Effects of exercise on L-carnitine and lipid metabolism in African catfish (*Clarias gariepinus*) fed different dietary L-carnitine and lipid levels

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African catfish (*Clarias gariepinus*) were fed four isonitrogenous diets (34 % crude protein), each containing one of two lipid (100 or 180 g/kg) and two L-carnitine (15 or 1000 mg/kg) levels. After 81 d of feeding, thirty-two fish (body weight 32 g) from each dietary group were randomly selected, sixteen fish were induced to a 3-h swim (speed of 1·5 body length (BL)/s), while the other sixteen fish were kept under resting condition. Fish fed 1000 mg L-carnitine accumulated 3·5 and 5 times more L-carnitine in plasma and muscle, respectively, than fish fed the 15 mg L-carnitine. Muscle L-carnitine content was significantly lower in exercised fish than in rested fish. High dietary lipid level (fish oil) led to an increase in muscle *n*-3 PUFA content and a decrease in SFA and MUFA content. In liver, the increase in dietary lipid level resulted in an increased levels of both *n*-6 and *n*-3 PUFA. L-carnitine supplementation significantly decreased *n*-3 PUFA content. Exercise decreased *n*-3 PUFA in both muscle and liver. Plasma lactate and lactate dehydrogenase, normally associated with increased glycolytic processes, were positively correlated with exercise and inversely correlated with dietary L-carnitine level. L-carnitine supplementation reduced significantly the RQ from 0·72 to 0·63, and an interaction between dietary L-carnitine and lipid was observed ($P < 0.03$). Our results indicate that an increase in fatty acids (FA) intake may promote FA oxidation, and both carnitine and exercise might influence the regulation of FA oxidation selectivity.

African catfish: L-carnitine metabolism: Lipid metabolism: Exercise

Fish are subjected to many changes in energy demand throughout their lifespan. As the lipid content of their diet increases so does the optimal dietary level of L-carnitine[1–3], a multi-physiological, bioactive additive compound, synthesised from two essential amino acids, protein-bound lysine and methionine. It is not entirely understood whether animals require exogenous L-carnitine, and over the past 20 years, the scientific discussion regarding this subject continued with ambiguous arguments.

L-Carnitine plays several important physiological roles like shuttling the long-chain fatty acid (FA) across the inner mitochondrial membrane for ATP production[4,5] and inhibition of superoxide radical formation[6]. According to Heo *et al.* [2,7], L-carnitine biosynthesis might be sufficient to maintain growth of 20-kg pigs during optimum husbandry conditions. Nevertheless, extra dietary L-carnitine is efficiently retained in body tissues, playing a role in nutrient utilisation and thus on growth performance and body composition. The rate of lipid oxidation is determined by the energy requirements of working muscles and by the availability of free L-carnitine delivery to muscle mitochondria[8,9].

Since exogenous L-carnitine may influence energy utilisation efficiency, several studies using birds and mammals as animal models tested the effect of dietary L-carnitine supplementation on the exercise performance and on lipid metabolism[10–13]. Extra dietary L-carnitine has shown to improve, to a certain degree, the professional sport performances[14] and has improved exercise endurance in rats exposed to short and long-term exercise[15,16]. However, little is known about the effects of dietary L-carnitine supplements on the energy metabolism of exercised fish. Ozório *et al.* [17] working with the African catfish fed high-fat level (190 g/kg diet), showed that dietary L-carnitine supplementation alleviated fatigue during short-term exhaustive exercise by facilitating the recovery of muscle high-energy phosphates content (ATP and phosphocreatine). Ozório *et al.* [17] suggested that muscle ATP recovery was stimulated from body lipids.

Simultaneous respirometric measurements using the differential concentration of O_2, CO_2 and total ammonia nitrogen is useful tool to accurately determine substrate preferences under forced swimming activity[18–20]. The oxygen consumption of swimming fish will increase considerably above its resting level. The level of the RQ ($RQ = CO_{2produced}/O_{2consumed}$) indicates the type of fuel being utilised under different metabolic conditions. The RQ for the catabolism of carbohydrates, lipids, and protein are 1·0, 0·7 and about 0·8, respectively. Under resting conditions, the RQ of rainbow trout (*Oncorhynchus mykiss*), goldfish and African catfish range from 0·83 to 1·0[21,22]. When African catfish is fed extra L-carnitine, RQ and total ammonia nitrogen tend to decrease, indicating better use of lipids and a decrease of protein catabolism, the so-called protein sparing action.

The aim of the present study was to study the combined effect of dietary L-carnitine and lipid levels on lipid metabolism in the African catfish (*Clarias gariepinus*) exposed to forced swimming exercise, a condition common both in nature and in intensive aquaculture systems.

Materials and methods

This experiment was approved by the Ethical Committee Judging Animal Experiments (DEC) of Wageningen University.

Fish and facilities

The experiment was conducted in the experimental facility, 'De Haar vissen' of the Wageningen University, The Netherlands. The African catfish (*C. gariepinus*) had a starting weight of 8·4 (SEM 0·3) g. The experimental animals were siblings with an identical nutritional history. The fish were kept in sixteen glass aquaria of 70 litres each, connected to a recirculation system. Water temperature (25·2 ± 0·6°C) and electric conductivity (6·5 ± 1·2 mS/cm) were daily checked.

Dissolved oxygen (11·1 (SEM 2·0) mg/l), pH (7·3 (SEM 0·3)), NH_4^+ (0·2 (SEM 0·2) mg/l), NO_3 (137·5 (SEM 78·9) mg/l) and NO_2 (0·2 (SEM 0·2) mg/l) were weekly checked.

Experimental diet and design

Four diets were selected to test two L-carnitine levels (15 or 1000 mg/kg) and two lipid levels (100 or 180 g/kg; Table 1). Carniking (50 % L-carnitine, 35 % silica and 15 % water, Lonza) was used to increase the dietary carnitine level. All the diets were isoproteic (336–340 g crude protein/kg diet) and had equal amounts of fishmeal. To obtain a basal diet with low L-carnitine level, fishmeal (main L-carnitine source ingredient) was kept at low level (153 g/kg diet). Thus, to obtain isoproteic diets at 340 g/kg, gluten, feather meal and blood meal was adjusted accordingly. To keep the diets close to isoenergetic (20 MJ/kg) at different lipid levels, capelin oil was exchanged by starch and wheat. Fat levels were set at the out margins of the accepted inclusion range for African catfish[23]. In addition, to enhance the effects of dietary L-carnitine supplementation, biosynthesis was set to a minimum by formulating diets with a low, but sufficient, lysine levels. The later was achieved by using a combination of wheat meal and maize gluten as primary protein sources.

