Ecotoxicology and Environmental Safety 205 (2020) 111127

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Protective effects of diet containing rutin against trichlorfon-induced muscle bioenergetics disruption and impairment on fatty acid profile of silver catfish *Rhamdia quelen*

Matheus D. Baldissera [a,*], Carine F. Souza [a], Belisa Parmeggiani [b], Raquel G. Vendrusculo [c], Lucila C. Ribeiro [d], Daniela K. Muenchen [d], Carla C. Zeppenfeld [a], Adriana D. Meinhart [e], Roger Wagner [c], Renato Zanella [d], Osmar D. Prestes [d], Aleksandro S. da Silva [f], Guilhian Leipnitz [g], Bernardo Baldisserotto [a]

[a] Department of Physiology and Pharmacology, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil
[b] Programa de Pós Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[c] Department of Food Science and Technology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[d] Department of Chemistry, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[e] Department of Food Science and Agrotechnology, Federal University of Pelotas (UFPEL), Pelotas, RS, Brazil
[f] Department of Animal Science, Universidade do Estado de Santa Catarina, Chapecó, RS, Brazil
[g] Programa de Pós Graduação em Ciências Biológicas: Bioquímica, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Pesticide
Creatine kinase
Energetics
Phosphotransfer network
Flavonoids
Fillet

ABSTRACT

Trichlorfon is an organophosphate insecticide that is widely used on fish farms to control parasitic infections. It has been detected in freshwater ecosystems as well as in fishery products. There is a growing body of evidence to suggest that certain feed additives may reduce or prevent pesticide-induced toxicity in fish. The aim of the present study was to determine whether acute exposure to trichlorfon would alter bioenergetic homeostasis and alter fatty acid profiles in muscles of silver catfish (*Rhamdia quelen*). We also sought to determine whether rutin prevents or reduces these effects. Cytosolic and mitochondrial creatine kinase (CK) and activities of complexes II-III and IV in muscle were significantly inhibited by exposure to 11 mg/L trichlorfon for 48 h compared to effects in the unexposed group. Total content of polyunsaturated fatty acids (omega-3 and omega-6) were significantly lower in muscle of silver catfish exposed to 11 mg/L trichlorfon for 48 h than in the unexposed group. Addition of 3 mg rutin/kg feed increased CK activity and prevented inhibition of complex IV activity, as well as preventing all alterations of muscle fatty acid profiles elicited by exposure to trichlorfon. No significant differences were observed between groups with respect to muscle adenylate kinase or pyruvate kinase activities, as well as total content of saturated and monounsaturated fatty acids. Our findings suggest that exposure (48 h) to 11 mg trichlorfon/L water inhibits cytosolic and mitochondrial CK activity in muscle. Trichlorfon also affects activities of complexes II-III and IV in respiratory chain, with important consequences for adenosine triphosphate production. The pesticide alters fatty acid profiles in the fish and endangers human consumers of the product. The most important finding of the present study is that inclusion of rutin improves bioenergetic homeostasis and muscle fatty acid profiles, suggesting that it reduces trichlorfon-induced muscle damage.

1. Introduction

Fish production is expanding rapidly in response to global demand; fish fillets are considered healthy foods that are a rich source of healthy-friendly oils, especially the polyunsaturated fatty acids (PUFAs) (Mohanty et al., 2019). Intensive production systems are commonly employed to expand fish production so as to meet this demand; however, fish culture under intensive and super intensive systems can pose

* Corresponding author.
E-mail addresses: matheusd.biomed@yahoo.com.br, matheus.baldissera@ufsm.br (M.D. Baldissera).

https://doi.org/10.1016/j.ecoenv.2020.111127
Received 24 May 2020; Received in revised form 30 July 2020; Accepted 1 August 2020
Available online 22 August 2020

stressful conditions that augment the risk of parasitic infection. Pesticides successfully combat external and internal infectious parasites; nevertheless, they represent an emerging threat to the environment and human health (López-Pacheco et al., 2019; Woo and Chung, 2020).

Trichlorfon is an organophosphate pesticide that is widely used to control various parasites in fish and other aquatic animals, including *Anancanthorus* spp., *Argulus* spp., *Dactylogyrus* spp., *Gyrodactylus* spp., *Dolops* spp., *Trichodina* spp., *Piscicola* spp., *Ichthyophthirius* spp., and *Piscinoodinium* spp. (Trujillo-González et al., 2018). It is currently the most widely disseminated organophosphate pesticide used by fish farmers (Duncan et al., 2020). According to the company that manufactures trichlorfon, the recommended concentration varies among species and type of parasitic infestation (0.25–25 mg/L) (Baldissera et al., 2019a; Woo and Chung, 2020). Despite its potent anti-parasitic effect and its short waterborne half-life (approximately 57 h), recent studies have suggested that trichlorfon contaminates aquatic environments via repeated, indiscriminate, and excessive use in fish farm management, representing a serious threat to the environment in the form of toxicity to target and non-targeted fish (Baldissera et al., 2019a; Yonar et al., 2020). The toxic effects of trichlorfon have been observed in freshwater fishes such as common carp (*Cyprinus carpio*) (Chang et al., 2020), tambaqui (*Colossoma macropomum*) (Duncan et al., 2020), and silver catfish (*Rhamdia quelen*) (Baldissera et al., 2019a). Trichlorfon tends to accumulate in edible tissues such as muscle (Venturini et al., 2015; Kang et al., 2019), thereby raising concerns not only for the fish itself but for human consumers. Venturini et al. (2015) found that exposure to sub-lethal concentrations of trichlorfon altered intermediary metabolism in muscle, evidenced by decreased free fatty acid and pyruvate levels, and increased glucose levels, leading us hypothesize that trichlorfon impairs bioenergetic homeostasis in muscle. Corroborating our hypothesis, Baldissera et al. (2019a) found that exposure to 11 mg/L triclorfon for 48 h impaired hepatic and branchial bioenergetic homeostasis via inhibition of enzymes belonging to the phosphotransfer network, including creatine kinase (CK), adenylate kinase (AK), and pyruvate kinase (PK). This network is essential for efficient intracellular energetic communication, enabling maintenance of homeostasis between adenosine triphosphate (ATP) consumption and production in organs with high energy requirements (Dzeja et al., 2004).

