acid method assumes that PUFA disappearance can be calculated from measurements of fatty acid intake and accumulation. Conversion of fatty acids like 20 : 5*n*-3 to 22 : 6*n*-3 may have occurred. Thus, this method only gives some references to fatty acid balance.

In conclusion, although the present study indicated a limited benefit of dietary carnitine supplementation on growth performance, our findings support the hypothesis that dietary carnitine can enhance the mobilisation of long-chain fatty acids towards oxidation.

Implications

Endogenous triacylglycerols represent an important source of fuel during physical activity, whereas triacylglycerol oxidation increases progressively during exercise. The oxidation rate of lipids is determined by energy requirements of working muscles and by the availability of

free-carnitine delivery to muscle mitochondria. Carnitine has been thoroughly investigated for its role in exercise performance in human subjects and terrestrial animals (Souffleux, 1994; Janssens *et al.* 1998; Kraemer & Volek, 2000; Sachan & Hongu, 2000). The effect of dietary carnitine supplements on the energy metabolism of fish during a physical stress (i.e. exhaustive swimming) is however unknown, and therefore it is a new and interesting subject for future research.

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References

Bamji MS (1984) Nutritional and health implications of lysine carnitine relationship. *World Review of Nutrition and Dietetics* **44**, 185–211.

Becker K, Schreiber S, Angoni C & Blum R (1999) Growth performance and feed utilization response of *Oreochromis niloticus* × *Oreochromis aureus* hybrids to L-carnitine measured over a full fattening cycle under commercial conditions. *Aquaculture* **174**, 313–322.

Bilinski E & Jonas REE (1970) Effects of coenzyme A and carnitine on fatty acid oxidation by rainbow trout mitochondria. *Canadian Journal of Fisheries and Aquatic Science* **27**, 857–864.

Brafield AE (1985) Laboratory studies of energy budget. In *Fish Energetics: New Perspectives*, pp. 257–281 [P Taylor and P Calow, editors]. London, UK: Croom Helm.

Brooks DE & McIntosh JE (1975) Turnover of carnitine by rat tissues. *Biochemical Journal* **148**, 439–445.

Broquist HP (1997) Memories of microbes and metabolism. *Annual Review of Nutrition* **17**, 1–18.

Cederblad G & Lindstedt S (1976) Metabolism of labeled carnitine in the rat. *Archives of Biochemistry Biophysics* **175**, 173–180.

Chatzifotis S, Takeushi T & Seikai T (1995) The effect of dietary carnitine on growth performance and lipid composition in Red Sea bream fingerlings. *Fisheries Science* **61**, 1004–1008.

Chatzifotis S, Takeushi T & Seikai T (1996) The effect of dietary carnitine supplementation on growth of Red Sea bream (*Pagrus major*) fingerlings at two levels of dietary lysine. *Aquaculture* **147**, 235–248.

Christiansen RZ & Bremer J (1988) Acetylation of Tris-(hydroxymethyl) amino-methane (Tris) and Tris derivatives by carnitine acetyltransferase. *FEBS Letters* **86**, 99–102.

Conceição LEC, Ozório ROA, Suurd EA & Verreth JAJ (1998*a*) Amino acid profiles and amino acid utilization in larval African catfish (*Clarias gariepinus*): effects of ontogeny and temperature. *Fish Physiology and Biochemistry* **19**, 43–57.

Conceição LEC, Verreth JAJ, Verstegen MWA & Huisman EA (1998*b*) preliminary model for dynamic simulation of growth in fish larvae: application to the African catfish (*Clarias gariepinus*) and turbot (*Scophthalmus maximus*). *Aquaculture* **163**, 215–235.

Cunnane SC & Yang J (1995) Zinc deficiency impairs whole-body accumulation of polyunsaturates and increases the utilization of [1-14 C]-linoleate for de novo lipid synthesis in pregnant rats. *Canadian Journal Physiology Pharmacology* **73**, 1246–1252.

Davis AT, Kruggel EM & Randall S (1993) Excess of dietary lysine increases skeletal muscle and plasma trimethyllysine in rats. *Journal of Nutrition* **6**, 1109–1116.

Erfanullah AKJ (1998) Effect of dietary carbohydrate-to-lipid ratio on growth and body composition of walking catfish (*Clarias batrachus*). *Aquaculture* **161**, 159–168.

Gaylord TG & Gatlin DM (2000) Dietary lipid level but not L-carnitine affects growth performance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture* **190**, 237–246.

Gropp JM, Schumacher A & Schweigert FJ (1994) Recent research in vitamin nutrition with special emphasis to vitamin A, β-carotene and L-carnitine. In *Proceedings of the Meeting of the Arkansas Nutrition Conference*, pp. 124–134. Fayetteville, AR: Arkansas Poultry Federation.

Harpaz S, Becker K & Blum R (1999) The effect of dietary L-carnitine supplementation on cold tolerance and growth of the ornamental cichlid fish (*Pelvicachromis pulcher*) – preliminary results. *Journal of Thermal Biology* **24**, 57–62.

Hecht T, Oellermann L & Verheust L (1996) Perspective on clariid catfish culture in Africa. In *The Biology and Culture of Catfishes* [M Legendre and JP Proteau, editors]. *Aquatic Living Resources* **9**, 197–206.

Heo K, Odle J, Han IK, Cho W, Seo S, van Heugten E & Pilkington DH (2000) Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. *Journal of Nutrition* **130**, 1809–1814.

