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Dietary L-carnitine supplementation changes lipid metabolism and glucose utilization of *Rhynchocypris lagowskii* fed diets with different lipid sources

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Abstract The widely available crop oil is an effective alternative to the increasingly scarce marine fish oil. However, simple alternative strategies have led to declining growth and the edible value of farmed fish. It is worthwhile to explore the effects of micro supplements in diets to improve the tolerance of fish to different dietary lipid sources, which finally optimizes the feeding strategies. This study aimed to investigate the regulation of L-carnitine and dietary oil conditions on nutrient composition, lipid metabolism, and glucose regulation of *Rhynchocypris lagowskii*. Four diets were prepared according to fish oil, fish oil supplemented with L-carnitine, corn oil, and corn oil supplemented with L-carnitine, and FO, LCFO, CO, and LCCO were labeled, respectively. *R. lagowskii* was fed experimental diets for 8 weeks, and the glucose tolerance test was performed. The CO diet significantly resulted in higher crude lipid content in muscle but a lower level of serum lipid parameters of *R. lagowskii* than the FO diet. However, dietary L-carnitine supplementation significantly reduced the crude lipid content in the hepatopancreas and muscle of the fish fed with the CO diet yet increased the serum lipid parameters. Additionally, the crude lipid content of muscle was reduced in the fish fed with an FO diet supplemented with L-carnitine. Compared with the FO diet, the CO diet significantly reduced the ratio of n3/n6 polyunsaturated fatty acid in the hepatopancreas and muscle of *R.lagowskii*. Dietary L-carnitine supplementation significantly reduced the contents of total saturated fatty acids and total monounsaturated fatty acids in hepatopancreas under both dietary lipid sources. The CO diet significantly up-regulated the expression of genes related to lipid uptake and adipogenesis in hepatopancreas, including lipoprotein lipase (*lpl*), acetyl-coenzyme A carboxylase alpha (*accα*), and sterol regulatory element binding protein-1 (*srebp1*), compared with the FO diet. While dietary L-carnitine supplementation significantly down-regulated the expressions of *lpl*, *accα*, *srebp1*, and fatty acid synthase in hepatopancreas and muscle of fish under both dietary lipid sources, along with up-regulated expression of carnitine palmitoyltransferase 1 in hepatopancreas. Moreover, the fish fed with a CO diet significantly increased the expression of glucose uptake and clearance and significantly

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down-regulated the expressions of glucose regulation-related genes, including glucose transporter 1, glycogen synthase 1, and phosphofructokinase in hepatopancreas and muscle, resulting in slower glucose uptake and clearance than fish fed with FO diet. Nevertheless, dietary L-carnitine supplementation up-regulated the expression of gluconeogenesis-related genes, including glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the hepatopancreas of *R. lagowskii* under both dietary lipid sources. In conclusion, a higher dietary n6 PUFA resulted in lipid deposition, decreased serum lipid parameters, and limited serum glucose utilization of *R. lagowskii*. While the regulatory effect of L-carnitine on lipid metabolism and glucose utilization of *R. lagowskii* varies with dietary lipid sources and tissues.

Keywords L-carnitine · Dietary lipid source · *Rhynchocypris lagowskii* · Lipid metabolism · Glucose tolerance

Introduction

Intensive aquaculture is considered an effective strategy to alleviate the conflict between the ever-increasing demand for aquatic products and limited marine resources (Farmaki et al. 2021). Fishmeal and fish oil are by-products of marine fish products (Khoomrung et al. 2014), and their increasing demand may be a potential limiting factor for aquaculture (Alhazzaa et al. 2019). Fish oil provides valuable C20 and C22 polyunsaturated fatty acids (PUFA) for aquatic diets (Merkle et al. 2017). Fish must utilize these long-chain polyunsaturated fatty acids (LC-PUFA) to achieve maximum growth performance, good health, and optimal physiological function (Alhazzaa et al. 2019; Tocher 2015). It is generally believed that many freshwater species have sufficient ability to synthesize LC-PUFA from C18 PUFA to achieve the minimum requirements for maintaining tissue structure and physiological functions (Tocher 2010). At present, the replacement of fish oil with plant-based ingredients is considered an effective strategy for maintaining the sustainability of fish farming (Sales and Glencross 2011; Tacon and Metian 2015) . Dietary replacement of fish oil in the diet with vegetable oils (VO) resulted in the reduction of LC-PUFA levels in the liver and muscle of farmed fish (Baoshan et al. 2019; Chen et al. 2020b; He et al. 2021). Accordingly, the content of saturated fatty acids (SFA), monounsaturated fatty acids, n3 PUFA, or n6 PUFA increased depending on the characteristics of the different plants (such as palm oil, canola oil, linseed oil, or soybean oil) (Chen et al. 2020b; Qin et al. 2022; Yu et al. 2021). There are two independent pathways to biosynthesize LC-PUFA from linolenic acid (ALA) or linoleic acid (LOA) by desaturation and elongation in fish (Tocher 2015). Plant-sourced n3 PUFA regulates lipid metabolism and restoration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by enhancing fatty acids (FA) desaturation in different species of fish, such as silver barb (Nayak et al. 2017), common carp (Ren et al. 2012), and Nile tilapia (de Almeida et al. 2021). However, there are contradictory studies available on plant-sourced n6 PUFA. On the one hand, fish oil and VO (rich in n6 PUFA) mixture reduced the lipid requirement of cultured fish (Zhou et al. 2014) without significant negative effects on the proximate composition and growth of fish (Chen et al. 2020b; Sarameh et al. 2019; Yu et al. 2021). On the other hand, a diet rich in n6 PUFA can induce abnormal lipid accumulation in teleost fish (Chen et al. 2020b; Du et al. 2017; Liu et al. 2022; Sun et al. 2020). Recently, a possible strategy has been reported in some recent studies to improve the tolerance of fish to VO through the usage of dietary micro supplements (Chen et al. 2020b; Huang et al. 2019; Jin et al. 2019a). Therefore, exploring the potential interactions of dietary lipid sources and micro supplements in regulating fish nutrient metabolism remains relevant for optimizing and refining culture strategies.

L-carnitine, a vital carrier for long-chain FA to β-oxidation (Harpaz 2005), is used in aquafeeds to exert growth-promoting, lipid-lowering, and protein-retaining effects on many different fish species (Harpaz 2005; Li et al. 2019). However, these positive effects of L-carnitine have also been reported to be affected by other dietary nutrient conditions. Studies on silver perch showed that the effects of L-carnitine on growth, proximate composition, and blood parameters were influenced by dietary lipid levels and protein type (Yang et al. 2012). Moreover, the lipid-lowering effect of L-carnitine on common carp was recorded at high dietary lipid levels (Sabzi et al. 2017). Furthermore, converse trends in the regulation of blood parameters by L-carnitine have been observed in largemouth bass-fed diets with

different dietary lipid sources (Chen et al. 2020b). In addition, recent studies have shown that the regulation of L-carnitine in the nutritional metabolism of fish is not only limited to protein and lipid but also related to carbohydrate utilization (Li et al. 2020, 2021). However, there is still a lack of research to explain how dietary nutritional factors affect the efficacy of L-carnitine. Therefore, the regulatory effects and correlations of exogenous L-carnitine in different nutritional environments still need to be further verified to clarify these changes.

In this study, we formulated four diets (fish oil, corn oil, fish oil with L-carnitine, and corn oil with L-carnitine) fed *R. laogwskii* for 8 weeks. The effects of L-carnitine and dietary lipid sources on serum lipid parameters, carnitine parameters, approximate composition, FA composition, glucose tolerance, and expressions of lipid metabolism and glucose regulation-related genes of *R. lagowskii* were evaluated. Thus, the objective of this study was to analyze the differences and interactions of L-carnitine on the regulation of lipid metabolism and glucose regulation in *R. lagowskii*-fed diets with different lipid sources. These results provide a theoretical basis for optimizing the application of L-carnitine supplementation under future dietary nutritional strategies for fish.

Materials and methods

Composition and preparation of experimental diets

The fatty acid and ingredients of diets were listed in Tables 1 and S1, respectively. Fish meal 20%, soybean meal 33%, corn gluten meal 15%, flour 10%, dextrin 7%, wheat bran 5.96%, oil (fish oil or corn oil) 4%, calcium phosphate 3%, vitamin and mineral premix 1%, methionine powder 0.5%, lysine powder 0.2%, choline chloride 0.3%, and micro-supplementation (L-carnitine or microcrystalline cellulose) 0.04% were used as the ingredients to formulate the experimental diets. The level of dietary L-carnitine supplementation (C0158, Sigma-Aldrich, China) is based on previous research (Yu et al. 2020). The fish oil diet, fish oil diet with L-carnitine, corn oil diet, and corn oil diet with L-carnitine were labeled as FO, LCFO, CO, and LCCO, respectively. The raw materials are mixed according to the order of content, from less to more. All ingredients were pressed into bars and dried at 55 °C. Finally, the strands are broken up and sifted into size pellets suitable for feeding the fish.