Fish fillets are critical sources of proteins, essential micronutrients, and fatty acids (Osmond and Colombo, 2019). For this reason, contamination by pesticides and bioaccumulation in edibles tissues represent serious threats to human health via alteration of fatty acid profiles (Sánchez-Muros et al., 2013; Ewere et al., 2019). According to these authors, pesticides alter fatty acid composition and consequently the use of lipids as alternative sources of energy under stressful conditions, as observed in muscle of gilthead sea bream (*Sparus aurata*) exposed to Diuron® (Sánchez-Muros et al., 2013) and in muscle of Sidney rock oysters (*Saccostrea glomerata*) exposed to imidacloprid (Ewere et al., 2019). Zhang et al. (2019) reported a correlation between elevated levels waterborne pesticides and reduction of muscle PUFAs; the authors concluded that individuals should pay attention to the quality of fish fillets for consumption associated with fatty acid composition. To the best of our knowledge, there have been no reports of effects of trichlorfon on fatty acid profiles in fish muscle. Because lipid sources are used as energy sources during exposure to pesticides, and because exposure to trichlorfon was recently associated with alterations in intermediary fat metabolism in muscle (Venturini et al., 2015), we generated a hypothesis that acute exposure to trichlorfon would impair fatty acid profile in muscles of silver catfish in so as to generate alternative energy sources. We also hypothesized that alterations in fillet fatty acid composition caused by this stress would have implications for human nutrition.

Recently, a growing body of evidence has suggested the use of feed additives with antioxidant and immunostimulatory properties to reduce or prevent pesticide-induced toxicity in fish (Abdelkhalek et al., 2015, 2017; Naiel et al., 2020; Bhattacharjee et al., 2020), including flavonoid compounds (Bhattacharjee et al., 2020). Rutin (3′,4′,5,7,-tetrahydroxy-flavone-3-rutinoside) is a flavonol glycoside, that is thought to be one of the most abundant flavonoids found in many foods such as red wine, tea and apples; it is used as supplement or additive in animal models because of its various pharmacological properties (Oboh et al., 2020). In fish, rutin-enriched diets showed several positive effects, including protection against T-2-toxin-induced liver and muscle oxidative damage in Nile tilapia (*Oreochromis niloticus*) (Deng et al., 2019), protection against *Aeromonas hydrophila*-induced muscle damage in silver catfish (Da Rosa et al., 2019), protection against oxytetracycline-induced liver damage in rainbow trout (*Oncorhynchus mykiss*) (Nazeri et al., 2017), and improvement in hematological properties and reduction of physiological stress in silver catfish (Pês et al., 2016). In the particular interest of study, Leffa et al. (2017) reported that mice fed 200 mg/kg/day rutin for 13 weeks showed improved bioenergetics via increased cerebral complex IV and CK activities; the authors concluded that the antioxidant action of rutin was responsible for enhancement of bioenergetic homeostasis. For these reasons, we hypothesized that rutin in fish feed would reduce or prevent trichlorfon-induced impairment of muscular bioenergetics.

Therefore, the aim of this study was to determine whether acute exposure to trichlorfon would impair bioenergetic homeostasis and would alter fatty acid profiles in muscles of silver catfish, as well as whether rutin would be capable of preventing or reducing these effects.

2. Material and methods

2.1. Chemicals

Rutin (95% purity; molecular weight 610.52 g/mol) was purchased from Sigma-Aldrich (St. Louis, MO, US), while trichlorfon [*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate](800 g/kg) was purchased in a Brazilian market with the commercial name Masoten® (Bayer, Brazil).

2.2. Experimental diet preparation

The formulation and nutritional composition of basal diet (Table 1) support the requirements of juvenile silver catfish (Zeppenfeld et al., 2017). All dried ingredients were separately ground to pass through a

Table 1
Formulation of basal diet.

Ingredient	g/kg
Soybean meal	300
Meat and bone meal	350
Rice brain	120
Corn	150
Canola oil	30
Common salt	10
Vitamin and mineral premix[a]	30
Dicalcium phosphate	10
Analyzed proximate composition	
Dry matter content	923.6
Protein	461.7
Ether extract	105.4
Crude fiber	29.4
Mineral matter	142.9
Acid detergent fiber	29.1
Neutral detergent fiber	164.1

[a] Vitamin and mineral mixture (per kilogram of product): 200 mg folic acid, 5000 mg pantothenic acid, 0.60 g antioxidant, 125 mg biotin, 25 mg cobalt, 2000 mg copper, 820 mg iron, 100 mg iodine, 3750 mg manganese, 5000 mg niacin, 75 mg selenium, 1,000,000 UI vitamin A, 1250 mg vitamin B1, 2500 mg vitamin B2, 2485 mg vitamin B6, 3,750 l mg vitamin B12, 28,000 mg vitamin C, 500,000 UI vitamin D3, 20,000 UI vitamin E, 500 mg vitamin K and 17,500 mg zinc.

185-μm mesh and then were combined with other ingredients. To prepare feed containing rutin, a nominal concentration of 3 mg rutin/kg feed was pre-mixed with the corn fraction before mixing with the other ingredients. Finally, basal and rutin-added diets were pelleted to 2.5-mm diameter, dried overnight in a forced air circulation at 35 °C, and finally stored in sample bags at 2 °C and protected from the light. Addition of nominal rutin concentration (3 mg/kg feed) followed the theoretical recommendation of Pês et al. (2016), who demonstrated the benefits of rutin for silver catfish health.