Table 1. Ingredients, proximate composition and fatty acid (FA) content of diets containing different L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels*

L-Carnitine	15		1000	
Lipid	100	180	100	180
Ingredients				
Fishmeal	153·1	152·9	153·1	152·9
Feather meal	102·0	–	102·0	–
Gluten	183·7	314·0	183·7	314·0
Wheat	133·7	213·0	133·7	213·0
Pregelatinised maize starch	255·1	86·6	255·1	86·6
Capelin oil	56·1	122·3	56·1	122·3
Vitamin and mineral premix†	5·1	5·1	5·1	5·1
Durabon binder	25·5	24·5	25·5	24·5
Cellulose	84·7	80·6	84·7	80·6
Carniking (mg/kg)‡	–	–	1600	1600
Chemical composition				
DM	985·1	988·3	985·1	988·3
Crude protein	335·9	339·2	335·9	339·2
Crude lipid	106·9	185·75	106·9	185·75
Ash	135·1	133·4	135·1	133·4
Carbohydrate§	326·0	240·3	326·0	240·3
Gross energy (MJ/kg)	19·2	21·6	19·4	21·5
Lys	11·4	11·5	11·7	11·4
Met	3·24	2·56	5·58	2·87
L-Carnitine (mg/kg)	14·3	17·1	972·0	1071·0
FA (g/kg)				
14:0	3·16	5·93	3·19	6·21
16:0	12·21	21·97	12·31	22·73
18:0	2·11	3·38	2·21	3·50
16:1	3·64	6·51	3·50	6·87
18:1	13·61	24·24	14·08	25·11
20:1	2·30	5·40	2·34	5·69
22:1	3·55	8·48	3·65	8·69
18:2n-6	6·62	10·53	6·76	11·04
18:3n-3	0·78	1·48	0·76	1·56
18:4n-3	1·42	2·89	1·39	3·08
20:5n-3	5·56	10·11	5·57	10·69
22:5n-3	0·56	0·87	0·57	1·03
22:6n-3	5·34	10·86	5·30	11·39
∑SFA	18·12	32·53	18·37	33·77
∑MUFA	23·73	45·97	24·23	47·67
∑(n-6)	7·50	12·03	7·54	12·64
∑(n-3)	13·99	27·00	13·96	28·59

* Values are expressed as g/kg as fed basis, unless otherwise stated.
† Ingredient supplied per kilogram of feed. Vitamins: 774 mg Vitamin A; 6·25 mg vitamin D; 20 000 mg vitamin E. Minerals: 501 mg $CuSO_4$; 15 000 mg $ZnSO_4$; 0·01 mg $MnSO_4$; 500 $CoSO_4$; 500 mg KI; 35 mg Na_2SeO_3.
‡ 65 % L-carnitine, 20 % silica and 15 % water. Provided by Lonza Group Ltd (Basel, Germany and Switzerland).
§ Gelatinised starch plus glucose was determined according to Goelema *et al.* [83].

The experiment was carried out according to a 2 × 2 factorial design with four replications each. Fish were acclimated for 7 d to the laboratory conditions before the feeding trial. During the acclimatisation period, fish were fed close to apparent satiation with the experimental diet containing 100 g lipid/kg diet and non-supplemented L-carnitine level (14 mg/kg). Thereafter, fish were randomly allocated to sixteen aquaria (forty-five fish/aquarium) and hand-fed twice a day with one of the four experimental diets for 81 d. Feed consumption and dead fish were recorded on a daily basis.

After the feeding trial, thirty-two fish from each dietary group were randomly selected, sixteen of them were induced to a 3-h swim (speed of 1·5 BL/s) in a Blazka swimming tunnel, previously described by Van Ginneken[24], and the
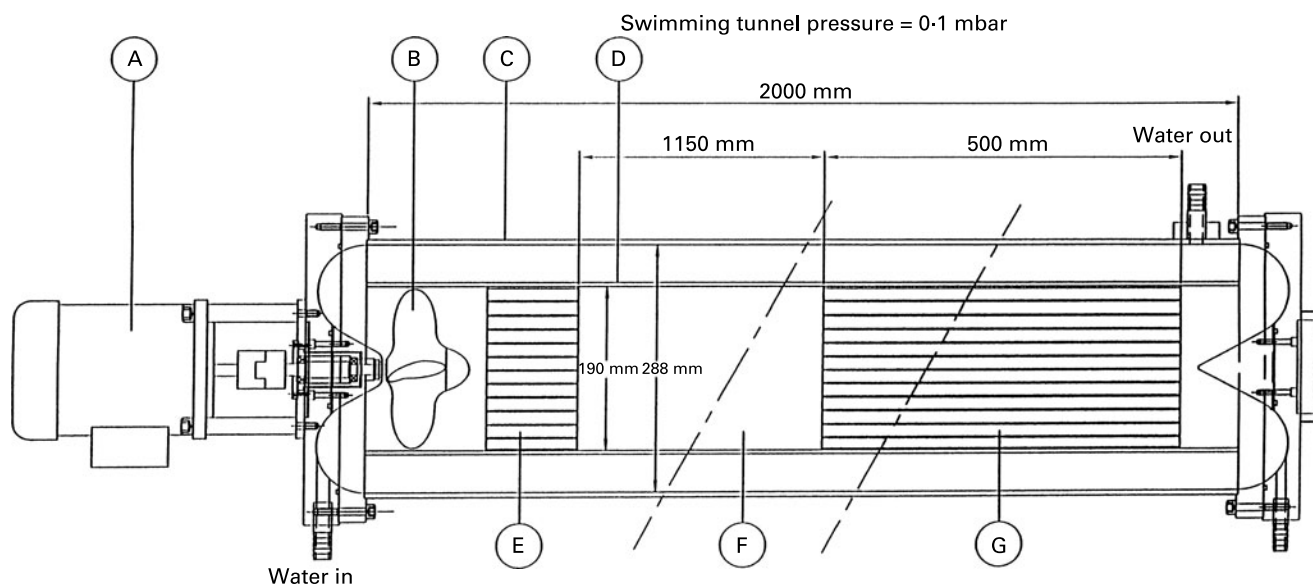
Swimming tunnel pressure = 0·1 mbar

Fig. 1. Schematic drawing of a 2·0-m swim-tunnel. The tunnel consists of two concentric perspex tubes of 2 m and two PVC end-caps. A, electromotor; B, propeller; C, perspex outer swim-tunnel tube; D, perspex inner swim-tunnel tube; E, PVC end-streamer; F, animal compartment; G, PVC front streamer. The propeller pushes water into the outer ring and 'sucks it' out from the inner tube. The cross-section area of the inner tube and of the outer ring has the same surface area. This results in equal flow rates at both sides. The turbulent water is pushed through streamers that have internal diameters of about 10 mm.

results were compared with the sixteen rested fish. The same handling procedures were performed on all fish to impose similar levels of stress on all animals before the swimming experiment.