Table 1 Fatty acid composition (% total lipids) of experimental diets

Fatty acids (%)	Groups			
	FO	LCFO	CO	LCCO
C12: 0	0.07	0.07	0.04	0.05
C14: 0	2.81	2.94	1.44	1.43
C15: 0	0.25	0.24	0.14	0.14
C16: 0	14.27	14.12	14.31	14.52
C17: 0	0.15	0.15	0.09	0.09
C18: 0	3.57	3.58	2.31	2.32
C20: 0	0.47	0.47	0.46	0.45
C24: 0	0.08	0.09	0.08	0.07
Total SFA	21.66	21.67	18.87	19.07
C16: 1	3.24	3.37	1.30	1.42
C17: 1	0.34	0.36	0.18	0.18
C18: 1n9t	0.14	0.12	0.03	0.04
C18: 1n9c	35.71	35.15	25.14	25.35
C20: 1n9	0.24	0.25	0.15	0.14
C22: 1n9	0.25	0.25	0.05	0.05
C24: 1n9	0.27	0.25	0.11	0.13
Total MUFA	40.20	39.74	26.96	27.30
C18: 3n3	4.31	4.38	1.43	1.39
C20: 5n3	4.34	4.41	2.54	2.60
C22: 5n3	1.10	1.12	0.32	0.33
C22: 6n3	4.66	4.76	2.59	2.65
Total n3 PUFA	14.41	14.67	6.88	6.97
Total n3 LC-PUFA	10.10	10.28	5.45	5.59
C18: 2n6t	0.11	0.12	0.64	0.64
C18: 2n6c	22.54	21.27	45.57	44.97
C18: 3n6	0.21	1.66	0.55	0.54
C20: 2n6	0.21	0.24	0.14	0.13
C20: 3n6	0.16	0.14	0.05	0.04
C20: 4n6	0.38	0.38	0.18	0.18
C22: 2n6	0.12	0.12	0.17	0.15
Total n6 PUFA	23.72	23.92	47.29	46.65
Total n6 LC-PUFA	0.53	0.52	0.23	0.22
Total PUFA	38.13	38.59	54.17	53.62
n3/n6 PUFA	0.61	0.61	0.15	0.15
Total LC-PUFA	10.63	10.80	5.68	5.81
n3/n6 LC-PUFA	18.97	19.85	23.80	25.10

FO, fish oil; *LCFO*, L-carnitine + fish oil; *CO*, corn oil; *LCCO*, L-carnitine + corn oil; *LC,* L-carnitine; *SFA*, saturated fatty acids; *MUFA*, monounsaturated fatty acids; *PUFA*, polyunsaturated fatty acids; *LC-PUFA*, long chain-polyunsaturated fatty acids

Experimental fish and feeding management

Niannianyouyu Fishery provided all experimental fish (Tonghua, Jilin, China). The culture system consists of 18 cylindrical tanks, each group containing 3 tanks. *R. lagowskii* were screened for the visually healthy and similar wet weight (12.21 ± 0.07 g), and 30 individuals were randomly assigned to each tank after sterilization with potassium permanganate solution. The daily diet quality for all groups was 3% of the average fish body weight for 8 weeks. The feeding amount was adjusted every week according to the total weight of fish in each tank. Dissolved oxygen (8 ± 1 mg/L), water temperature (22 ± 1 °C), and transparency are observed daily.

Sample collection

R. lagowskii fasted for 24 h when reaching the 8-week trial. Plasma, hepatopancreas, and muscle were isolated after the fish were anesthetized. Serum was collected by centrifugation of plasma (1000 × *g*, 10 min, 4 °C). The samples were stored at – 80 °C after being snap frozen with liquid nitrogen.

Proximate composition and FA composition analysis

Proximate composition of the experimental diets, hepatopancreas, and muscle were executed referring to standard methodology (AOAC 2005). Crude protein was confirmed by the Kjeldahl method, and crude lipid was confirmed by the Soxhlet extraction method (Michelato et al. 2016). The moisture of samples was determined by calculating the weight loss using a drying oven (BG2-246, Boxun, China) at 105 °C. Ash was calculated by the weight of the residue using a muffle furnace (SX2-6-13 T, OLABO, China) at 500 °C for 6 h.

The lyophilized samples and solvent (chloroform: methanol, 2:1, *v*/*v*) were mixed, and then FAs were methylated using a 2% KOH–methanol solution. The FAs composition was analyzed by gas chromatograph (6890 N, Agilent, USA) (Dong et al. 2014).

Serum lipid parameters and total carnitine concentration analysis

High-density lipoprotein cholesterol (HDL-C) (A112-1–1), total cholesterol (T-CHO) (A045-4), low-density lipoprotein cholesterol (LDL-C) (A113-1–1), and total triglyceride (TG) (A110-1–1) concentrations in serum were quantified by analyzing suite (Nanjing Jiancheng Bioengineering Institute, China).

Hepatopancreas, muscle, and serum concentrations of total carnitine (YX-030118F) were analyzed using a commercial kit (Sinobestbio, Shanghai, China) based on the competitive enzyme-linked immunosorbent assay (ELISA) method. Antibodies were produced by immune reaction induced by incomplete antigen formed by carnitine coupling with bovine serum albumin and were pre-fixed in micropores. Samples and standards were added to the wells to bind their antigen to the antibody. Then the antigen labeled by horseradish peroxidase (HRP) was added to the micropores to further bind the remaining antibody. The color reaction was carried out by HRP-catalyzed urea hydrogen peroxide and 3, 3′, 5, 5′-tetramethylbenzene.

Glucose metabolic profile

A glucose tolerance test (GTT) was used to evaluate the glucose metabolic profile in serum of *R. lagowskii* fed experimental diets of 8 weeks. After 24 h fast, glucose administration by intraperitoneal (I.P.) injection for 6 fish per group (1.67 g/kg body weight) (Du et al. 2020; Zhang et al. 2020), and glucose in serum was detected at 0, 0.5, 1, 2, 4, 6, 8, and 10 h using a commercial suite (F006-1–1, Nanjing Jiancheng Bioengineering Institute, China) based on glucose oxidase method.

Analysis of relative mRNA expression of genes

Total RNA of samples were obtained using the trizol extractant method (Wissen, China). The quality and concentration of total RNA were identified by agarose gel electrophoresis and NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, USA), respectively. Reaction systems were prepared according to the instructions of the reverse transcription reaction (RR047A, Takara, China) and RT-qPCR (RR820A, Takara, China) product kits, respectively. The cDNA acquisition and real-time quantitative PCR procedures were referred to in previous studies (Wang et al. 2020). The results were calculated by the $2^{-\Delta\Delta CT}$ protocols, based on the CT value of the target genes and β-actin of each sample (Livak and Schmittgen 2001) . The primers were designed by NCBI online tools and listed in Table S2.

Statistical analysis of data

The results of all items were analyzed by SPSS 22 (IBM, Chicago, USA). After the homogeneity of variances and normality of distribution, the main effect, simple effect, and interaction of different items were concluded using a two-way analysis of variance. Tukey's method of multiple range test was corrected for differences between groups ($P < 0.05$). Results have been listed as means with standard deviation (mean ± SD).

Results

Proximate composition

The CO diet significantly reduced the crude protein content in the hepatopancreas of *R. lagowskii* compared with the FO diet, but crude lipid content in the muscle was significantly increased ($P < 0.05$) (Fig. 1A and F). Both L-carnitine supplementation and dietary lipid sources had no significant effect on the crude protein content in the muscle of *R. lagowskii* ($P > 0.05$) (Fig. 1E). LCCO diet significantly decreased the content of crude lipid in hepatopancreas and muscle than fish fed CO diet ($P < 0.05$) (Fig. 1B and F). The lowest content of moisture in the hepatopancreas was observed in the FO group (Fig. 1C), while there was no significant difference in the moisture content of muscle among FO, LCFO, CO, and LCCO groups ($P > 0.05$) (Fig. 1G). No significant difference was observed in the ash content of hepatopancreas and muscle among FO, LCFO, CO, and LCCO groups ($P > 0.05$) (Fig. 1D and H).

FA composition in hepatopancreas and muscle

FA composition in the hepatopancreas and muscle of *R. lagowskii* were listed in Tables 2 and 3, respectively. The CO diet had significantly increased the total n6 PUFA content in muscle and hepatopancreas than the fish-fed FO diet ($P < 0.05$), while the total n3 PUFA content was significantly decreased ($P < 0.05$). CO diet significantly reduced total MUFA content in hepatopancreas ($P < 0.05$) than the FO diet. There was no significant effect was observed in the total MUFA content in the muscle ($P > 0.05$). L-carnitine supplementation significantly reduced total SFA and total MUFA content in the hepatopancreas ($P < 0.05$), whereas total n6 PUFA content was significantly increased ($P < 0.05$). L-carnitine had no significant effect on the content of total n3 PUFA content in the hepatopancreas and muscle of *R. lagowskii* fed diets with fish oil and corn oil ($P > 0.05$).

Serum lipid parameters and total carnitine concentrations

Compared with the FO diet, the dietary CO diet had significantly lower TG, T-CHO, and LDL-C concentrations in the serum ($P < 0.05$) (Fig. 2A, B, and D). The LCCO diet significantly increased serum TG, T-CHO, HDL-C, and LDL-C concentrations more than the CO diet ($P < 0.05$) (Fig. 2A–D). However, no significant differences in these serum lipid parameters were observed between the FO and LCFO diets ($P > 0.05$) (Fig. 2A–D).

R. lagowskii fed CO diet had significantly lower total carnitine concentrations in hepatopancreas and muscle than the FO diet ($P < 0.05$) (Fig. 3A and B). LCFO diet significantly increased total carnitine concentrations in hepatopancreas and muscle than the FO diet ($P < 0.05$) (Fig. 3A and B). Moreover, LCFO and LCCO diets significantly increased total carnitine concentration in the serum of *R. lagowskii* than fish-fed FO and CO diets, respectively ($P < 0.05$) (Fig. 3C).