2.3. Rutin content in silver catfish diet

Methanol and formic acid were acquired from Sigma Aldrich (St. Louis, MO, USA), and acetonitrile was acquired from Avantor™ (Radnor, PA, US), both in chromatographic grade. Water was purified using MegaPurity® system (MecLab, Brazil), and rutin standard was purchased from Sigma-Aldrich (Darmstadt, Germany). A rutin stock solution was prepared in methanol (1 mg/mL) and stored at −70 °C. All solutions were filtered through 0.22-μm pore polyvinylidene fluoride membranes.

The sample preparation method was based on a recent study by. In 50-mL tubes, 1 g of feed was dissolved in 10 mL of methanol:water solution (80:20 v/v), maintained heated (30 °C) and spun (200×*g*) for 15 min. The supernatants were collected and the pellets were subjected to the same process twice more. The pellets were combined, the volume was checked and filtered using 0.22-μm pore nylon membrane, and subjected to analysis. Rutin analysis was performed using high-performance liquid chromatography with diode array detector (Shimadzu, Japan) operating at 350 nm, equipped with an automatic injector, a quaternary pump, and a column C18 Zorbax Eclipse Plus (Agilent Technologies, Germany) with 4.6-mm i.d., 150-mm length and 3.5-μm particle size, maintained at 40 °C, as recommended by Meinhart et al. (2017). The method was validated using detection limit, quantification, linearity, and precision, following the guidelines of the International Union of Pure and Applied Chemistry (Thompson et al., 2002). The real rutin content in the diets containing rutin was 1.56 ± 0.05 mg/g feed, while rutin was not detected in the basal feed. The chromatograms of rutin quantification in fish feed can be found in Fig. 1 of supplementary material.

2.4. Experimental fish: collection, maintenance and water quality variables

Juvenile silver catfish were acquired from a commercial fish farm (Santa Maria, Brazil) and transported to Fish Physiology Laboratory of Universidade Federal de Santa Maria (UFSM, Santa Maria, Brazil). The fish were acclimated (15 days) in fiberglass tanks containing 500 L static water under the following conditions: dissolved oxygen 6.1 ± 0.3 mg/L, temperature 20.3 ± 0.2 °C, pH 7.0 ± 0.3, and ammonia 0.81 ± 0.05 mg/L, measured every three days. Dissolved oxygen was measured using an oximeter (oxygen meter Y5512, USA), temperature was measured using a thermometer, pH was measured using a pH meter (DMPH-2 pH meter, Digimed, SP, Brazil), and ammonia levels were quantified following the method described by Verdouw et al. (1978). During the acclimation period, silver catfish were fed basal feed once a day (2 p.m.), in a proportion to 5% total biomass in order to acclimatize to feed conditions used in the experiment. Feces and excess feed were cleaned, and a quarter of water total volume was renewed 60 min after feeding.

2.5. Experimental set up

A total of 72 healthy silver catfish of similar size (20.09 ± 1.99 g; 23

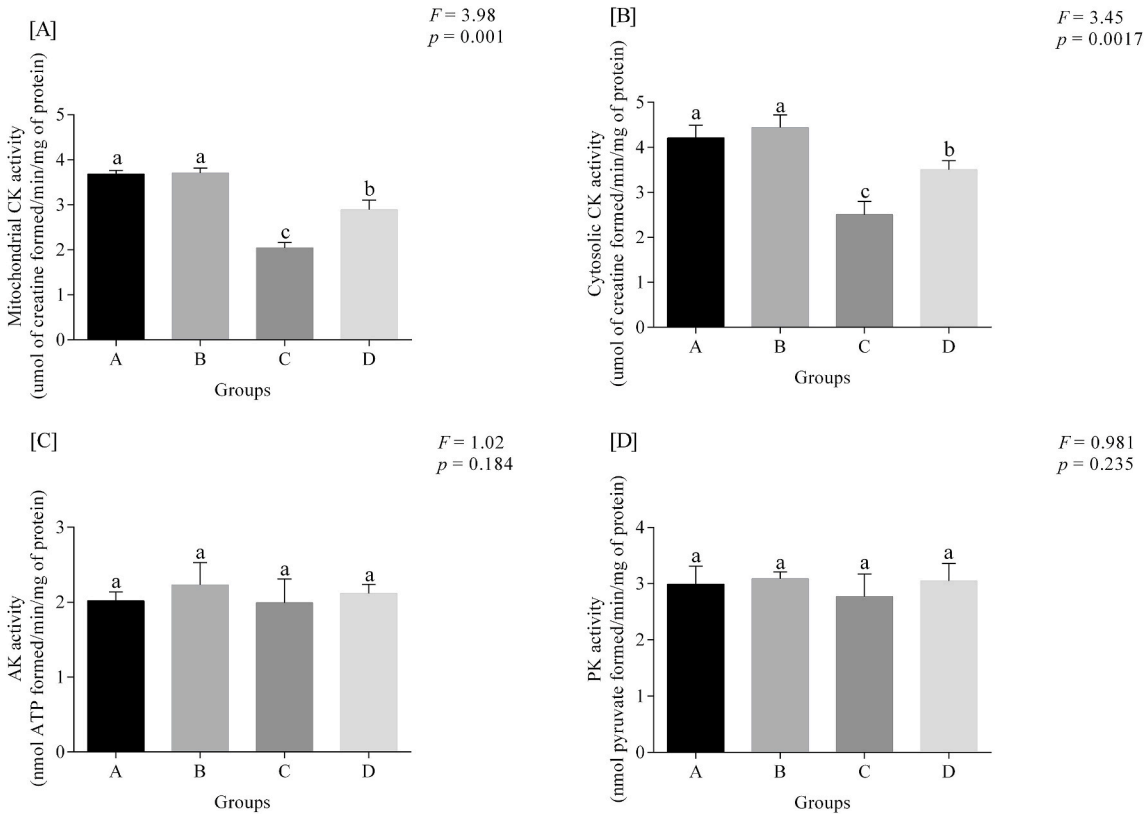

Fig. 1. Muscle mitochondrial [A] and cytosolic [B] creatine kinase (CK), adenylate kinase (AK) [C], and pyruvate kinase (PK) [D] activities of silver catfish (*Rhamdia quelen*) fed with nominal concentration of 3 mg rutin/kg of feed for 21 days and exposed to nominal concentration of 11 mg trichlorfon/L water for 48 h. Bars that do not share a common superscript letter ($^{a-b}$) differ significantly using a bilateral two-way analysis of variance (ANOVA) for independent samples followed by Tukey's post-hoc test ($p < 0.05$; $n = 6$ per group).