The Blazka swimming tunnel (Fig. 1) had a length of 200 cm and consists of two tubes. The diameter of the outer and inner (the actual swimming tube) tubes were 28·8 and 19·0 cm, respectively, with a total volume of 127 litres. The engine power was 400 W and the propeller consisted of three blades of 7·5 in. with a pitch of 7 in. The motor speed corresponding to 1·5 BL/s was calculated by the following equation: $Y = 0.001307 \times X - 0.01724$, elucidated by Laser-Doppler techniques at the Hydraulics Laboratory TU (Delft, The Netherlands), where: $Y =$ water velocity in meter per second; $X =$ rpm of the engine of the Blazka swim tunnel. Here 0·001307 and 0·01724 are constants relating to the size of the tunnel and motor. This equation relates the number of rpm of the motor with the mean water velocity in the tunnel.

Sampling, measurements and analytical procedure

During the feeding trial, animals were weighed at the start and at the end of the experiment and total feed intake were determined for each aquarium at the end of the trial. Before every sampling, fish were fasted for 24-h, anaesthetised with tricaine methane sulphonate (Crescent Research Chemicals, Phoenix, AZ, USA), individually weighed and measured. The initial (twenty fish from the initial stock population) and final samples (thirty fish/treatment) of whole body were taken and stored at −20°C. During the final sampling, dorsal muscle sample and whole liver were withdrawn from nine fish per treatment, individually weighed and stored at −80°C pending analyses.

Diet and muscle tissue were homogenised with a meat mincer, liver samples were pooled per aquarium before the homogenisation using an Ultra Torax. Blood (0·5 ml/fish) was collected from the caudal vein, centrifuged (10 min at 1500 g, 4°C) and plasma immediately stored at −20°C. After blood sampling, fish were sacrificed with an overdose of tricaine methane sulphonate buffered with NaHCO$_3$ (0·3 g + 0·4 g/l water).

L-Carnitine was determined for diets, muscle and plasma according to Christiansen & Bremer[25]. In brief, L-carnitine analyses were performed by radiometric detection of free and L-carnitine esters. The assay is based on the reaction of free L-carnitine with acetyl-CoA catalysed by L-carnitine acetyl-transferase with the production of acetyl L-carnitine and coenzyme A. All the samples were deproteinated with perchloric acid, subsequently neutralised with KOH, centrifuged and the supernatant was collected. L-carnitine was extracted by step-wise heating, ultrasonic treatment or extraction with various detergents. Additionally, alkaline hydrolysis was performed for the determination of L-carnitine esters.

DM (4 h at 103°C), ash (6 h at 550°C), protein (macro-Kjeldahl N × 6·25), lipid (petroleum diethyl ether extraction using Soxlet) and energy (bomb calorimetry) analyses were determined for diets, muscle and liver samples.

FA concentrations of the diets, muscle and liver were determined as FA methyl esters according to Ozorio et al.[26]. Freeze-dried sample (0·2 g/fish) and internal standard (1·99 mg Tricosanoic acid methyl ester (23:0)/ml hexane) were saponified and esterified (dry bath, 22°C for 90 min) with methanolic KOH and the methyl esters extracted into hexane (1 hexane:1 KOH). Supernatant hexane was then separated and taken for automatic injection (2 μl/sample) in a gas chromatograph (Varian 3300) with a cross linked 30 m × 0·25 mm column silicate type, internal diameter of 0·53 mm and H$_2$ as the carrier gas. The injection and detector temperatures were 280°C and peak identification and quantification was done by using the relative retention times of the reference FA methyl esters standards.

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Table 2. Effects of dietary L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels and exercise on free and esterified L-carnitine content in muscle (mg/kg) and plasma (μmol/l) of African catfish (*Clarias gariepinus*)

	Resting					Exercised					ANOVA			
	15		1000			15		1000						
L-Carnitine														
Lipid	100	180	100	180	SEM	100	180	100	180	SEM	L-C	L	E	C×L
Muscle														
Free	111	138	432	599	7	102	111	403	564	13	<0·0001	<0·0001	0·04	<0·0001
Esterified	120	206	565	623	10	124	139	572	568	12	<0·0001	0·03	–	–
Plasma														
Free	1·75	1·73	5·6	7·25	0·28	1·88	1·78	5·7	7·05	0·26	<0·0001	0·03	–	0·02
Esterified	1·83	2·23	4·73	8·15	0·67	2·38	2·05	5·35	6·8	0·46	<0·0001	–	–	–

SEM, pooled standard error of mean: standard deviation/$\sqrt{n}$; L-C, L-carnitine; L, lipid; E, exercise; –, NS.
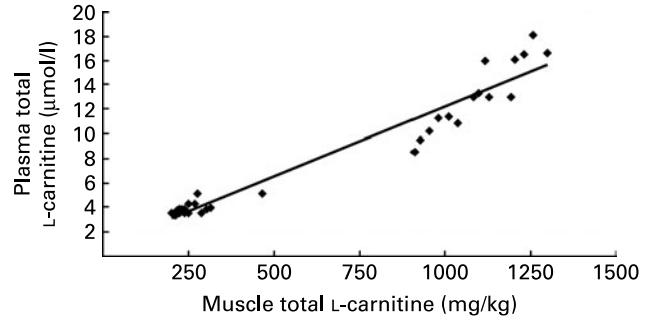

Fig. 2. Relationship between muscle and plasma total L-carnitine content (thirty-two observations). $y = 0{\cdot}0115x + 0{\cdot}7474$; R^2 0·94.

Plasma analyses were carried out for total protein (colorimetric method, Roche Diagnostics GmbH, Mannheim, Germany), TAG (colorimetric method, Boehringer Mannheim Gmbh Diagnostica, Mannheim, Germany), cholesterol (cholesterol esterase colorimetric method, Boehringer Mannheim Gmbh Diagnostica), lactate dehydrogenase (kinetic determination of the LDH activity, Boehringer Mannheim Gmbh Diagnostica), lactate (enzymatic method, Roche Diagnostics GmbH), urea (urease–glutamate dehydrogenase reaction, Boehringer Mannheim). All the above plasma assays were carried out using a Hitachi 717 Automatic Analyser from Boehringer Mannheim. Plasma glucose levels were determined with deproteinated samples by the GOD method (Boehringer Mannheim Gmbh Diagnostica) and NEFA were determined by NEFA-C-Test kit (WAKO, GmbH, Germany) using ELISA reader at 420 nm-wave length. Plasma cortisol levels were measured by RIA[27].