Higgs DA, Dosanjh BS, Uin LM, Himick BA & Eales JG (1992) Effects of dietary-lipid and carbohydrate-levels and chronic 3,5,3′-triiodo-L-thyronine treatment of growth, appetite, food and protein-utilization and body composition of immature rainbow-trout, *Oncorhynchus-mykiss*, at low-temperature. *Aquaculture* **105**, 175–190.

Huisman EA & Richter CJJ (1987) Reproduction, growth, health control and aquaculture potential of the African catfish, *Clarias gariepinus* (Burchell 1822). *Aquaculture* **63**, 1–14.

Infante JP & Huszagh VA (2000) Secondary carnitine deficiency and impaired docosahexaenoic (22 : 6*n*-3) acid synthesis: a common denominator in the pathophysiology of diseases of oxidative phosphorylation and beta-oxidation. *FEBS Letters* **469**, 1–5.

International Organization for Standardization ISO 5483 (1979) *Animal Feeding Stuffs – Determination of Nitrogen Content and Calculation of Crude Protein Content*, Geneva: ISO.

International Organization for Standardization ISO 5984 (1978) *Animal Feeding Stuffs – Determination of Crude Ash*, Geneva: ISO.

International Organization for Standardization ISO 6496 (1983) *Animal Feeding Stuffs – Determination of Moisture Content*, Geneva: ISO.

Janssens GPJ, Buyse J, Seynaeve M, Decuypere E & De Wilde R (1998) The reduction of heat production in exercising pigeons after L-carnitine supplementation. *Poultry Science* **77**, 578–584.

Jayaprakas V & Sambhu C (1996) Growth response of white prawn, *Penaeus indicus*, to dietary L-carnitine. *Asian Fisheries Science* **9**, 209–219.

Ji H, Bradley TM & Tremblay GC (1996) Atlantic salmon (*Salmo salar*) fed L-carnitine exhibit altered intermediary metabolism and reduced tissue lipid, but no change in growth rate. *Journal of Nutrition* **126**, 1937–1950.

Kraemer WJ & Volek JS (2000) L-Carnitine supplementation for

the athlete: a new perspective. *Annals of Nutrition and Metabolism* **44**, 88–89.
Krajcovicova-Kudlackova M, Simoncic R, Bederova A, Babinska K & Beder I (2000) Correlation of carnitine levels to methionine and lysine intake. *Physiological Research* **49**, 399–401.
Lepage G & Roy CC (1984) Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research* **25**, 1391–1396.
Li MH & Robinson EH (1998) Effects of supplemental lysine and methionine in low protein diets on weight gain and body composition of young channel catfish *Ictalurus punctatus*. *Aquaculture* **163**, 297–307.
Munsiri P & Lovell RT (1993) Comparison of satiate and restricted feeding of Channel catfish with diets of varying protein quantity in production ponds. *Journal of the World Aquaculture Society* **24**, 459–465.
Odle J (1997) New insights into the utilization of medium-chain triglycerides by the neonate: observations from a piglet model. *Journal of Nutrition* **127**, 1061–1067.
Rabie MH & Szilagyi M (1998) Effects of L-carnitine supplementation of diets differing in energy levels on performance, abdominal fat content, and yield and composition of edible meat of broilers. *British Journal of Nutrition* **80**, 391–400.
Rebouche CJ & Seim H (1998) Carnitine metabolism and its regulation in microorganisms and mammals. *Annual Review of Nutrition* **18**, 39–61.
Robinson EH, Wilson RP & Poe WE (1980) Re-evaluation of the lysine requirement and lysine utilization by fingerling channel catfish. *Journal of Nutrition* **110**, 2313–2316.
Rodehutscord M (1995) Effects of supplemental dietary L-carnitine on the growth and body composition of rainbow trout (*Oncorhynchus mykiss*) fed high-fat diets. *Journal of Animal Physiology and Animal Nutrition* **73**, 276–279.
Sachan DL & Mynatt RL (1993) Wheat gluten-based diet retarded ethanol metabolism by altering alcohol-dehydrogenase and not carnitine status in adult rats. *Journal of the American College of Nutrition* **12**, 170–175.
Sachan DS & Hongu N (2000) Increases in $VO_{2\ max}$ and metabolic markers of fat oxidation by caffeine, carnitine, and choline supplementation in rats. *Journal of Nutritional Biochemistry* **11**, 521–526.
Souffleux G (1994) Benefit of using Rossovet carnitine for horses in preparation for amateur carriage-racing. *Pratique Vétérinaire Équine* **26**, 241–248.
Torreele E, van der Sluiszen A & Verreth J (1993) The effect of dietary L-carnitine on the growth performance in fingerlings of the African catfish (*Clarias gariepinus*) in relation to dietary lipid. *British Journal of Nutrition* **69**, 289–299.
Uys W (1989) Aspects of the nutritional physiology and dietary requirements of juvenile and adult sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae). PhD Thesis, Rhodes University.
Van Kempen TATG & Odle J (1995) Carnitine affects octanoate oxidation to carbon dioxide and dicarboxylic acids in colostrum-deprived piglets: In vivo analysis of mechanisms involved based on CoA- and carnitine-ester profiles. *Journal of Nutrition* **125**, 238–250.
Wynn JP & Ratledge C (2000) Evidence that the rate-limiting step for the biosynthesis of arachidonic acid in *Mortierella alpina* is at the level of the 18 : 3 to 20 : 3 elongase. *Microbiology-UK* **146**, 2325–2331.