The expression of lipid metabolism-related genes in hepatopancreas and muscle

L-carnitine supplementation significantly up-regulated hepatopancreatic carnitine palmitoyltransferase 1 (*cpt1*) expression in *R. lagowskii* under both dietary conditions ($P < 0.05$), and *cpt1* expression in muscle was not significantly changed ($P > 0.05$) (Fig. 4A). The CO diet significantly up-regulated lipoprteinlipase (*lpl*) expression in hepatopancreas and muscle of *R. lagowskii* than fish fed FO diet, while LCCO diet significantly decreased *lpl* expression in muscle compared with fish fed CO diet (Fig. 4B). No significant difference in fatty acid synthase (*fas*) expression of hepatopancreas and muscle were observed between fish fed FO and CO diets ($P > 0.05$), while LCCO diet significantly down-regulated *fas* expression in hepatopancreas and muscle than fish fed CO diet ($P < 0.05$) (Fig. 4C). The CO diet significantly

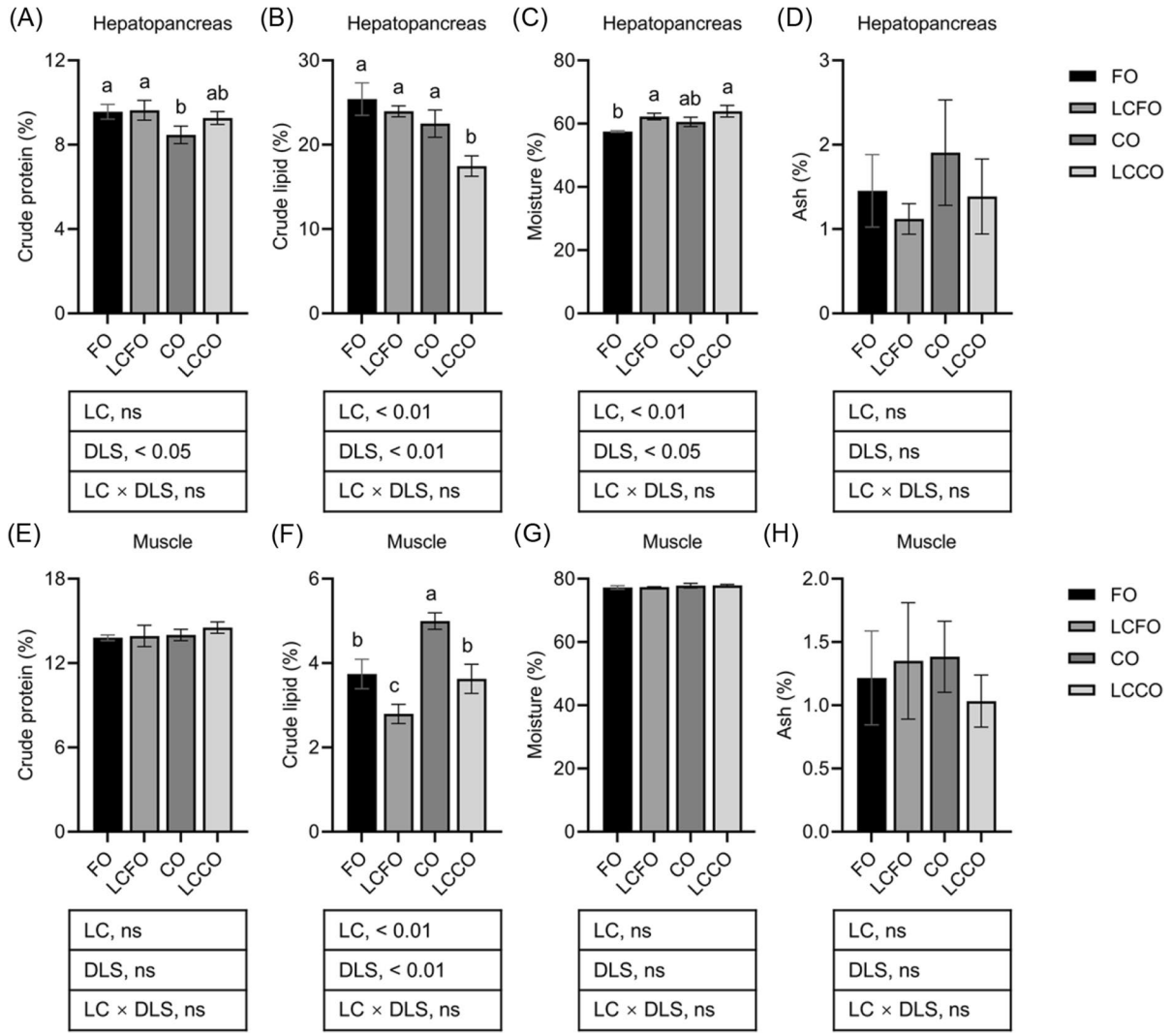

Fig. 1 Effect of the L-carnitine supplementation on the proximate composition (wet weight) in hepatopancreas and muscle of *R. lagowskii* under different dietary lipid sources. **A** Crude protein in hepatopancreas; **B** crude lipid in hepatopancreas; **C** moisture in hepatopancreas; **D** ash in hepatopancreas; **E** crude protein in muscle; **F** crude lipid in muscle; **G** moisture in muscle; **H** ash in muscle. Values are expressed as means ± SD ($n = 3$). Values with different lowercase letters represent statistical differences at $P < 0.05$. The significance of simple effects (LC, L-carnitine and DLS, dietary lipid source) and interaction effects (LC × DLS) of two-way ANOVA are listed at the bottom of the figure. No significant differences ($P \geq 0.05$) in two-way ANOVA are marked as "ns"

up-regulated hepatic acetyl-coenzyme A carboxylase alpha (*accα*) and sterol regulatory element binding protein 1 (*srebp1*) expressions compared to fish fed FO diet ($P < 0.05$), whereas L-carnitine significantly decreased *accα* and *srebp1* expressions in hepatopancreas of *R. lagowskii* fed CO diet ($P < 0.05$) (Fig. 4D and E).

The expression of glucose metabolism-related genes and GTT of *R. lagowskii*

In the hepatopancreas, the CO diet significantly down-regulated the expression of glucose transporter 1 (*glut1*) and glycogen synthase 1 (*pfkla*) of *R. lagowskii* than fish fed FO diet ($P < 0.05$) (Fig. 5A and E), while

Table 2 Effect of the L-carnitine supplementation on the fatty acid composition (% total lipids) in hepatopancreas of *R. lagowskii* under different dietary lipid sources