During the forced swimming trial, the RQ (RQ $= CO_{2\text{produced}}/O_{2\text{consumed}}$) and nitrogen quotient (NQ $= N_{\text{produced}}/O_{2\text{consumed}}$) were determined by using an automated auto analyser (Skalar B.V., Breda, The Netherlands) according to Ozório *et al.* [22].

Table 3. Effects of dietary L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels on growth performance and feed utilisation of African catfish (*Clarias gariepinus*)

| Dietary L-carnitine | 15 | | 1000 | | |
Dietary lipid	100	180	100	180	SEM
Initial weight (g)	8·4	8·4	8·5	8·4	0·1
Final weight (g)	29·2	31·7	27·1	29·6	0·8
DGI (% BW/d)*	1·27	1·38	1·18	1·29	0·03
VFI (% BW/d)†	2·40	2·31	2·11	1·99	0·06
FCR (g/g) ‡	1·79	1·63	1·65	1·48	0·05
PER (g/g)§	1·67	1·83	1·83	2·05	0·06
HSI (% BW)‖	4·1	4·4	3·7	4·1	0·1
Condition factor¶	0·6	0·6	0·6	0·6	0·0
Mortality (%)	13·9	9·4	11·1	11·7	3·2

DGI, daily growth index; BW, body weight; VFI, voluntary feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; HSI, hepatosomatic index.
SEM, pooled standard error of mean: standard deviation/$\sqrt{n}$.
* DGI $= (FBW^{1/3} - IBW^{1/3})/(t)$, where IBW, initial BW; FBW, final BW; t, trial duration.
† VFI $=$ feed [(100) (intake/average BW (ABW)/(t)], where ABW $= (IBW + FBW)/2$.
‡ FCR $=$ total dry feed intake (g)/total wet weight gain (g).
§ PER $=$ wet weight gain (g)/crude protein intake (g).
‖ HSI $=$ liver weight $\times$ 100/BW (%).
¶ $K = [(100)(FBW, g)/(Lt^3, cm)]$, where Lt is the final standard length.

Table 4. Effects of dietary L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels and exercise on proximate composition* of muscle and liver of African catfish (*Clarias gariepinus*)

	Resting					Exercised					ANOVA				
L-Carnitine	15		1000			15		1000							
Lipid	100	180	100	180	SEM	100	180	100	180	SEM	L-C	L	E	L×E	L-C × L × E
Liver															
DM	501·9	537	491	502	10	502	534	485	520	7	0·009	0·0003	–	–	–
Crude protein	77·3	63·2	74·8	70·8	1·0	72·8	64·2	75·8	66·8	0·7	–	0·0001	–	–	0·048
Crude lipid	354·8	373·9	323·7	364·4	10·1	363·9	376·4	337·3	345·3	8·4	0·0007	0·0003	–	–	–
Ash	7·0	6·0	7·0	6·5	0·1	6·0	5·0	6·5	5·5	0·1	0·02	0·0001	0·0001	–	–
Gross energy (MJ/kg)	16·6	17·1	16·1	17·1	0·3	16·1	16·6	15·6	16·1	0·3	–	–	0·01	–	–
Muscle															
DM	237	242	235	246	4	248	256	252	251	4	–	–	0·0009	–	–
Crude protein	156·9	155·5	157·4	149·1	1·0	153·3	150·0	151·2	152·6	0·7	–	–	0·0002	–	–
Crude lipid	54·5	56·2	54·3	62·1	2·9	61·4	60·9	59·7	55·5	2·6	–	–	0·02	0·045	–
Ash	19·0	18·7	18·7	18·2	0·2	17·5	17·5	17·5	17·8	0·1	–	–	–	–	–
Gross energy (MJ/kg)	5·9	5·9	5·9	5·9	0·1	5·5	5·7	5·5	5·5	0·1	–	–	–	–	–

SEM, pooled standard error of mean: standard deviation/√n; L-C, L-carnitine; L, lipid; E, exercise; –, NS.
*Values are g/kg unless otherwise stated, wet weight basis.

Statistical analysis

The data were analysed as 3×2 factorial using a general linear model (Proc GLM, SAS, Cary, NC, USA) that included the effect of exercise (resting and swimming), dietary L-carnitine (15 and 1000 mg/kg diet) and dietary lipid (100 and 180 g/kg diet) as main factors and their interactions. Differences between means were reported as significant if $P < 0.05$, using Bonferroni's multiple t tests. 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 before further statistical analysis.

Results

Fish fed 1000 mg L-carnitine accumulated 3·5-fold more L-carnitine in plasma than fish fed 15 mg L-carnitine did (Table 2). Exercise had no detectable effect on total, free or esterified L-carnitine levels in plasma. Dietary L-carnitine significantly increased the L-carnitine levels in muscle by 5-fold. There was a strong interaction between dietary L-carnitine and lipid levels, resulting in a steep increase in the free L-carnitine content with increasing dietary lipid level. Exercised fish retained significantly less free L-carnitine in muscle when compared with rested fish. Muscle total L-carnitine content was positively correlated to plasma total L-carnitine content (Fig. 2). The relationship between muscle and plasma L-carnitine content was fitted using a linear regression, described as $Y = 0.0115X + 0.7474$, R^2 0·94. Plasma L-carnitine content also increased moderately when muscle L-carnitine accumulated from 200 to 500 mg/kg, thereafter a sharp increase in plasma L-carnitine with increase in muscle L-carnitine was observed.

Depending upon the dietary treatment, fish grew from the initial 8·4 g to a mean final body weight of 27–32 g (Table 3). Growth rates varied between 1·18 and 1·38 % body weight/d. The average mortality during the experiment ranged from 9 to 14 % with no effect of diet. Dietary L-carnitine and lipid content had no significant effect on growth performance and feed utilisation.

Liver and muscle proximate composition was significantly affected as a result of experimental treatments (Table 4). DM content increased with the dietary lipid ($P < 0.0003$) and decreased with the dietary L-carnitine ($P < 0.009$). Protein content was inversely related with the dietary lipid ($P < 0.0001$) and a three-way interaction was observed. Liver lipid content increased with increasing dietary lipid ($P < 0.0003$) and decreased with the L-carnitine levels ($P < 0.0007$). Exercise did not alter the lipid content in liver. Liver energy was significantly affected by all experimental treatments.

The effects of dietary composition and exercise were less evident in muscle proximate composition than in liver. Dietary lipid and L-carnitine levels did not affect muscle lipid content. Exercised fish tended to have higher DM content (248–256 g/kg) than non-exercised fish (235–246 g/kg). Exercised fish retained significantly more lipid than non-exercised fish, and an interaction between dietary lipid and exercise was observed.

FA content (mg/g lipids) in muscle (Table 5) and liver (Table 6) were influenced by exercise and dietary treatments.