± 2 cm; mean and standard deviation) were randomly allocated to 12 plastic tanks containing 60 L-static water and divided in four groups (n = 6 each) with three replicates, as follows: groups A and C received basal feed, while groups B and D received feed containing rutin. All groups received experimental feed once a day, at 2 pm, for 21 consecutive days, in proportion to 5% of total biomass (Pês et al., 2016). After 21 days, groups C and D were exposed for 48 h to a nominal concentration of 11 mg trichlorfon/L water, as previously described by Baldissera et al. (2019a) for juvenile catfish. During this period, the animals continued receiving experimental diets, and the water was no longer changed. During the experimental period, the environmental conditions and daily management were mostly the same as that of the acclimation period.

2.6. Trichlorfon and dichlorvos water levels

The true concentration of trichlorfon and its main metabolite, dichlorvos, were measured in tank water at the beginning (0 h) and end (48 h) of exposure using ultra-high-performance liquid chromatography coupled to mass spectrophotometry (UHPLC-MS), as recently published in detail by Baldissera et al. (2018a) (Table 2).

2.7. Silver catfish performance

After 48 h trichlorfon exposure, final weight was measured to calculate weight gain (WG) - WG (g) = (final mean fish weight (g) – initial mean fish weight (g)); growth rate (SGR) – SGR [((ln final weight) – (ln initial weight)/experiment duration) x 100]; percentage fish survival (% survival) - % survival = (number of fish at end of experiment/ initial number of fish) x 100.

2.8. Sample collection

After measurement of final weight, muscles of two juveniles from each tank (n = 6 per treatment) were collected under anesthesia with eugenol (50 mg/L) (Cunha et al., 2010) followed by spinal cord sectioning.

2.9. Muscle phosphotransfer network

Muscular tissue was washed in SET buffer (0.32 M sucrose, 1 mM EGTA, 10 mM Tris-HCl, pH 7.4) and homogenized (1:10 w/v) in the same SET buffer using a Potter-Elvehjem glass homogenizer. The homogenates were centrifuged at 800 × g for 10 min at 4 °C, and some of the supernatants was used to measure AK activity. The remaining supernatants from the first centrifugation were again centrifuged at 10.000 × g for 15 min at 4 °C, and the new supernatants were collected to measure PK and cytosolic CK activities. The pellets were washed with SET buffer, then resuspended in 100 mM Trizma and 15 mM $MgSO_4$ buffer (pH 7.5) to measure mitochondrial CK activity. The supernatants were stored for no more than 1 week at −80 °C. Muscle CK activity was assayed based on the method described by Hughes (1962), as reported in detail by Baldissera et al. (2019a), and the results were expressed as μmol of creatine formed/min/mg of protein. Muscle AK activity was measured according to Dzeja et al. (1999), as published in detail by Baldissera et al. (2019a), and the activity was expressed as nmol ATP formed/min/mg of protein. Muscle PK activity was assayed according Leong et al. (1981), and published in detail by Baldissera et al. (2019a). Activity was expressed as nmol pyruvate formed/min/mg of protein.

Table 2
Trichlorfon and dichlorvos concentration in water after exposure to 11 mg/L trichlorfon under experimental conditions at 0 and 48 h of exposure.

Time/Group	Trichlorfon (mg/L)	Dichlorvos (mg/L)
0 h		
Group C	8.041 ± 0.57	0.066 ± 0.01
Group D	8.123 ± 0.89	0.072 ± 0.02
48 h		
Group C	0.06 ± 0.03	0.155 ± 0.03
Group D	0.080 ± 0.05	0.116 ± 0.05

Note 1: Values expressed as mean ± standard deviation.
Note 2: Limit of trichlorfon detection: 0.006 mg/L; limit of trichlorfon quantification: 0.02 mg/L; Limit of dichlorvos detection: 0.001 mg/L; limit of dichlorvos quantification: 0.004 mg/L.

2.10. Muscle respiratory chain complexes

To measure respiratory chain complex activity, muscle was homogenized (1:20 w/v) in SETH buffer (250 mM sucrose, 2.0 mM EGTA, 10 mM Trizma base, pH 7.4), and centrifuged at 750×g for 10 min at 4 °C. After centrifugation, the supernatant was collected and subjected to three freeze-thawing procedures before use for measurement of the activity of complexes II-III (succinate/cytochrome c oxidoreductase), while the same homogenization process was conducted to evaluate complex IV activity (cytochrome c oxidase), but without three freeze-thawing procedures.

Muscle complexes II-III activities were evaluated according to the method described by Fisher et al. (1985), while muscle complex IV activity was determined following the technique described by Rustin et al. (1994), both methods described in details by Grings et al. (2018). All enzymatic activities were expressed as nmol/min/mg of protein.