Fatty acid (%)	Groups				*P*-value		
	FO	LCFO	CO	LCCO	LC	DLS	LC × DLS
C12: 0	0.08 ± 0.01^{c}	0.09 ± 0.01^{bc}	0.10 ± 0.01^{ab}	0.12 ± 0.01^{a}	< 0.01	< 0.01	ns
C14: 0	1.81 ± 0.05^{a}	1.80 ± 0.05^{a}	1.61 ± 0.05^{b}	1.35 ± 0.04^{c}	< 0.01	< 0.01	< 0.01
C15: 0	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	ns	< 0.05	ns
C16: 0	16.16 ± 0.19^{a}	15.15 ± 0.22^{b}	15.55 ± 0.19^{b}	14.37 ± 0.22^{c}	< 0.01	< 0.01	ns
C17: 0	0.11 ± 0.01^{b}	0.14 ± 0.01^{a}	0.09 ± 0.01^{b}	0.10 ± 0.01^{b}	< 0.01	< 0.01	ns
C18: 0	4.19 ± 0.13^{a}	3.50 ± 0.14^{b}	3.64 ± 0.10^{b}	3.72 ± 0.11^{b}	< 0.01	< 0.05	< 0.01
C20: 0	0.03 ± 0.01^{c}	0.15 ± 0.01^{b}	0.17 ± 0.01^{b}	0.21 ± 0.01^{a}	< 0.01	< 0.01	< 0.01
C21: 0	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.06 ± 0.03	ns	ns	ns
C22: 0	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	ns	ns	ns
C24: 0	0.10 ± 0.01	0.09 ± 0.03	0.10 ± 0.01	0.06 ± 0.01	< 0.05	ns	ns
Total SFA	22.77 ± 0.42^{a}	21.23 ± 0.50^{bc}	21.54 ± 0.39^{b}	20.22 ± 0.45^{c}	< 0.01	< 0.01	ns
C14: 1	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.06 ± 0.00^{b}	< 0.05	< 0.01	< 0.01
C16: 1	7.17 ± 0.04^{a}	7.49 ± 0.06^{a}	6.28 ± 0.15^{b}	4.54 ± 0.22^{c}	< 0.01	< 0.01	< 0.01
C17: 1	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}	0.07 ± 0.01^{b}	0.07 ± 0.01^{b}	ns	< 0.01	ns
C18: 1n9t	0.15 ± 0.01^{a}	0.10 ± 0.01^{b}	0.10 ± 0.01^{b}	0.10 ± 0.01^{b}	< 0.01	< 0.01	< 0.01
C18: 1n9c	46.86 ± 0.21^{a}	43.76 ± 0.19^{b}	38.99 ± 0.13^{c}	39.43 ± 0.22^{c}	< 0.01	< 0.01	< 0.01
C20: 1n9	0.18 ± 0.01^{a}	0.15 ± 0.01^{b}	0.10 ± 0.01^{c}	0.11 ± 0.01^{c}	ns	< 0.01	< 0.01
C22: 1n9	0.21 ± 0.01^{a}	0.15 ± 0.01^{b}	0.16 ± 0.03^{b}	0.18 ± 0.01^{ab}	ns	ns	< 0.01
Total MUFA	54.67 ± 0.22^{a}	51.43 ± 0.35^{b}	45.62 ± 0.24^{c}	44.16 ± 0.48^{d}	< 0.01	< 0.01	< 0.01
C18: 3n3	1.62 ± 0.08^{a}	1.43 ± 0.04^{b}	1.29 ± 0.06^{b}	1.41 ± 0.06^{b}	ns	< 0.01	< 0.01
C20: 3n3	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	ns	ns	ns
C20: 5n3	0.77 ± 0.04^{a}	0.78 ± 0.04^{a}	0.57 ± 0.01^{b}	0.59 ± 0.05^{b}	ns	< 0.01	ns
C22: 5n3	0.25 ± 0.01^{a}	0.18 ± 0.01^{b}	0.14 ± 0.01^{c}	0.18 ± 0.01^{b}	< 0.05	< 0.01	< 0.01
C22: 6n3	2.23 ± 0.09^{b}	2.65 ± 0.11^{a}	1.70 ± 0.14^{c}	1.72 ± 0.12^{c}	< 0.05	< 0.01	< 0.05
Total n3 PUFA	5.02 ± 0.23^{a}	5.19 ± 0.18^{a}	3.85 ± 0.19^{b}	4.04 ± 0.19^{b}	ns	< 0.01	ns
Total n3 LC-PUFA	3.40 ± 0.14^{a}	3.76 ± 0.17^{a}	2.56 ± 0.16^{b}	2.62 ± 0.17^{b}	ns	< 0.01	ns
C18: 2n6t	0.19 ± 0.01^{c}	0.34 ± 0.02^{b}	0.39 ± 0.03^{a}	0.38 ± 0.01^{ab}	< 0.01	< 0.01	< 0.01
C18: 2n6c	16.10 ± 0.46^{d}	20.01 ± 0.57^{c}	27.15 ± 0.42^{b}	29.53 ± 0.54^{a}	< 0.01	< 0.01	< 0.05
C18: 3n6	0.17 ± 0.01^{a}	0.12 ± 0.01^{b}	0.11 ± 0.01^{b}	0.10 ± 0.01^{b}	< 0.01	< 0.01	< 0.05
C20: 2n6	0.12 ± 0.01^{b}	0.14 ± 0.01^{b}	0.17 ± 0.01^{a}	0.17 ± 0.01^{a}	ns	< 0.01	ns
C20: 3n6	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	ns	ns	ns
C20: 4n6	0.59 ± 0.09^{b}	0.87 ± 0.09^{a}	0.78 ± 0.02^{a}	0.83 ± 0.01^{a}	< 0.01	ns	< 0.05
C22: 2n6	0.23 ± 0.01^{a}	0.18 ± 0.01^{b}	0.16 ± 0.02^{b}	0.17 ± 0.01^{b}	< 0.05	< 0.01	< 0.01
Total n6 PUFA	17.46 ± 0.40^{d}	21.72 ± 0.69^{c}	28.83 ± 0.53^{b}	31.24 ± 0.55^{a}	< 0.01	< 0.01	< 0.05
Total n6 LC-PUFA	0.65 ± 0.09^{b}	0.94 ± 0.08^{a}	0.85 ± 0.01^{a}	0.89 ± 0.02^{a}	< 0.01	ns	< 0.05
Total PUFA	22.48 ± 0.18^{d}	26.91 ± 0.52^{c}	32.68 ± 0.36^{b}	35.27 ± 0.37^{a}	< 0.01	< 0.01	< 0.01
n3/n6 PUFA	0.29 ± 0.02^{a}	0.24 ± 0.02^{b}	0.13 ± 0.01^{c}	0.13 ± 0.01^{c}	< 0.05	< 0.01	< 0.05
Total LC-PUFA	4.05 ± 0.22^{b}	4.70 ± 0.09^{a}	3.41 ± 0.15^{c}	3.51 ± 0.18^{c}	< 0.01	< 0.01	< 0.05
n3/n6 LC-PUFA	5.33 ± 0.69^{a}	4.04 ± 0.52^{b}	3.02 ± 0.22^{b}	2.95 ± 0.18^{b}	< 0.05	< 0.01	< 0.05

All data are expressed as means ± SD ($n = 3$). Different lowercase letters after the data in the same line indicate significant differences ($P < 0.05$). Individual fatty acid concentrations are expressed as a percentage of the total lipids. The significance of simple effects (LC, L-carnitine and DLS, dietary lipid source) and interaction effects (LC × DLS) of two-way ANOVA are listed in the table. No significant differences in two-way ANOVA are marked as "ns." *SFA*, saturated fatty acids; *MUFA*, monounsaturated fatty acids; *PUFA*, polyunsaturated fatty acids; *LC-PUFA*, long chain-polyunsaturated fatty acids

Table 3 Effect of the L-carnitine supplementation on the fatty acid composition (% total lipids) in muscle of *R. lagowskii* under different dietary lipid sources

Fatty acid (%)	Groups				*P*-value		
	FO	LCFO	CO	LCCO	LC	DLS	LC×DLS
C14: 0	2.43 ± 0.07^{a}	2.20 ± 0.03^{b}	1.85 ± 0.01^{c}	1.62 ± 0.05^{d}	< 0.01	< 0.01	ns
C15: 0	0.21 ± 0.04	0.22 ± 0.02	0.23 ± 0.05	0.23 ± 0.05	ns	ns	ns
C16: 0	14.70 ± 0.22^{ab}	15.01 ± 0.43^{ab}	14.41 ± 0.32^{b}	15.47 ± 0.52^{a}	< 0.05	ns	ns
C17: 0	0.10 ± 0.01	0.16 ± 0.03	0.10 ± 0.03	0.13 ± 0.02	< 0.05	ns	ns
C18: 0	1.72 ± 0.08^{b}	1.73 ± 0.06^{b}	1.86 ± 0.08^{b}	2.18 ± 0.09^{a}	< 0.01	< 0.01	< 0.01
C20: 0	0.21 ± 0.04	0.23 ± 0.04	0.20 ± 0.02	0.21 ± 0.05	ns	ns	ns
C22: 0	0.41 ± 0.01	0.39 ± 0.06	0.41 ± 0.03	0.39 ± 0.03	ns	ns	ns
Total SFA	19.78 ± 0.44	19.93 ± 0.61	19.06 ± 0.48	20.23 ± 0.76	ns	ns	ns
C16: 1	7.73 ± 0.29^{a}	7.50 ± 0.59^{a}	7.01 ± 0.55^{ab}	6.18 ± 0.17^{b}	ns	< 0.01	ns
C17: 1	0.36 ± 0.05	0.35 ± 0.06	0.32 ± 0.03	0.34 ± 0.05	ns	ns	ns
C18: 1n9c	31.06 ± 0.67	29.76 ± 0.70	29.86 ± 0.29	30.82 ± 0.28	ns	ns	< 0.01
C18: 1n9t	0.16 ± 0.05	0.17 ± 0.04	0.21 ± 0.04	0.13 ± 0.04	ns	ns	ns
C20: 1n9	1.31 ± 0.25^{bc}	1.76 ± 0.12^{a}	1.60 ± 0.05^{ab}	1.15 ± 0.18^{c}	ns	ns	< 0.01
C22: 1n9	0.28 ± 0.04	0.26 ± 0.04	0.27 ± 0.04	0.24 ± 0.05	ns	ns	ns
C24: 1n9	0.08 ± 0.01	0.10 ± 0.03	0.09 ± 0.04	0.09 ± 0.03	ns	ns	ns
Total MUFA	40.99 ± 0.88	39.91 ± 1.47	39.36 ± 1.01	38.94 ± 0.54	ns	ns	ns
C18: 3n3	3.28 ± 0.02^{a}	3.09 ± 0.10^{b}	2.27 ± 0.04^{c}	2.24 ± 0.02^{c}	< 0.01	< 0.01	< 0.05
C20: 5n3	1.65 ± 0.08^{a}	1.50 ± 0.08^{a}	1.04 ± 0.06^{b}	0.92 ± 0.09^{b}	< 0.05	< 0.01	ns
C22: 6n3	4.08 ± 0.07^{a}	4.04 ± 0.06^{a}	2.91 ± 0.09^{b}	2.72 ± 0.06^{c}	< 0.05	< 0.01	ns
Total n3 PUFA	9.01 ± 0.17^{a}	8.64 ± 0.23^{a}	6.22 ± 0.18^{b}	5.88 ± 0.16^{b}	< 0.05	< 0.01	ns
Total n3 LC-PUFA	5.73 ± 0.15^{a}	5.54 ± 0.13^{a}	3.95 ± 0.14^{b}	3.64 ± 0.14^{b}	< 0.05	< 0.01	ns
C18: 2n6c	25.71 ± 0.55^{b}	26.93 ± 0.80^{b}	30.82 ± 0.49^{a}	30.30 ± 0.47^{a}	ns	< 0.01	< 0.05
C18: 3n6	0.77 ± 0.01^{b}	0.68 ± 0.02^{c}	0.76 ± 0.03^{b}	0.93 ± 0.02^{a}	< 0.05	< 0.01	< 0.01
C20: 2n6	1.21 ± 0.05	1.15 ± 0.07	1.25 ± 0.02	1.27 ± 0.07	ns	< 0.05	ns
C20: 3n6	0.83 ± 0.02	0.84 ± 0.06	0.87 ± 0.05	0.85 ± 0.04	ns	ns	ns
C20: 4n6	1.00 ± 0.04^{b}	1.23 ± 0.09^{a}	0.93 ± 0.08^{b}	0.89 ± 0.03^{b}	< 0.05	< 0.01	< 0.01
C22: 2n6	0.69 ± 0.03	0.70 ± 0.04	0.73 ± 0.03	0.72 ± 0.05	ns	ns	ns
Total n6 PUFA	30.21 ± 0.69^{b}	31.53 ± 1.05^{b}	35.36 ± 0.68^{a}	34.95 ± 0.52^{a}	ns	< 0.01	ns
Total n6 LC-PUFA	1.83 ± 0.06^{ab}	2.07 ± 0.15^{a}	1.80 ± 0.13^{ab}	1.74 ± 0.08^{b}	ns	< 0.05	< 0.05
Total PUFA	39.22 ± 0.60^{b}	40.17 ± 0.94^{ab}	41.58 ± 0.53^{a}	40.83 ± 0.48^{ab}	ns	< 0.01	ns
n3/n6 PUFA	0.30 ± 0.01^{a}	0.27 ± 0.01^{a}	0.18 ± 0.01^{b}	0.17 ± 0.01^{b}	< 0.05	< 0.01	ns
Total LC-PUFA	7.56 ± 0.11^{a}	7.61 ± 0.08^{a}	5.75 ± 0.07^{b}	5.37 ± 0.07^{c}	< 0.05	< 0.01	< 0.01
n3/n6 LC-PUFA	3.14 ± 0.18^{a}	2.69 ± 0.26^{ab}	2.21 ± 0.24^{bc}	2.10 ± 0.18^{c}	ns	< 0.01	ns