Table 5. Effects of dietary L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels and exercise on total fatty acid (FA) concentration (mg/g muscle and mg/g lipids) and individual FA composition (mg/g lipids) of African catfish (*Clarias gariepinus*) muscle

	Carnitine		Lipid		Exercise					
	15	1000	100	180	Resting	Exercise	SEM	C	L	E
FA										
FA (mg/g muscle)	174·0	184·0	178·0	179·0	181·0	177·0	0·0	–	–	–
FA (mg/g Lipids)	718·0	785·0	768·0	734·0	773·0	729·0	15·2	0·004	–	0·050
14:0	24·0	26·4	23·8	26·6	25·9	24·5	0·51	0·003	<0·001	0·050
16:0	172·0	191·0	197·0	167·0	187·0	176·0	3·7	0·001	<0·001	–
18:0	39·9	44·8	46·6	38·1	43·5	41·2	1·0	0·002	<0·001	–
16:1n-7	36·8	41·0	42·2	35·6	39·8	38·0	0·8	0·001	<0·001	–
18:1n-9	151·0	166·0	173·0	145·0	163·0	155·0	3·5	0·005	<0·001	–
18:1n-7	16·8	18·6	18·7	16·7	18·4	17·0	0·4	0·002	<0·001	0·013
20:1n-9	20·1	21·6	18·6	23·1	21·6	20·1	0·4	0·015	<0·001	0·022
22:1n-11	15·4	17·0	13·3	19·0	16·6	15·7	0·4	0·007	<0·001	–
18:2n-6	59·3	63·7	61·6	61·4	63·9	59·0	1·4	0·037	–	0·019
18:3n-6	1·36	1·34	1·25	1·44	1·37	1·33	0·04	–	0·002	–
20:2n-6	2·64	2·85	2·61	2·87	2·68	2·82	0·08	–	0·027	–
20:3n-6	2·93	3·24	3·69	2·47	3·21	2·96	0·09	0·021	<0·001	–
20:4n-6	3·21	3·57	3·48	3·29	3·59	3·19	0·10	0·019	–	0·010
22:2n-6	3·92	4·21	4·38	3·76	3·37	4·77	0·25	–	–	<0·001
18:3n-3	6·10	6·58	5·75	6·92	6·54	6·13	0·14	0·018	<0·001	0·040
18:4n-3	8·47	8·77	7·21	10·0	8·89	8·34	0·21	–	<0·001	–
20:3n-3	0·69	0·74	0·60	0·82	0·73	0·70	0·03	–	<0·001	–
20:4n-3	3·49	3·86	3·17	4·19	3·78	3·57	0·09	0·011	<0·001	–
20:5n-3	30·3	31·1	27·6	33·8	32·1	29·4	0·6	–	<0·001	0·006
22:5n-3	8·38	9·11	8·38	9·10	9·00	8·47	0·18	0·007	0·008	0·043
22:6n-3	74·1	79·9	69·6	84·4	80·1	73·8	1·6	0·018	<0·001	0·011
Total SFA	242·0	269·0	273·0	237·0	263·0	248·0	5·2	0·001	<0·001	–
Total MUFA	252	278	277	253	272	258	5·5	0·004	0·004	–
n-6	73·4	78·9	77·0	75·3	78·2	74·1	1·70	0·032	–	–
n-3	131	140	122	149	141	130	2·7	0·037	<0·001	0·010
Total PUFA	205	219	199	225	219	204	4·4	0·033	<0·001	0·025

SEM, pooled standard error of mean: standard deviation/$\sqrt{n}$; n-3 and n-6, total FA of (n-3) or (n-6) series; L-C, L-carnitine; L, lipid; E, exercise; –, NS.

In muscle, L-carnitine supplementation increased the content of most FA, whereas high dietary lipid level increased the n-3 PUFA and decreased the SFA and MUFA. The effect of exercise on muscle FA content was less pronounced but exercise decreased n-3 PUFA and tended ($P<0·06$) to decrease SFA. None of the interactions between main factors were significant.

In liver, L-carnitine supplementation decreased the n-3 PUFA and tended ($P<0·07$) to increase MUFA, whereas high dietary lipid level increased the n-3 PUFA and decreased the SFA and MUFA. However, a significant interaction between dietary levels of L-carnitine and lipids were observed for most of the n-3 PUFA, and also 18:2n-6 and 22:1n-11. All these FA increased with the level of lipid in the diet, but the increase was higher in fish fed non-supplemented L-carnitine diet (Fig. 3). Exercised fish had lower n-3 PUFA levels in liver than non-exercised fish.

Table 7 shows the means of selected plasma parameters of fish fed different experimental diets, with or without swimming exercise. Dietary L-carnitine combined with exercise evoked significant changes on several plasma metabolites, such as cholesterol, lactate, lactate dehydrogenase, NEFA and cortisol. Cholesterol levels increased with increasing dietary L-carnitine. Plasma lactate decreased with increasing dietary L-carnitine. Plasma lactate and lactate dehydrogenase showed a significant increase during exercise. Plasma cortisol was significantly lower in the resting fish (63 µg/l) when compared with exercised fish (166 µg/l). There were interactions between dietary L-carnitine, lipid and exercise

($P<0·02$) on plasma cortisol and NEFA levels. Plasma protein and glucose levels were not affected by dietary treatments or exercise also L-carnitine and exercise tended to decrease plasma urea. Plasma TAG were slightly higher in fish fed 1000 mg L-carnitine and tended to decrease with exercise.

The effects of dietary L-carnitine and lipid supplements on gas exchange, respiratory and nitrogen quotients in exercised African catfish is depicted in Table 8. During exercise, the O_2 uptake tended to increase with dietary L-carnitine supplementation. The changes in O_2 uptake led to a RQ ranging from 0·62 to 0·72. Dietary L-carnitine supplementation significantly reduced the RQ from 0·72 to 0·63, and an interaction between dietary L-carnitine and lipid was observed ($P<0·03$).

Nitrogen (N-NH$_4^+$) excretion was significantly influenced by the dietary L-carnitine and lipid level ($P<0·01$). In groups supplemented with 1000 mg L-carnitine, nitrogen excretion was significantly reduced when fed 180 g lipid/kg diet (0·93 and 1·06 mmol/kg per h) compared with fish fed 100 g lipid (1·03 and 1·63 mmol/kg per h), and an interaction between dietary L-carnitine and lipid levels was observed ($P<0·05$). The nitrogen quotient ranged from 0·07 to 0·12 and was not affected by the dietary treatment.