2.11. Muscle protein determination

Protein content in muscle homogenates was evaluated by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.12. Muscle fatty acid profile

Lipids were extracted according to the method described by Bligh and Dyer (1959) with modifications for fish samples according to Baldissera et al. (2020). A total of 0.5 g of grounded sample were weighed, and 3 mL methanol, 1.5 mL chloroform and 0.9 mL distilled water were added in a 15-mL polypropylene conical tube. The extraction occurred by homogenization on orbital shaker table (Q225M, Quimis, BR) at 150 rpm for 30 min. Then, 1.5 mL chloroform and 1.5 mL aqueous sodium sulfate solution (1.5%) were added. After 2 min of additional homogenization, the samples were centrifuged at 698.75×g for 2 min (MTD III PLUS, Metroterm, BR). The organic phase was removed, the solvent was evaporated under nitrogen, and the lipids were subjected to derivatization according to the method proposed by Hartman and Lago (1973). We added 1 mL of methanolic solution of potassium hydroxide (0.4 mol L^{-1}) to the lipid fraction, which was then kept in a water bath at boiling point for 10 min. Subsequently, 3 mL methanolic solution of sulfuric acid (1.0 mol L^{-1}) were added and once again was maintained in a water bath at the boiling point for 10 min. The tubes were cooled and 2 mL of hexane were added. The upper phase containing fatty acid methyl esters (FAME) dissolved in hexane underwent chromatographic analysis; 1 μL of FAME extract were injected in 20:1 split mode in a gas chromatograph with a flame ionization detector (Varian, Star 3600, USA). The carrier gas was hydrogen ultrapure at constant pressure of 25 psi. The FAME were separated into HP-88 capillary column (Agilent Technologies, USA) (100 m × 0.25 mm; 0.20 μm of thickness film) with initial temperature of 50 °C for 1 min, increasing to 185 °C at 15 °C/min and then at 0.5 °C/min increased to 195 °C and finally up to 230 °C to 15 °C/min, remaining for 5 min. Injector and detector were maintained at 250 °C. The identification of analytes was performed by comparing retention times with standards of FAME Mix 37 (P/N 47885-U), vaccenic acid methyl ester (P/N 46905-U) and docosapentaenoic methyl ester – DPA (P/N 47563-U) (Sigma-Aldrich, USA). Results were expressed as a percentage of the total area considering the factors of FID correction and the conversion of esters to acids according to Visentainer (2012). The chromatograms of fatty acids quantification in fish muscle can be found

in Fig. 2 of supplementary material.

2.13. Statistical analysis

The data were subjected to the homogeneity of variance test (Levene test) and assessed for the normality of residuals (Shapiro–Wilk test). Data that did not meet normality and homoscedasticity were transformed (lg) and then analyzed using bilateral two-way analyses of variance (ANOVA) followed by the Tukey post hoc analysis with significance set at $p < 0.05$. All results were expressed as mean ± standard deviation.

3. Results

3.1. Silver catfish performance computations

No significant differences were observed between groups with respect to final weight, WG, SGR, or survival (Table 3).

3.2. Muscle phosphotransfer network

There was a significant interaction between rutin dietary consumption and trichlorfon exposure in terms of muscle mitochondrial and cytosolic CK activities. Muscle mitochondrial and cytosolic CK activities were significantly lower in silver catfish exposed to trichlorfon (group C) than in the unexposed group (group A). Feed containing rutin in the unexposed group (group B) did not significantly affect muscle mitochondrial and cytosolic CK activities compared to the effects on group A. Muscle mitochondrial and cytosolic CK activities were significantly higher in fish fed rutin and exposed to trichlorfon (group D) compared to those of group C, but remained lower than those of group A (Fig. 1AB).

No significant difference was observed between groups with respect to muscle AK and PK activities (Fig. 1CD).

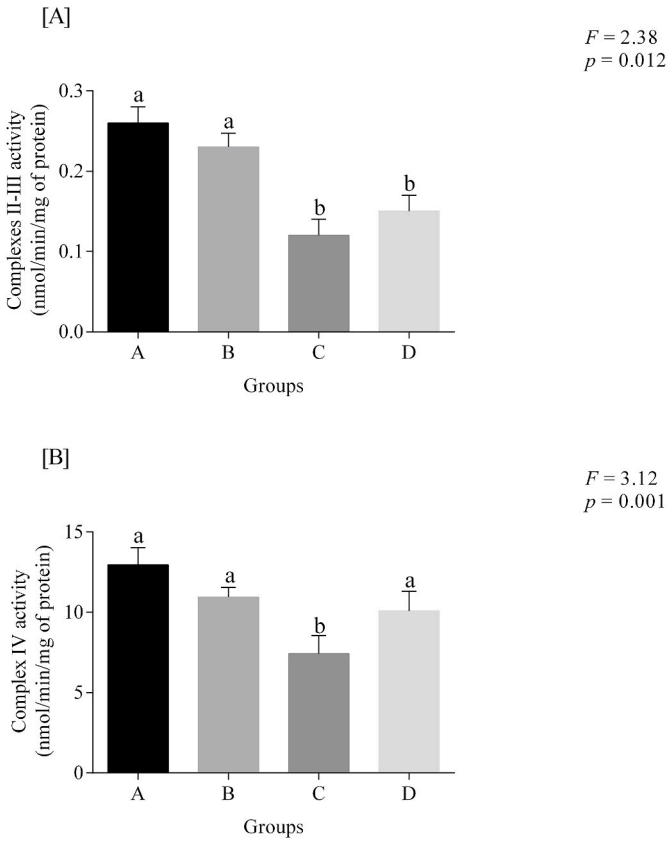

Fig. 2. Muscle complexes II-III (succinate/cytochrome *c* oxidoreductase) [A] and complex IV (cytochrome *c* oxidase) [B] activities of silver catfish (*Rhamdia quelen*) fed with nominal concentration of 3 mg rutin/kg of feed for 21 days and exposed to nominal concentration of 11 mg trichlorfon/L water for 48 h. Bars that do not share a common superscript letter ($^{a\text{-}b}$) differ significantly using a bilateral two-way analysis of variance (ANOVA) for independent samples followed by Tukey's post-hoc test ($p < 0.05$; $n = 6$ per group).

Table 3
Performance and survival of silver catfish (*Rhamdia quelen*) juveniles fed basal diet or containing nominal concentrations of 3 mg rutin/kg of feed over 21 days and exposed 48 h to 11 mg trichlorfon/L water.