All data are expressed as means ± SD ($n = 3$). Different lowercase letters after the data in the same line indicate significant differences ($P < 0.05$). Individual fatty acid concentrations are expressed as a percentage of the total lipids. The significance of simple effects (LC, L-carnitine and DLS, dietary lipid source) and interaction effects (LC × DLS) of two-way ANOVA are listed in the table. No significant differences in two-way ANOVA are marked as "ns." *SFA*, saturated fatty acids; *MUFA*, monounsaturated fatty acids; *PUFA*, polyunsaturated fatty acids; *LC-PUFA*, long chain-polyunsaturated fatty acids

glucose-6-phosphatase (*g6pase*) and phosphoenolpyruvate carboxykinase (*pepck*) expressions were significantly up-regulated ($P < 0.05$) (Fig. 5C and D). Furthermore, LCFO and LCCO diets significantly up-regulated the expression of *g6pase* and *pepck* in the hepatopancreas than fish-fed FO and CO diets ($P < 0.05$), respectively (Fig. 5C and D). In the muscle, the CO diet significantly down-regulated *glut1*, glycogen synthase 1 (*gys1*), *g6pase*, *pepck*, and *pfkla* expression in *R. lagowskii* than dietary FO ($P < 0.05$) (Fig. 5A–E).

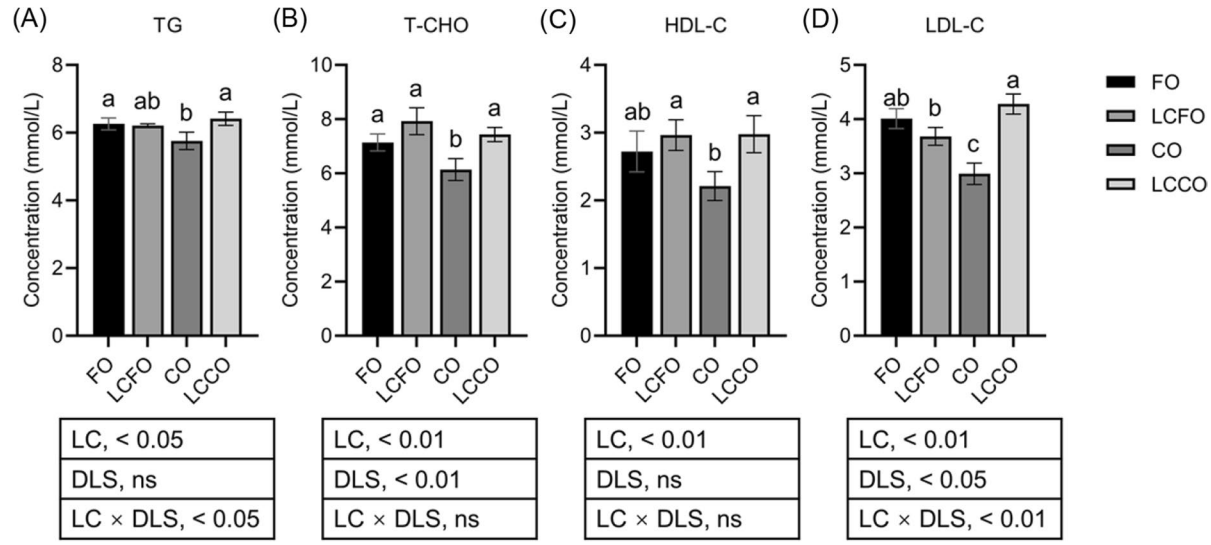

Fig. 2 Effect of the L-carnitine supplementation on the lipid parameters in the serum of *R. lagowskii* under different dietary lipid sources. Values are expressed as means ± SD ($n = 3$). **A** TG in serum; **B** T-CHO in serum; **C** HDL-C in serum; **D** LDL-C in serum. Values with different lowercase letters represent statistical differences at $P < 0.05$. The significance of simple effects (LC, L-carnitine and DLS, dietary lipid source) and interaction effects (LC × DLS) of two-way ANOVA are listed at the bottom of the figure. No significant differences ($P \geq 0.05$) in two-way ANOVA are marked as "ns." TG, triglycerides; T-CHO, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

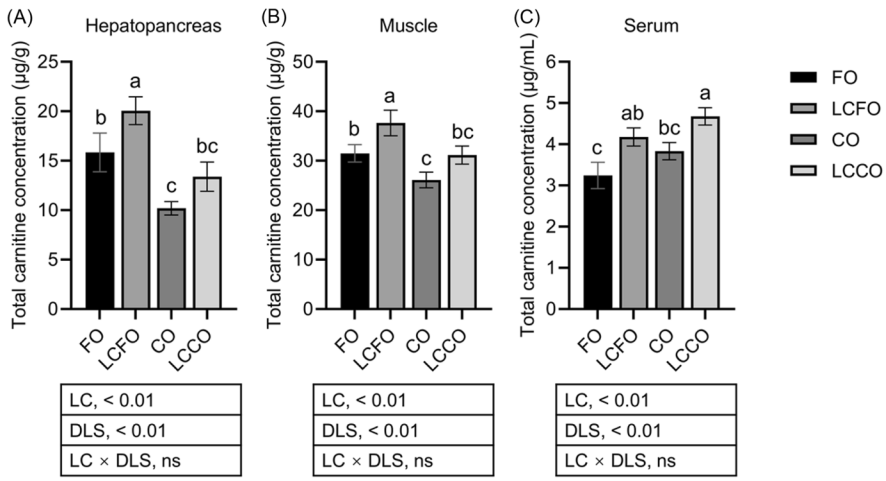

Fig. 3 Effect of the L-carnitine supplementation on the carnitine concentration in tissues (wet weight) and serum of *R. lagowskii* under different dietary lipid sources. **A** Total carnitine in hepatopancreas; **B** total carnitine in muscle; **C** total carnitine in serum. All values are means ± SD ($n = 3$). Values with different lowercase letters represent statistical differences at $P < 0.05$. The significance of simple effects (LC, L-carnitine and DLS, dietary lipid source) and interaction effects (LC × DLS) of two-way ANOVA are listed at the bottom of the figure. No significant differences ($P \geq 0.05$) in two-way ANOVA are marked as "ns"