Discussion

L-Carnitine is available from endogenous biosynthesis and from dietary sources. The body distribution of L-carnitine is

Table 6. Effects of dietary L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels and exercise on total fatty acid (FA) concentration (mg/g tissue and mg/g lipids) and individual FA composition (mg/g lipids) of African catfish (*Clarias gariepinus*) liver

| | Carnitine | | Lipid | | Exercise | | | | | |
	15	1000	100	180	Resting	Exercise	SEM	C	L	E
FA										
FA (mg/g liver)	448	438	418	467	447	439	8	–	<0·001	–
FA (mg/g lipids)	611	643	609	645	635	619	14·1	–	–	–
14:0	12·1	12·2	10·4	13·8	12·4	11·9	0·269	–	<0·001	–
16:0	188	206	202	192	200	194	4·5	0·008	–	–
18:0	41·7	44·9	44·3	42·3	43·0	43·6	1·50	–	–	–
16:1n-7	41·7	46·0	48·2	39·6	44·8	43·0	1·09	0·008	<0·001	–
18:1n-9	186	199	202	183	193	192	5·62	–	0·027	–
18:1n-7	14·3	16·1	15·5	15·0	15·4	15·1	0·30	<0·001	0·252	–
20:1n-9	13·6	14·1	11·9	15·9	13·9	13·8	0·31	–	<0·001	–
22:1n-11	2·39	2·25	1·27	3·37	2·37	2·26	0·046	0·039	<0·001	–
18:2n-6	23·8	22·4	15·9	30·3	23·7	22·5	0·66	–	<0·001	–
18:3n-6	1·05	0·96	0·76	1·25	1·04	0·97	0·035	0·054	<0·001	–
20:2n-6	3·05	3·04	2·40	3·68	3·05	3·03	0·055	–	<0·001	–
20:3n-6	4·66	4·70	4·11	5·26	4·81	4·55	0·101	–	<0·001	–
20:4n-6	1·60	1·58	1·41	1·76	1·71	1·46	0·059	–	<0·001	0·006
22:2n-6	7·47	7·53	8·13	6·89	6·85	8·16	0·485	–	–	–
18:3n-3	1·70	1·46	0·75	2·40	1·64	1·52	0·057	0·005	<0·001	–
18:4n-3	0·66	0·52	0·31	0·87	0·64	0·54	0·032	0·005	<0·001	0·028
20:3n-3	0·53	0·58	0·35	0·75	0·57	0·53	0·030	–	<0·001	–
20:4n-3	1·88	1·52	0·65	2·75	1·81	1·59	0·072	0·001	<0·001	0·034
20:5n-3	7·17	5·30	2·98	9·49	6·61	5·87	0·198	<0·001	<0·001	0·014
22:5n-3	5·75	4·49	2·65	7·59	5·28	4·96	0·154	<0·001	<0·001	–
22:6n-3	31·2	28·1	17·1	42·1	31·8	27·4	0·89	0·020	<0·001	0·002
Total SFA	244	266	259	252	258	253	6·1	0·018	–	–
Total MUFA	266	285	285	266	277	274	7·1	–	–	–
Total n-6	41·6	40·2	32·7	49·1	41·2	40·7	0·85	–	<0·001	–
Total n-3*	48·9	41·9	24·8	66·0	48·3	42·4	1·27	<0·001	<0·001	0·003
Total PUFA	90·5	82·2	57·5	115	89·5	83·1	1·90	0·005	<0·001	0·024

SEM, pooled standard error of mean: standard deviation/√*n*; L-C, L-carnitine; L, lipid; E, exercise; –, NS.
* Significant (*P* < 0·05) interaction between L-carnitine and lipid.

determined by a series of systems that transport L-carnitine into cells against a concentration gradient, and the liver plays a unique role in the whole-body L-carnitine homeostasis. In the present study, the levels of dietary L-carnitine (15 and 1000 mg/kg) were selected to represent the below and above optimal level for catfish[26,28]. L-Carnitine deficiency can be obtained by decreasing synthesis[5], increasing excretion[29] or by reducing dietary L-carnitine availability. In the present study, L-carnitine deficiency was induced by limiting dietary lysine level to a sub-optimum level, so as to restrict biosynthesis and reducing dietary L-carnitine availability[5]. Ozório[30,31] and Harpaz[4] suggested that when limiting the level of L-carnitine precursor, L-carnitine supplementation might compensate for the low level of a dietary precursor. The optimal L-carnitine level seems to vary and depends on several biotic and abiotic factors, such as age, diet, metabolic conditions and tissue dependence on FA oxidation.

In the present study, free and acyl L-carnitine contents in muscle and plasma were linearly correlated with dietary L-carnitine level (3 to 5 times greater in the L-carnitine supplemented groups than in the non-supplemented groups). The ability to take up and retain dietary L-carnitine was previously observed in African catfish[17,26], as well as in Atlantic salmon[32], red sea bream[33], rabbits[34], piglets[35], rats[36], among others.

L-Carnitine concentrations are typically higher in tissues than in extracellular fluid compartments[5]. Indeed, in the

present study the concentration of L-carnitine was over 50 times higher in skeletal muscle than that in plasma. Free and acyl L-carnitine content in skeletal muscle was linearly correlated (*R*² 0·91) with plasma L-carnitine, indicating that L-carnitine supplements were taken up by tissues and available for intermediary metabolism. Since L-carnitine determination
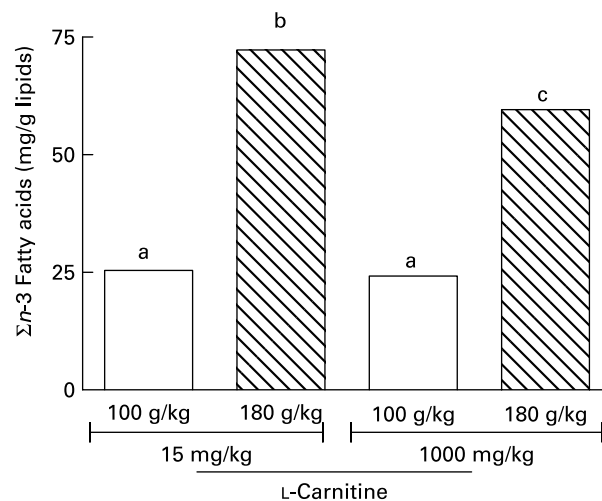

Fig. 3. Combined effects of dietary lipid (100 × 180 g/kg) and L-carnitine levels (15 × 1000 mg L-carnitine/kg diet) on liver total n-3 PUFA. a,b,c Values with unlike letters were significantly different (*P* < 0·05).