Parameters/ Groups	A	B	C	D	*F* and *p* values
Initial mean weight (g)	20.11 ± 1.14[a]	21.08 ± 0.19[a]	20.11 ± 1.44[a]	20.94 ± 0.96[a]	0.456; 0.893
Final mean weight (g)	23.10 ± 1.29[a]	24.01 ± 1.11[a]	23.22 ± 1.12[a]	23.85 ± 1.21[a]	1.02; 0.325
WG (g)	2.99 ± 0.15[a]	2.93 ± 0.92[a]	3.11 ± 0.48[a]	2.91 ± 0.62[a]	1.11; 0.210
SGR (%)	0.619 ± 0.003[a]	0.619 ± 0.006[a]	0.666 ± 0.01[a]	0.627 ± 0.01[a]	1.22; 0.134
Survival (%)	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	0.125; 0.968

Note 1: weight gain (WG); specific growth rate (SGR); **Note 2:** group A (basal diet; without rutin addition); group B (diet containing nominal concentration of 3 mg rutin/kg of feed); group C (basal diet; without rutin addition and exposed to nominal concentration of 11 mg trichlorfon/L water); group D (diet containing nominal concentration 3 mg rutin/kg of feed and exposed to nominal concentration of 11 mg trichlorfon/L water); **Note 3:** Values accompanied by different letters in the same row are statistically different considering $p < 0.05$.

3.3. Muscle respiratory chain complexes

A significant effect of trichlorfon exposure was observed for muscle complex II-III activities. These activities decreased significantly in silver catfish exposed to trichlorfon (groups C and D) compared to those of the control group (group A). However, addition of rutin (groups B and D) did not significantly affect this activity compared to control and trichlorfon groups (groups A and C, respectively) (Fig. 2A).

There was a significant interaction between rutin dietary consumption and trichlorfon exposure for muscle complex IV activity. Muscle complex IV activity decreased significantly in silver catfish exposed to trichlorfon (group C) compared to the unexposed group (group A). Rutin addition in the unexposed group (group B) did not significantly affect muscle complex IV activity compared to the effects on group A. Rutin given to the trichlorfon-exposed group (group D) prevented decreases in muscle complex IV activity observed in silver catfish exposed to trichlorfon (group C) (Fig. 2B).

3.4. Muscle fatty acid profile

There was a significant interaction between rutin consumption and trichlorfon exposure for total muscle PUFA levels, as well as for total contents of n3 and n6. Levels of muscle PUFA (n3 and n6) decreased significantly in silver catfish exposed to trichlorfon (group C) compared to the unexposed group (group A). Inclusion of rutin for the unexposed group (group B) did not significantly affect muscle PUFA levels compared to group A. Rutin fed to the trichlorfon-exposed group (group D) prevented the decrease in total content of muscle PUFA observed in silver catfish exposed to trichlorfon (group C). No significant differences were observed between groups with respect to total content of muscle SFA and MUFA (Table 4).

There was a significant interaction between rutin dietary consumption and trichlorfon exposure in terms of muscle levels of C14:1 (myristylic acid), C16:1 (palmitoleic acid), C18:2n6 cis (linoleic acid), C18:3n6 (gamma-linolenic acid), C20:2n6 (eicosadienoic acid), and

C20:3n6 (di-homo-y-linolenic acid). Levels of all these fatty acids were significantly lower in muscle of silver catfish exposed to trichlorfon (group C) compared to the unexposed group (group A). Rutin given to unexposed fish (group B) did not significantly affect levels of any of these fatty acids compared to group A. Rutin given to the trichlorfon-exposed group (group D) prevented the decrease in levels of all these fatty acids in fish exposed to trichlorfon (group C). By contrast, there were no significant differences between groups with respect to muscle levels of C12:0 (dodecanoic acid), C13:0 (tridecanoic acid), C14:0 (myristic acid), C15:0 (pentadecanoic acid), C16:0 (palmitic acid), C17:0 (heptadecanoic acid), C18:0 (stearic acid), C20:0 (eicosanoic acid), C22:0 (docosanoic acid), C23:0 (tricosanoic acid), C17:1 (heptadecenoic acid), C18:1n9 cis (cis-9-octadecenoic acid), C20:1n9 (eicosenoic acid), C22:1n9 (erucic acid), C24:1n9 (nervonic acid), C18:3n3 (alpha-linolenic acid), C20:3n3 (eicosatrienoic acid), C20:5n3 (eicosapentaenoic acid), C22:5n3 (docosapentaenoic acid), or C22:6n3 (docosahexaenoic acid) (Table 5).

4. Discussion

This study was the first to show that acute exposure to an environmentally-relevant concentration of trichlorfon caused mitochondrial dysfunction in muscle of silver catfish due to reduction of: (1) activity cytosolic and mitochondrial CK, a central controller of cellular bioenergetics in tissues with high and fluctuating energy demands; (2) complexes II-III and IV of the mitochondrial respiratory chain, a route that provides biological energy for intracellular metabolic pathways. This exposure also negatively altered fatty acid profiles in muscle via decreases in PUFA levels, suggesting reduction of fillet quality with respect to consumers of fish. The most important contribution of this study is the finding that rutin added to fish feed reduced trichlorfon-induced CK inhibition and reversed inhibition of complex IV activity, as well as preventing changes in fatty acid profiles in muscle. Taken together, the data suggest that rutin reduces trichlorfon-induced muscle bioenergetic imbalance.

The energetic demand of muscle tissue is primarily met by the phosphotransfer network, mainly CK, and by the mitochondrial respiratory chain (Sundberg and Fitts, 2019). The evaluation of these pathways provides compelling data that contributes to the understanding the changes in muscular energetic metabolism during trichlorfon exposure. Acute exposure to 11 mg/L trichlorfon for 48 h significantly inhibited cytosolic and mitochondrial CK activities, suggesting inhibition of key

Table 4
Total of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in meat of silver catfish (*Rhamdia quelen*) juveniles fed with basal diet or containing the nominal concentration of 3 mg rutin/kg of feed during 21 days and exposed 48 h to 11 mg trichlorfon/L water.