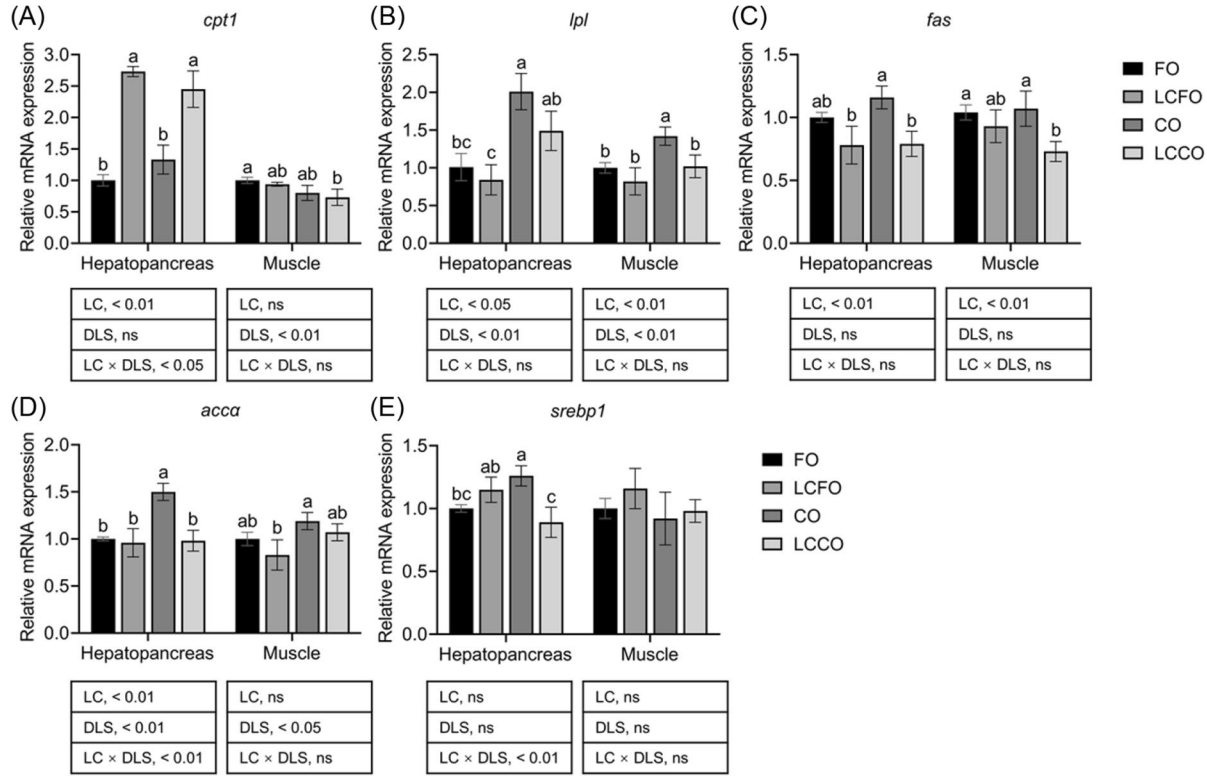

Fig. 4 Effect of the L-carnitine supplementation on the expression of lipid metabolism-related genes of *R. lagowskii* under different dietary lipid sources. Values are expressed as means ± SD ($n = 3$). **A** *cpt1* relative expression; **B** *srebp1* relative expression; **C** *lpl* relative expression; **D** *fas* relative expression; **E** *accα* relative expression. Values with different lowercase letters represent statistical differences at $P < 0.05$. The significance of simple effects (LC, L-carnitine and DLS, dietary lipid source) and interaction effects (LC × DLS) of two-way ANOVA are listed at the bottom of the figure. No significant differences ($P \geq 0.05$) in two-way ANOVA are marked as "ns." *cpt1*, carnitine palmitoyltransferase 1; *srebp1*, sterol regulatory element binding protein-1; *lpl*, lipoprteinlipase; *fas*, fatty acid synthase; *accα*, acetyl-coenzyme A carboxylase alpha

However, adding L-carnitine to the CO diet significantly up-regulated *pfkla* expression in muscle relative to the fish-fed CO diet ($P < 0.05$) (Fig. 5E). There was no significant difference in the expression of hexokinase-4 (*hk4*) in hepatopancreas and muscle between *R. lagowskii* fed FO and CO diets ($P > 0.05$) (Fig. 5F). Moreover, L-carnitine supplementation in FO and CO diets had no significant effect on the expression of *hk4* in hepatopancreas and muscle of *R. lagowskii* ($P > 0.05$) (Fig. 5F). The serum glucose curve of *R. lagowskii* after GTT was shown in Fig. 5G. During glucose I.P. administration, initial glucose concentration was significantly higher in the serum of *R.lagowskii* fed LCCO diet, compared with fish fed FO, LCFO, and CO diets ($P < 0.05$) (fasted for 24 h). The serum glucose concentration of *R. lagowskii* fed FO and LCFO diets peaked at 0.5 h and decreased significantly at 1 h after the I.P. ($P < 0.05$). The peak glucose concentration in the serum of *R. lagowskii* fed the LCFO diet was significantly reduced at 0.5 h than the FO diet. At 0.5 h, the peak serum glucose concentration was significantly reduced in *R. lagowskii* fed LCFO diet than fish fed FO diet ($P < 0.05$). Differently, the serum glucose concentration of *R. lagowskii* fed CO and LCCO diets peaked at 1 h and significantly reduced at 1.5 h ($P < 0.05$). However, no significant difference was observed in the peak concentration of serum glucose between dietary CO and LCCO at 1 h ($P > 0.05$). The serum glucose concentration of *R. lagowskii* fed FO, LCFO, and LCCO diets recovered to the initial concentration at 6 h after glucose I.P., while fish fed CO diet returned to the initial serum glucose concentration after 10 h.

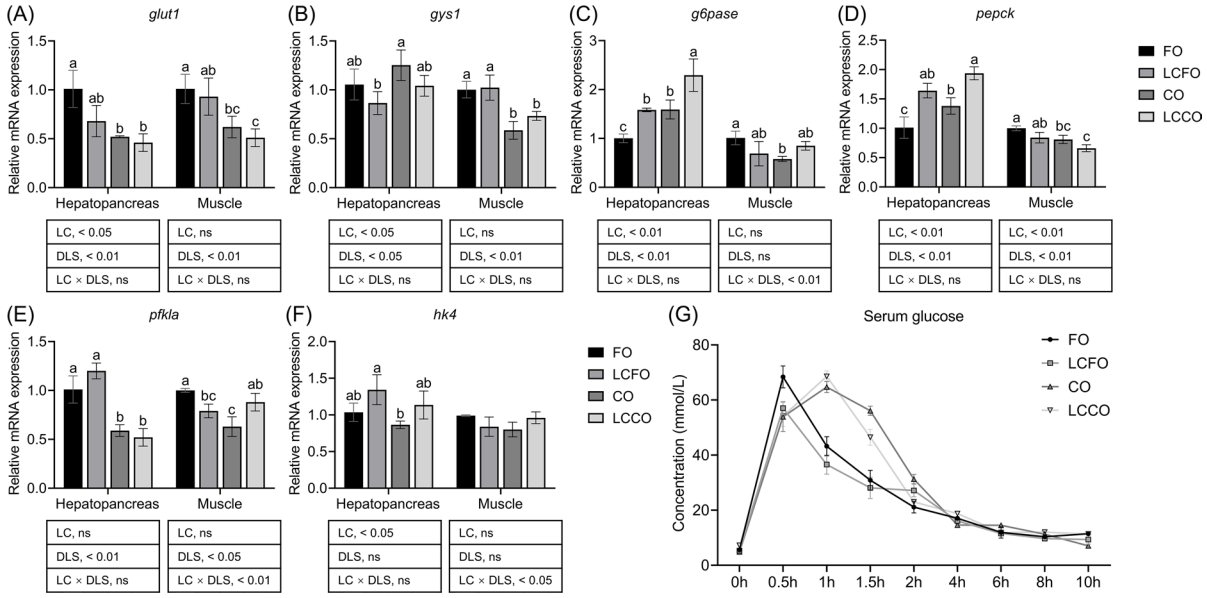

Fig. 5 Effect of the L-carnitine supplementation on the expression of glucose metabolism-related genes and glucose tolerance test of *R. lagowskii* under different dietary lipid sources. Values are expressed as means ± SD ($n = 3$). **A** *glut1* relative expression; **B** *gys1* relative expression; **C** *g6pase* relative expression; **D** *pepck* relative expression; **E** *pfkla* relative expression; **F** *hk4* relative expression; **G** serum glucose curves after intraperitoneal injection. Values with different lowercase letters represent statistical differences at $P < 0.05$. The significance of simple effects (LC, L-carnitine and DLS, dietary lipid source) and interaction effects (LC × DLS) of two-way ANOVA are listed at the bottom of the figure. No significant differences ($P \geq 0.05$) in two-way ANOVA are marked as "ns." *glut1*, glucose transporter 1; *gys1*, glycogen synthase 1; *g6pase*, glucose-6-phosphatase; *pepck*, phosphoenolpyruvate carboxykinase; *pfkla*, phosphofructokinase; *hk4*, hexokinase-4

Discussion

In the present study, dietary n6 PUFA-rich corn oil resulted in lipid accumulation in muscle and protein loss in the hepatopancreas of *R. lagowskii* compared with the fish oil diet. Similarly, the replacement of soybean oil for fish oil significantly increased crude lipid content in the muscle of grass carp (Liu et al. 2022) and black seabream (Peng et al. 2008). In addition, lipid accumulation resulting from n6 PUFA-rich VO replacing fish oil has also been reported to be enriched in the liver of large yellow croaker (Du et al. 2017), Totoaba macdonaldi (Maldonado-Othón et al. 2020), and the whole body of rainbow trout (Yıldız et al. 2018). For fish, the ingested lipids are decomposed by the liver and resynthesized into FA, while the non-oxidized FA is mainly accumulated in the liver, muscle, and adipose tissue (He et al. 2015; Wang et al. 2022; Yin et al. 2021). Replacement of dietary fish oil by n6 PUFA-rich VO (such as soybean oil, corn oil, rapeseed oil, etc.), which greatly reduced the ratio of n3/n6 PUFA in the diet (Chen et al. 2020b; Yu et al. 2021; Zhou et al. 2014). In this study, the total n6 PUFA content in hepatopancreas and muscle of *R. lagowskii* fed corn oil diet was increased by 65.12 and 17.05% compared to fish fed fish oil diet, respectively. Previous mammalian studies have indicated that n3 PUFA reduces obesity by inhibiting fat deposition (Duwaerts and Maher 2019; Martínez-Fernández et al. 2015), whereas dietary n6 PUFA did not effectively inhibit the hypertrophy and hyperplasia of adipose tissue (Sharma and Agnihotri 2020). Studies on grass carp have shown that soybean oil instead of fish oil promotes lipid utilization by up-regulated β-oxidation, elongation, and desaturation of FA (Liu et al. 2022). Likewise, studies on black sea bream have also confirmed that lipolysis and FA synthesis increase with decreasing dietary n3 LC-PUFA/n6 C18 PUFA ratios. Therefore, the increased crude lipid content in the muscle of *R.lagowskii* fed corn oil diet is closely related to the reduced dietary n3/n6 PUFA ratio (Bandarra et al. 2011; Li et al. 2015; Liu et al. 2022). However, the tolerance of different fish to n6 PUFA-rich VO is also related to the requirement of essential FA and the basic nutrition of the diet (Miller et al. 2008). As in previous studies, the substitution of VO for dietary fish

oil has no significant effect on the crude lipid content of some fish species, including the liver and muscle of largemouth bass (Chen et al. 2020b), the whole body and muscle of black seabream (Jin et al. 2019b; Peng et al. 2008), and the whole body of sharp snout seabream (Piedecausa et al. 2007). In this study, dietary L-carnitine supplementation resulted in lower crude lipid content in the hepatopancreas and muscle of *R. lagowskii*. Dietary L-carnitine exhibits systemic or tissue lipid-lowering effects in different fish (Li et al. 2019). However, previous studies reported that neither the liver nor muscle of largemouth bass (Chen et al. 2020b), hybrid striped bass (Twibell and Brown 2000) , and tilapia (Yang et al. 2010) was affected by L-carnitine supplementation. Differences in the dose of L-carnitine supplementation, dietary lipid content, and protein source can explain differences in the lipid-lowering effect of L-carnitine, such as common carp (Sabzi et al. 2017), silver perch (Yang et al. 2012), Nile tilapia (Sanchez et al. 2021; Zhang et al. 2022b), and black seabream (Jin et al. 2019a; Ma et al. 2008). Therefore, the corn oil diet resulted in lipid accumulation in the muscle of *R.lagowskii*, and the lipid-lowering effect of L-carnitine supplementation in the hepatopancreas and muscle of *R.lagowskii* was independent of the dietary lipid source.