Table 7. Effects of dietary L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels and exercise on selected plasma indices in African catfish (*Clarias gariepinus*)

L-Carnitine	Resting					Exercised					ANOVA				
	15		1000			15		1000							
Lipid	100	180	100	180	SEM	100	180	100	180	SEM	L-C	E	C×L	C×E	C×L×E
Total protein (g/l)	31·7	31·6	29·5	30·1	1·4	30·6	31·0	31·1	30·8	0·8	–	–	–	–	–
TAG (mmol/l)	9·6	9·4	10·1	11·3	1·1	8·0	9·6	7·3	9·1	1·9	–	–	–	–	–
Cholesterol (mmol/l)	4·9	5·0	5·3	5·5	0·2	4·5	5·0	5·0	5·3	0·3	0·02	–	–	–	–
Lactate dehydrogenase (U/l)	965·8	1039·0	1045·5	1130·5	80·9	1686·3	1411·5	1203·3	1322·8	139·2	–	0·005	–	–	–
Lactate (mmol/l)	7·0	7·4	5·8	5·6	0·3	7·5	7·6	7·1	7·0	0·6	0·01	0·02	–	–	–
Urea (mmol/l)	0·6	0·6	0·6	0·6	0·1	0·9	0·6	0·6	0·8	0·1	–	–	–	–	–
Glucose (mmol/l)	6·3	6·3	6·3	5·4	0·6	5·9	5·8	5·4	5·8	0·4	–	–	–	–	–
NEFA (mmol/l)	0·6	0·6	0·6	0·6	0·0	0·7	0·8	0·6	0·6	0·1	–	–	0·02	0·04	–
Cortisol (μg/ml)	71·9	76·8	63·2	86·4	4·7	86·2	128·7	166·2	106·4	11·4	–	<0·0001	0·02	0·02	0·009

SEM, pooled standard error of mean: standard deviation/√*n*; L-C, L-carnitine; L, lipid; E, exercise; –, NS.

in plasma samples is a non-invasive method (favouring repeated measurements throughout the experimental period), the relation between plasma and muscle L-carnitine levels was validated from the results obtained from previous studies[3,17,22,26], and because muscle tissue is the main storage site of L-carnitine, it is plausible to infer that plasma L-carnitine content can be a good indicator of muscle L-carnitine content in African catfish.

Dietary lipid supplementation appears to elevate L-carnitine levels in the African catfish. Changes in optimal L-carnitine level are correlated with changes in the amount of circulating L-carnitine. The size of L-carnitine pool in animals is maintained by a combination of L-carnitine from supplemental sources, a modest rate of biosynthesis and an efficient reabsorption[5]. In the present study, an increase in the dietary lipid level caused a 30 % increase in muscle and in plasma L-carnitine content. In human subjects, plasma L-carnitine concentration is higher after a high-fat or high-protein diet[37]. As a consequence, more L-carnitine is reabsorbed, however, at lower efficiency rate. Berger & Sachan[38] observed that the efficiency of L-carnitine reabsorption in rats decreased with increasing levels of circulating L-carnitine, thus more L-carnitine is excreted. These observations suggest that dietary lipid levels may increase L-carnitine deposition, although body esterified L-carnitine level is often sub-estimated due to losses from excretion.

In fish, plasma and muscle L-carnitine contents increased with dietary L-carnitine level[3,22,39], as has been also observed for other species[2,40]. Gaylord & Gatlin[39] showed that hybrid striped bass fed 3000 mg L-carnitine/kg diet, had differences ranged from 11 to 61 mg L-carnitine/g muscle for fish fed 5 and 20 % lipid, respectively. The mechanism by which this occurs is not clear, but it seems that L-carnitine transport and utilisation may be influenced by dietary lipid level.

Exercise significantly altered the free L-carnitine levels in muscle tissue. From rest to 3-h of swimming at 1-BL/s, muscle free L-carnitine content decreased from 6 to 20 %. Hiatt *et al.*[41] observed in human subjects that from rest to 10 min of high-intensity exercise, free L-carnitine content decreased by 66 %. According to Hiatt *et al.*[41], the alteration of the ratio esterified-to-free is dependent on the workload and is poorly reflected in the plasma and urine carnitine pools. In the present study, however, exercise did not affect the ratio esterified-to-free L-carnitine. Presumably, the workload applied to catfish was not very intense, and an extension of the swimming trial would have caused a more drastic alteration.

In the present study, the amount of lysine was 1·1 %, in comparison to the optimal 1·5 % as determined for catfish[42]. Burtle & Liu[43] investigated the effects of 0·1 % dietary L-carnitine in diets containing 1·1, 1·4 and 1·7 % dietary lysine in channel catfish. L-Carnitine did not cause any effects on the growth rate, but a combination of L-Carnitine and lysine reduced body lipid content and visceral fat and increased body protein.

In the present study, L-Carnitine supplements had no clear effect on growth, but clearly affected the lipid and energy content in liver. The limited influence of L-Carnitine on African catfish growth and feed efficiency has been noted in a previous study in African catfish[26], as well as in other fish species[33,44,45].

Table 8. Effects of dietary L-carnitine (15 or 1000 mg/kg) and lipid (100 or 180 g/kg) levels on respiratory gas exchange (mmol/kg per h) and respiratory and nitrogen quotients in exercised African catfish (*Clarias gariepinus*)

L-Carnitine	15		1000			ANOVA		
Lipid	100	180	100	180	SEM	L-C	L	C × L
O_2 consumed (O_2)	21·3	20·3	22·0	21·0	0·6	–	–	–
CO_2 excretion (CO_2)	13·1	14·7	14·3	13·2	0·6	–	–	–
$N-NH_4^+$ excretion (N)	1·03	1·06	1·63	0·93	0·08	0·01	0·01	0·05
RQ	0·62	0·72	0·65	0·63	0·01	0·03	–	0·03
Nitrogen quotient (NQ)	0·07	0·1	0·12	0·08	0·02	–	–	–

SEM, pooled standard error of mean: standard deviation/$\sqrt{n}$; L-C, L-carnitine; L, lipid; –, NS.

Some other studies have shown dramatic influences of dietary L-carnitine on body composition and on growth performance. Ji *et al.*[32] noted that Atlantic salmon fed diets supplemented with L-carnitine had reduced fillet lipid. Chatzifotis *et al.*[46] reported an increase in growth rate and feed efficiency in red sea bream when L-carnitine was supplemented at 1087 mg/kg diet. In a classical dose–response study, Torreele *et al.*[28] clearly observed that dietary L-carnitine and lipid supplementation significantly affected performance of 5 g African catfish. In contrast, no significant effect on performance of African catfish juveniles growing from 9–32 g was observed in the present study. The difference in fish size might explain the different results obtained by Torreele *et al.*[28] working in the same laboratory and with the same species as in the present study. During early life stages when fish are building up new tissues at very fast rate, a very high-energy demand has to be covered. Since endogenous L-carnitine synthesis is less developed in younger animals, dietary L-carnitine requirement might be more evident in 5 g than in 9 g African catfish.