Fatty acids/ Groups	A	B	C	D	*F* and *p* values
∑ SFA	41.17 ± 1.64[a]	40.78 ± 1.35[a]	42.81 ± 1.85[a]	42.44 ± 0.91[a]	0.456; 0.893
∑ MUFA	36.02 ± 1.24[a]	37.17 ± 2.21[a]	37.47 ± 1.87[a]	34.47 ± 1.29[a]	1.02; 0.125
∑ PUFA	24.03 ± 0.44[a]	21.88 ± 1.00[a]	20.48 ± 1.20[b]	23.87 ± 1.02[a]	**4.98; 0.0001**
∑ n-3	10.43 ± 0.83[a]	9.02 ± 1.13[a]	6.58 ± 0.46[b]	9.56 ± 0.95[a]	**3.87; 0.0002**
∑ n-6	15.53 ± 0.33[a]	14.99 ± 0.60[a]	10.67 ± 0.36[b]	14.75 ± 1.34[a]	**4.02; 0.0001**

Note 1: group A (basal diet; without rutin addition); group B (diet containing nominal concentration of 3 mg rutin/kg of feed); group C (basal diet; without rutin addition and exposed to nominal concentration of 11 mg trichlorfon/L water); group D (diet containing nominal concentration 3 mg rutin/kg of feed and exposed to nominal concentration of 11 mg trichlorfon/L water); **Note 2:** Values accompanied by different letters in the same row are statistically different considering $p < 0.05$.

Table 5
Fillet fatty acid profile (g/kg) of silver catfish (*Rhamdia quelen*) juveniles fed with basal diet or containing the nominal concentration of 3 mg rutin/kg of feed during 21 days and exposed 48 h to 11 mg trichlorfon/L water.

Fatty acids/ Groups	A	B	C	D	*F* and *p* values
C12:0	0.09 ± 0.011[a]	0.066 ± 0.010[a]	0.069 ± 0.010[a]	0.071 ± 0.014[a]	0.678; 0.123
C13:0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.00 ± 0.0[a]	0.234; 0.321
C14:0	0.97 ± 0.06[a]	0.84 ± 0.11[a]	0.79 ± 0.11[a]	0.82 ± 0.16[a]	0.341; 0.444
C15:0	0.39 ± 0.04[a]	0.30 ± 0.06[a]	0.36 ± 0.04[a]	0.35 ± 0.05[a]	0.542; 0.298
C16:0	29.69 ± 1.68[a]	30.74 ± 1.11[a]	31.39 ± 1.45[a]	30.54 ± 1.09[a]	0.800; 0.100
C17:0	0.69 ± 0.07[a]	0.59 ± 0.06[a]	0.60 ± 0.07[a]	0.46 ± 0.005[a]	0.111; 0.987
C18:0	8.73 ± 0.32[a]	8.15 ± 0.37[a]	9.22 ± 0.57[a]	9.42 ± 0.28[a]	0.450; 0.500
C20:0	0.32 ± 0.022[a]	0.32 ± 0.022[a]	0.39 ± 0.047[a]	0.35 ± 0.055[a]	0.901; 0.099
C22:0	0.09 ± 0.008[a]	0.07 ± 0.012[a]	0.09 ± 0.011[a]	0.10 ± 0.013[a]	0.678; 0.126
C23:0	0.22 ± 0.013[a]	0.19 ± 0.036[a]	0.19 ± 0.031[a]	0.19 ± 0.016[a]	1.03; 0.091
C14:1	0.047 ± 0.04[a]	0.040 ± 0.002[a]	0.031 ± 0.003[b]	0.048 ± 0.008[a]	**3.89; 0.0001**
C16:1	3.05 ± 0.26[a]	4.01 ± 0.61[a]	2.55 ± 0.40[b]	3.44 ± 0.51[a]	**6.54; 0.00001**
C17:1	0.077 ± 0.009[a]	0.060 ± 0.006[a]	0.068 ± 0.013[a]	0.074 ± 0.005[a]	1.02; 0.089
C18:1n9 cis	27.97 ± 1.89[a]	30.01 ± 1.79[a]	27.88 ± 2.22[a]	28.76 ± 1.44[a]	0.345; 0.430
C20:1n9	0.33 ± 0.03[a]	0.32 ± 0.03[a]	0.29 ± 0.015[a]	0.31 ± 0.05[a]	0.567; 0.200
C22:1n9	2.85 ± 0.22[a]	2.48 ± 0.51[a]	2.47 ± 0.25[a]	2.96 ± 0.38[a]	0.333; 0.190
C24:1n9	0.31 ± 0.02[a]	0.35 ± 0.04[a]	0.30 ± 0.04[a]	0.33 ± 0.03[a]	0.983; 0.104
C18:2n6 cis	14.01 ± 0.40[a]	11.89 ± 0.84[a]	9.39 ± 0.56[b]	12.56 ± 1.38[a]	**4.56; 0.0023**
C18:3n3	0.15 ± 0.023[a]	0.13 ± 0.017[a]	0.12 ± 0.008[a]	0.15 ± 0.13[a]	0.431; 0.320
C18:3n6	0.16 ± 0.02[a]	0.13 ± 0.017[a]	0.10 ± 0.008[b]	0.17 ± 0.013[a]	**5.56; 0.00001**
C20:2n6	0.27 ± 0.01[a]	0.22 ± 0.02[a]	0.18 ± 0.01[b]	0.22 ± 0.01[a]	**5.01; 0.00012**
C20:3n3	0.15 ± 0.01[a]	0.12 ± 0.02[a]	0.13 ± 0.006[a]	0.15 ± 0.01[a]	0.201; 0.984
C20:3n6	1.26 ± 0.08[a]	1.26 ± 0.23[a]	0.83 ± 0.07[b]	1.13 ± 0.05[a]	**4.99; 0.0019**
C20:5n3	0.88 ± 0.06[a]	0.79 ± 0.09[a]	0.72 ± 0.05[a]	0.77 ± 0.07[a]	0.876; 0.101
C22:5n3	1.08 ± 0.12[a]	0.99 ± 0.09[a]	1.05 ± 0.07[a]	1.02 ± 0.09[a]	0.763; 0.100
C22:6n3	6.25 ± 1.42[a]	5.09 ± 1.39[a]	6.01 ± 1.27[a]	6.58 ± 1.12[a]	1.23; 0.081