Our study showed that *R. lagowskii* fed the diet with corn oil had markedly higher total n6 PUFA content in hepatopancreas and muscle than fish fed the fish oil diet, whereas total n3 PUFA content was significantly reduced. The FA enrichment in the hepatopancreas and muscle of *R. lagowskii* reflected the FA composition of the diets consumed, like that higher LOA and lower oleic acid (OA), EPA, and DHA contents were observed in the fish-fed corn oil diet. Freshwater fish are generally considered to have the ability to regulate LC-PUFA biosynthesis, converting C18 PUFA (such as LOA and ALA) to EPA, DHA, and arachidonic acid (ARA) through desaturation and elongation (Hossain et al. 2021; Ren et al. 2012; Yu et al. 2021). The previous study demonstrated that common carp, rainbow trout, and Nile tilapia fed diet with LOA-rich corn oil or soybean oil had markedly higher ARA content in the liver and muscle (Fawole et al. 2021; Godoy et al. 2019; Ren et al. 2012). In this study, *R. lagowskii*-fed diet supplemented with corn oil had markedly higher ARA content in the hepatopancreas, rather than in the muscle. However, due to limited desaturation capacity, largemouth bass-fed soybean oil diet replacement of fish oil had higher LOA content and lower ARA content in the hepatopancreas and muscle (Chen et al. 2020a; Subhadra et al. 2006). We speculate that the freshwater *R. lagowskii* may have some ability to biosynthesis LC-PUFA based on C18 PUFA as informed in some previous studies on the role of FA desaturation and elongation in most fish in the *Cyprinidae* family (Tocher 2015). In the present study, when *R. lagowskii* was fed fish oil and corn oil diets, L-carnitine reduced total SFA and MUFA contents in the hepatopancreas. According to past studies, medium-chain and long-chain FA are transferred by L-carnitine to the mitochondrial matrix and inner membrane for β-oxidation (Jones et al. 2006), while very long-chain FA is the breakdown in peroxisomes (Ali et al. 2022). In the present study, SFA (C14:0, C16:0, and C18:0) and MUFA (C16:1 and C18:1n9c) in the hepatopancreas were observed to be reduced to varying degrees in *R. lagowskii*-fed corn oil and fish oil diets supplemented with L-carnitine. In addition, some evidence also shows that LC-PUFA is mainly caused by the dependence on carnitine, and α-tocopherol is synthesized through mitochondrial desaturation, which is based on LOA-carnitine (as substrate), α-tocopherol quinone (as electron donor) and acetylcarnitine (as acyl donor) is used to synthesize EPA and DHA through desaturation and elongation (Infante and Huszagh 2000; Takeno et al. 2005). In the present study, L-carnitine supplementation in a fish oil diet increased the total LC-PUFA content in the hepatopancreas of *R.lagowskii*. Similarly, a high dosage of L-carnitine significantly increased the total PUFA content in the liver of African catfish fed a diet with low-lysine and low fat (Ozorio et al. 2001). However, in this study, dietary L-carnitine showed no effects on the FA composition in the muscle of *R. lagowski*. These results are also consistent with the study of largemouth bass (Chen et al. 2020b). In contrast, L-carnitine supplementation significantly increased the total LC-PUFA content in the muscle of common carp (Zhang et al. 2022a). Furthermore, L-carnitine supplementation significantly promoted the accumulation of total SFA and MUFA contents in the muscle of rainbow trout without significantly affecting total PUFA content (Dikel et al. 2010). According to the above studies, these contradictory results are not only related to the dosage of L-carnitine supplementation, dietary lipid source, and

tissue specificity but also the selective enrichment of FA by different fish species (Li et al. 2019). Therefore, the corn oil diet significantly reduced the n3/n6 PUFA ratio of hepatopancreas and muscle of *R. lagowskii* compared with the fish oil diet. Supplementation of L-carnitine in fish oil and corn oil diets significantly decreased total SFA and MUFA content in *R. lagowskii* hepatopancreas and increased total n6 PUFA content, while L-carnitine had a limited effect on FA composition in muscle.

High concentrations of TG and HDL-C and disorderly distribution of HDL-C are considered significant dyslipidemia markers caused by metabolic disorders and insulin resistance in obesity, diabetes, and cardiovascular disease (Rothblat et al. 1992; Sharma and Agnihotri 2020; Shearer et al. 2012). Moreover, serum T-CHO concentrations indicate the ability of the liver to utilize acetyl-coenzyme A to synthesize cholesterol (Bae et al. 2013). In mammals, the corn oil diet induced an increase in plasma TG concentration than the fish oil diet (Ribeiro et al. 1991; Sharma and Agnihotri 2020), which differs from the findings in some fish species. Previous studies reported that dietary replacement of fish oil by n6 PUFA-rich VO could reduce plasma cholesterol (CHO) and TG concentrations in largemouth bass (Chen et al. 2020b), black seabream (Peng et al. 2008), and rainbow trout (Richard et al. 2006). Consistent results were also achieved in this study, including that dietary corn oil instead of fish oil significantly increased T-CHO and LDL-C concentrations in the serum of *R. lagowskii*, while TG and HDL-C concentrations in serum were slightly increased. Furthermore, both dietary safflower oil (rich in LOA) and linseed oil (rich in ALA) have shown significant plasma CHO clearance in the past study (Garg et al. 1988). In the present study, the content of LOA was increased by 11.05% and 5.11% in muscle and hepatopancreas of *R. laogwskii* fed the corn oil diet compared with fish fed fish oil diet, respectively. Studies on Japanese sea bass fed ALA diet showed hepatic transcriptional repression of multiple genes for apolipoproteins, FA-binding proteins, FA de novo synthesis, and triglyceride synthesis compared with fish fed LOA diet (Xu et al. 2019). Therefore, we reasoned that LOA enrichment and ALA loss in the hepatopancreas and muscle of *R. lagowskii* might be one of the contributing factors to the decrease in T-CHO, TG, LDL-C, and HDL-C concentrations in the serum. In the present study, dietary corn oil supplemented with L-carnitine significantly increased TG, T-CHO, HDL-C, and LDL-C concentrations in serum of *R. lagowskii*, but dietary fish oil supplemented with L-carnitine had no significant effect on serum lipid parameters of *R. lagowskii*. Similarly, dietary soybean oil supplemented with L-carnitine showed higher plasma TG, CHO, HDL-C, and LDL-C concentrations in the plasma of largemouth bass, but a complex lipid diet (containing soybean oil and fish oil) supplemented with L-carnitine showed the hypolipidemic effect (Chen et al. 2020b). Previous studies have shown that L-carnitine supplementation and dietary lipid levels (containing 3 and 8% sunflower oil) have significant interaction effects on CHO, high-density lipoprotein, and low-density lipoprotein concentrations in the plasma of common carp (Sabzi et al. 2017). However, L-carnitine supplementation had no significant effect on lipid parameters in the plasma of silver perch and tilapia under the condition of a complex lipid source diet (fish oil and soybean oil mixture) (Yang et al. 2012, 2010). Therefore, compared with the fish oil diet, the corn oil diet significantly reduced the serum lipid parameters of *R. lagowskii*. L-carnitine significantly increased the serum lipid parameters of the *R. lagowskii*-fed corn oil diet, while L-carnitine had no significant effect on the serum lipid parameters of the *R. lagowskii*-fed fish oil diet.