In liver, DM, energy and lipid content increased, and protein decreased with increasing dietary lipid level. As a consequence, protein to lipid ratio also decreased. Since liver is the major site of lipid and glycogen deposition in fish, it is expected to observe a positive relationship between dietary lipid content and body lipid[47]. Dietary L-carnitine supplementation significantly reduced DM, energy and lipid content of the liver. The effects of dietary L-carnitine on proximate composition of several fish species have been inconsistent. Dietary L-carnitine did not alter tissue composition of rainbow trout[45] or hybrid tilapia[48]. On the other hand, dietary L-carnitine reduced tissue lipid concentrations in rohu[49], tilapia[50], channel catfish[43], European sea bass[51] and Atlantic salmon[32]. Opposite to the latter studies, dietary L-carnitine increased lipid concentrations in liver and muscle of red sea bream[46].

In teleost fish, the FA composition and content of muscle and liver is mainly dependent of the profile of dietary FA source. This effect might, however, be modulated by selective FA oxidation and modification of absorbed FA[52]. In the present experiment, increased fish oil incorporation in the diets resulted in an increased FA intake but had no major effects on FA profile of diets. Moreover, the intake of long-chain PUFA was quite high even with lower fat diets, so minimal endogenous modification of absorbed PUFA can be expected. Thus, in the present study, the FA oxidation might become the most important factor determining the FA composition of tissues. To our best knowledge, the selectivity mechanism regulating FA oxidation in fish are poorly understood[52], and our data suggest that increased FA intake may promote FA oxidation and both carnitine and exercise might influence the regulation of FA oxidation selectivity, as discussed later.

Supplementing high fish oil level did not change the total FA content of muscle, but changed the FA profile with increased PUFA coupled with decreased MUFA and SFA concentration. The increased FA intake might led to an increase FA oxidation, as observed in rainbow trout[53] and in mice[54], and because both SFA and MUFA are expected to be oxidised preferentially to PUFA[52], these will result in an altered FA composition of muscle.

In liver, the enrichment on both *n*-6 and *n*-3 PUFA induced by elevated fish oil level was also observed but was highest in animals fed non-supplemented L-carnitine diets. L-carnitine might facilitate the oxidation of *n*-3 PUFA, thus attenuating its accumulation in the liver. This is consistent with earlier studies on red sea bream, where is suggested that, L-carnitine supplementation affected FA metabolism by shifting the oxidation from C14–C18 to C20–C22 FA[46,55].

Muscle of exercised fish had lower total FA content (mg/g lipid) and lower total lipids than the rested animals. This may indicate increased FA oxidation in muscles to couple with increased energy demand in exercised fish. In rested animals, *n*-3 PUFA are probably less oxidised than the other FA, but that selectivity mechanism regulating FA oxidation might be by-passed with increasing energy demand, such as in exercised fish. In that case, *n*-3 PUFA, SFA and MUFA are oxidised at similar rates.

Dietary L-carnitine levels did only influence cholesterol and lactate as plasma biochemical parameters. Exercise significantly elevated lactate and lactate dehydrogenase, which may indicate the activation of the anaerobic glycolysis to provide energy during exercise[56–58]. These findings are consistent with previous studies[59–64]. The nitrogen excretion can be divided in two different components: endogenous and exogenous (postprandial) excretion[65]. The endogenous excretion reflects the 'turn-over' body protein and the exogenous excretion, the result of the catabolism of dietary protein.

It is generally observed that the postprandial nitrogen excretion rates represent approximately 35–40 % of the digestible nitrogen ingestion and may vary with the dietary protein to lipid ratio, the quality of the ingredients and the feed processing technique[66,67]. As demonstrated in previous studies with salmonids, ammonia excretion represents about 90 % of the total excretion of the nitrogenous compounds[68–72]. The lowest values were observed in unfed fish, during which urea excretion represented 20 % of the nitrogen excretion.

Fuel oxidation preferences are often realised in changes of the RQ rates[73]. According to Kleiber[18], 1·0 is the upper limit for aerobic catabolism and usually ranges between 0·65 and 1·0. The lower value indicates lipid oxidation and the higher value carbohydrate oxidation. In the present study, the RQ rates vary between 0·62 and 0·72, and the pattern is in agreement with the variation observed in our previous study with African catfish. Ozório *et al.* [22] reported a reduction in RQ in fish fed L-carnitine supplementation, particularly when dietary lipid level was high. In the present study, dietary L-carnitine supplementation significantly reduced RQ and nitrogen (N-NH$_4^+$) excretion in fish fed 180 g/kg. There were interaction effects between L-carnitine and lipid for RQ and nitrogen excretion, indicating that dietary L-carnitine effects on lipid, and indirectly on protein catabolism, may be conditioned by the dietary lipid level.

We assessed an experimental set up with isonitrogenous diets containing two lipid levels, two L-carnitine levels and a resting or a moderate swimming protocol (1·5 BL/s). Swimming activity causes fish to have less lipid reserves than those of sedentary fish. Plasma cortisol level was also lower in the resting fish when compared with exercised fish. Catfish is an air-breathing fish species and swimming does prevent this species from this additional source of oxygen supply because vertical migration is prevented and the swim tunnel is a closed system. However, the higher cortisol levels in the swimming group can be an advantageous condition because cortisol acts to mobilise fuel stores to maintain metabolic homeostasis and thus exerts effects on metabolism directly[74]. In addition, Milligan *et al.* [75] observed that trout are able to recover much faster when they are allowed to swim at low speeds after collapse.

Swimming performance may change as a result of nutritional status[76]. The rate of cellular lipid oxidation is determined by the energy requirements of the working muscles and by the availability of free L-carnitine delivery to muscle mitochondria[17]. L-Carnitine may result in an elevated capacity for lipid oxidation in muscle mitochondria. During exercise, lipids oxidised in locomotory muscles can come from the circulation or from intramuscular reserves. Circulatory lipids and cholesterol are transported from the gut and from hepatic or adipose tissue stores[77] by lipoproteins[78].

The increase in concentrations of plasma lactate observed in the present study, normally associated with increased glycolytic processes, was correlated with exercise and is consistent with other studies[79–81].

Little information is available about the release and timescale for excretion of lactic acid from the active tissues in circulation. In addition, lactic acid may not solely be considered as an end product causing fatigue and acid–base disturbances, but also may be considered as a substrate for oxidative tissues, such as red and cardiac muscle. In principle, using lactate as a substrate, hepatic gluconeogenesis (i.e. the Cori cycle) and *in situ* glyconeogenesis in white and red muscle may also occur[79]. Swimming has the advantage that it activates blood perfusion of most tissues, and thus allows rapid oxidisation of the accumulated lactate that quickly replenishes the HCO$_3^-$ pool[82]. The re-synthesis of lactate to glycogen will also help to restore metabolic acidosis. So, possibly slow swimming may be the natural way of recovery from a bout of exercise.

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