Dietary L-carnitine supplementation enriched total carnitine content in the hepatopancreas and muscle of *R. lagowskii* under both dietary lipid sources, which was the same as studies on zebrafish and Nile tilapia (Li et al. 2017, 2020). The CPT1 is essential for controlling the rate of mitochondrial FA oxidation and is located in the outer mitochondrial membrane, where it performs transesterification of medium-chain and long-chain acyl-coenzyme A (McGarry and Brown 1997; van der Hoek et al. 2018). Previous studies have demonstrated that L-carnitine supplementation up-regulated the expression of *cpt1* in the liver of black seabream (Jin et al. 2019a) and the liver and muscle of zebrafish (Li et al. 2017). A comparative study reported that L-carnitine supplementation decreased the expression of *cpt1* in the liver and increased the expression of *cpt1* in the muscle of *Cyprinus carpio* var Jian (Zhang et al. 2022a). In the present study, fish oil and corn oil diets supplemented with L-carnitine significantly up-regulated *cpt1* expression in the hepatopancreas of *R. lagowskii*, while L-carnitine supplementation had no significant effect on *cpt1* expression in the muscle of *R. lagowskii* in both dietary lipid sources. The effect of L-carnitine supplementation on

the expression of *cpt1* varies with different fish species and different tissue types. Moreover, no significant effect on the expression of *cpt1* in hepatopancreas and muscle was observed between *R. lagowskii*-fed fish oil and corn oil diets. Similarly, the expression of *cpt1* in the liver of turbo (Peng et al. 2017) and the activity of CPT1 in the liver of largemouth bass was not affected by dietary VO instead of fish oil (Chen et al. 2020b). In contrast, the complex lipid source diet (linseed oil and sunflower oil) significantly increased the expression of *cpt1* in the liver of rainbow trout compared with the linseed oil diet (Vestergren et al. 2013). FA β-oxidation of different fish species varies with dietary lipid sources. The LPL regulates the catabolism of chylomicrons and very low-density lipoproteins (Eckel 1989). It is the rate-limiting process for HDL formation and FA derivation, which in turn affects plasma TG and lipoprotein particle concentrations (Merkel et al. 2002; Preiss-Landl et al. 2002). Corn oil diet significantly up-regulated *lpl* expression in the hepatopancreas of *R. lagowskii* than fish oil diet in this study. A study on Nile tilapia showed a negative correlation between hepatic *lpl* expression and plasma TG and HDL-C concentration, which was similar to the trend in this study (Tian et al. 2013). The SREBP1 mediates the uptake, synthesis, and deposition of FA, TG, and CHO by regulating gene transcription for adipogenesis and lipid absorption (Cha and Repa 2007; Schoonjans et al. 2000). During FA de novo adipogenesis, ACCα regulates acetyl-coenzyme A to generate malonyl-coenzyme A, while FAS regulates all reaction steps of synthesis palmitate using acetyl-coenzyme A and malonyl-CoA (Sul et al. 1998). Previous studies showed that the expression of *fas* and *accα* was significantly increased in the liver of black seabream with the decrease of dietary n3 LC-PUFA/n6 C18 PUFA ratio (Jin et al. 2019b). Expressions of hepatic *lpl* and *fas* of turbot significantly increased with increasing levels of soybean oil replacing dietary fish oil (Peng et al. 2014). The expression of *fas* was significantly increased in the liver of rainbow trout-fed soybean oil diet compared with the fish-fed fish oil diet (Fawole et al. 2021). In our study, the *srebp1* and *accα* expressions were significantly up-regulated in the hepatopancreas of *R. lagowskii* fed corn oil diet than fish oil diet. These results indicate that high dietary n6 PUFA content (source of VO) induces FA uptake and FA de novo synthesis. Unlike hepatopancreas and adipose tissue, muscle is not the primary adipogenesis tissue. In this study, *lpl* expression was significantly up-regulated in the muscle of *R. lagowskii* fed a corn diet than a fish oil diet. It is possible that corn oil-induced lipid deposition in the muscle of *R. lagowskii* may originate from FA uptake and storage rather than de novo synthesis of FA. Therefore, the corn oil diet promoted the FA intake and synthesis in the hepatopancreas and the FA intake in the muscle of *R. lagowskii* (including significantly up-regulating the expression of *lpl*, *fas*, *accα*, and srebp1 in the liver and *lpl* expression in muscle). L-carnitine supplementation promoted FA β-oxidation (significantly up-regulated the expression of *cpt1*) in the liver of *R. lagowskii* under two dietary lipid sources but inhibited FA uptake and synthesis in hepatopancreas and muscle of *R. lagowskii* fed corn oil diet (including significantly down-regulated the expression of *fas*, *accα*, *srebp1* in liver and expression of *lpl* and *fas* in muscle).

Glucose homeostasis is the comprehensive regulation of proteins, lipids, and carbohydrates by the animal body to stabilize blood glucose concentration within a narrow range (Kramer 2016; Seward et al. 2021). In the present study, there were no significant differences in the initial (0 h) serum glucose concentrations were observed between *R. lagowskii*-fed corn oil or fish oil diets. Similarly, there were no significant differences in serum or plasma glucose concentrations were observed in largemouth bass, grouper, Nile tilapia, and sturgeon-fed VO diet compared with fish fed fish oil diet (Chen et al. 2020b; de Almeida et al. 2021; Qin et al. 2022; Sarameh et al. 2019). Some studies reported that much of the glucose has already been cleared at ~ 1 h postinjection during the GTT test in mice (Andrikopoulos et al. 2008; Small et al. 2022). However, in some fish species, blood showed slow blood glucose changes clearance and decreased to a basal level about 6–8 h post-injection (Castro et al. 2015; Zhang et al. 2020). In our study, dietary corn oil resulted in delayed uptake and metabolism of exogenous glucose in the blood circulation of *R. lagowskii* challenged with GTT compared with dietary fish oil. This glucose profile was supported by the significantly down-regulated expression of glucose transport (*glut1*) and glycolysis (*pfkla*) related gene expressions, while significantly up-regulated gluconeogenesis (*g6pase* and *pepck*) related gene expressions of *R. lagowskii* fed corn oil diet compared with fish oil diet. Similarly, high dietary soybean oil levels significantly up-regulated *g6pase* and *pepck* expression in large yellow croaker, whereas *glut2*, *gys*, and *hk* expressions

were significantly down-regulated (Gu et al. 2019). In addition, the soybean oil diet up-regulated gluconeogenesis-related genes of grass carp compared with the fish oil diet through differential analysis of metabolic signals (Liu et al. 2022). In previous studies, L-carnitine has been linked to the glucose utilization of fish (Li et al. 2017, 2020; Sharifzadeh et al. 2017). In this study, dietary L-carnitine significantly increased serum glucose concentration at 0 and 10 h during the GTT of the *R. laogwskii*-fed corn oil diet. Similarly, other fish species, like silver perch, common carp, and Nile tilapia fed a diet with L-carnitine supplementation and showed higher serum glucose concentration (Li et al. 2020; Sabzi et al. 2017; Yang et al. 2012). Moreover, in the present study, L-carnitine up-regulated *g6pase* and *pepck* expressions in the hepatopancreas of *R. lagowskii* under both dietary lipid sources. This is also consistent with previous studies that L-carnitine up-regulated the expression of gluconeogenesis-related genes in the liver of zebrafish (Li et al. 2017). L-carnitine has also been confirmed to elevate serum glucose concentrations by enhancing lipid catabolism to reduce energy utilization of glucose in Nile tilapia (Li et al. 2020). This is also supported by L-carnitine up-regulation of the expression of lipid metabolism-related genes and down-regulation of the expression of adipogenic-related genes in this study. Therefore, corn oil diet inhibited glucose transport, glycogen synthesis, and glycolysis (including *glut1* and *pfkla* in hepatopancreas and muscle, and *gys1* in muscle) and promoted gluconeogenesis (including *g6pase* and *pepck* in hepatopancreas) of *R. lagowskii* compared with fish oil diet. L-carnitine supplementation enhanced the gluconeogenesis (including *g6pase* and *pepck* in hepatopancreas) of *R. lagowskii* under two dietary lipid sources.

Conclusion

This study confirmed that the CO diet-induced lipid accumulation in the muscle of *R. lagowskii*, along with reduced serum lipid parameters than the FO diet. However, the CO diet supplemented with L-carnitine decreased crude lipid content in hepatopancreas and muscle while restoring serum lipid parameters. Correspondingly, lipid uptake and lipogenesis were inhibited of *R. lagowskii* was fed a CO diet with L-carnitine, along with FA β-oxidation was activated. The CO diet instead of the FO diet increased the content of total n6 PUFA while decreasing the content of total n3 PUFA in the hepatopancreas and muscle of *R. lagowskii*. L-carnitine decreased the contents of total SFA and MUFA in the hepatopancreas of fish fed with FO and CO diets but increased the content of total n6 PUFA in the hepatopancreas. However, L-carnitine supplementation failed to improve n3 PUFA accumulation in the hepatopancreas and muscle of fish fed with FO and CO diets. The CO diet inhibited glucose uptake and clearance in *R. lagowskii* due to inhibited glucose transport, glycogen synthesis, and glycolysis compared with the FO diet. However, L-carnitine supplementation activated gluconeogenesis of *R. lagowskii* fed with a CO diet, but weakly affected glucose transport, glycolysis, and glycogenesis.

Author contribution Dongming Zhang, Qiuju Wang, and Sen Wang contributed to the study's conception and design. Funding acquisition and resources were provided by Dongming Zhang, Qiuju Wang, Zhixin Guo, and Yuke Chen. Material preparation was performed by Sen Wang, Rongxin Zheng, Nan Zheng, and Wenhao Fang. Data collection was performed by Sen Wang, Xin Wang, Jiajing Wang, and Ning Wang. Data analysis was performed by Sen Wang. Project administration and supervision were performed by Dongming Zhang, Qiuju Wang, Zhixin Guo, and Yuke Chen. The first draft of the manuscript was written by Wang Sen. Writing—review and editing were performed by Dongming Zhang, Qiuju Wang, Zhixin Guo, and Yuke Chen. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary materials.

Declarations

Ethics approval This experiment animal care was performed conformity with NIH Guide for the Care and Use of Laboratory Animals. All fish used in this study were approved by the Institutional Animal Care and Use Committee of Jilin Agricultural University.

Consent to participate Participation in this article has been consented to by all authors.

Consent for publication All authors approved the manuscript for publication.

Conflict of interest The authors declare no competing interests.

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