Note 1: dodecanoic acid (C12:0); tridecanoic acid (C13:0); myristic acid (C14:0); pentadecanoic acid (C15:0); palmitic acid (C16:0); heptadecanoic acid (C17:0); stearic acid (C18:0); eicosanoic acid (C20:0); docosanoic acid (C22:0); tricosanoic acid (C23:0); myristylic acid (C14:1); palmitoleic acid (C16:1); heptadecenoic acid (cis-10) (C17:1); cis-9-octadecenoic acid (oleic acid) (C18:1n9 cis); eicosenoic acid (C20:1n9); erucic acid (C22:1n9); nervonic acid (C24:1n9); cis-9,12-octadecadienoic acid (linoleic acid) (C18:2n6 cis); alpha-linolenic acid (C18:3n3); gamma-linolenic acid (C18:3n6); eicosadienoic acid (C20:2n6); eicosatrienoic acid (C20:3n3); di-homo-y-linolenic acid (C20:3n6); eicosapentaenoic acid (C20:5n3); docosapentaenoic acid (C22:5n3); docosahexaenoic acid (C22:6n3). **Note 2:** group A (basal diet; without rutin addition); group B (diet containing nominal concentration of 3 mg rutin/kg of feed); group C (basal diet; without rutin addition and exposed to nominal concentration of 11 mg trichlorfon/L water); group D (diet containing nominal concentration 3 mg rutin/kg of feed and exposed to nominal concentration of 11 mg trichlorfon/L water); **Note 3:** Values accompanied by different letters in the same row are statistically different considering $p < 0.05$.

enzymes associated with maintenance of cellular energetic homeostasis. This occurs via the use of creatine for reversible phosphoryl transfer between ATP and phosphocreatine (PCr), where PCr acts as an alternative energy source (Schlattner et al., 2018). This enzyme generates and uses PCr, a concentrated and highly diffusible cellular "high energy" intermediate to maintain cellular energy balance during conditions of spatial mismatch between ATP generation and ATP consumption (Janssen et al., 2000). The inhibition of mitochondrial and cytosolic CK activity avoids the synthesis of large PCr pools that could be used to regenerate ATP during a temporal mismatch between ATP utilization and generation. Similar to our observations, Baldissera et al. (2018b) found that exposure to 1.125 and 3.75 μg/L thiamethoxam, a neonicotinoid pesticide, significantly inhibited branchial cytosolic and mitochondrial CK activities, contributing to energetic imbalance in gills secondary to disequilibrium between ATP/ADP and PCr/Cr ratios and decreased branchial ATP levels. It is interesting and relevant to highlight the existence of a compensatory relationship between cytosolic and mitochondrial CK in order to avoid the mismatch between ATP generation and ATP consumption (Janssen et al., 2000). In the current study, there was no compensation between cytosolic and mitochondrial CK, and both fractions were inhibited by trichlorfon, and this may have directly contributed to bioenergetic dysregulation in muscle, as recently observed by Baldissera et al. (2018b) in gills of silver catfish exposed to thiamethoxam. Similarly, Baldissera et al. (2019a) found that exposure to 11 mg/L trichlorfon for 48 h significantly reduced both CK fractions in gills of silver catfish, suggesting absence of a compensatory relationship between CK fractions, contributing to impairment of bioenergetics. In the present study, muscle AK activity was not significantly affected by 11 mg/L trichlorfon, in agreement with observations by Baldissera et al. (2019a) in gills and liver of silver catfish exposed to the same conditions of the present study. Moreover, muscular PK activity was also not affected by the pesticide, in disagreement to observations by Baldissera et al. (2019a) showing significant reduction of branchial and hepatic PK activity in silver catfish exposed to 11 mg/L trichlorfon for 48 h; the authors concluded that reduction of PK activity may impair ATP content because this enzyme produces the second of two ATP molecules generated in the glycolytic pathway. In summary, the inhibition of both CK fractions, as well as the absence of a compensatory relationship between them, contributed to muscle bioenergetic imbalance during exposure to trichlorfon.

Many lines of evidence point to a critical role of mitochondrial activity in impairment of bioenergetic homeostasis during exposure to pesticides (Pereira et al., 2018; Ko et al., 2020); however, its involvement in the toxic effects of trichlorfon related to energetic balance remains unknown. For the first time (to our knowledge), we have demonstrated that exposure to an environmentally-relevant concentration of trichlorfon inhibited muscle complex II-III and IV activities, which may have caused reduction in ATP content, because mitochondria are the key players in ATP generation (Depaoli et al., 2018). In accordance to our observations, Pereira et al. (2018) found that exposure to 0.065 and 1.0 mg/L glyphosate for 7 days reduced activity of the respiratory chain complexes I and IV, suggesting that dysfunction of the respiratory chain plays a role in the toxic effects of glyphosate on energetics. According to Quinlan et al. (2012), the main function of mitochondrial complexes is to produce a proton gradient across the cristae membrane to produce ATP by ATP-synthase; the inhibition of complex II-III activities impairs the oxidation of succinate to fumarate in the Krebs cycle, reduces the transfer of electrons from succinate directly to ubiquinol, and impairs the transfer of electrons from reduced ubiquinol to a soluble electron carrier (cytochrome *c*), that consequently affects the physiological function of complex IV which is the establishment of suitable proton gradient via transfer of electrons from cytochrome *c* to molecular oxygen. In summary, inhibition of muscle complex II-III and IV activities of the mitochondrial respiratory chain impairs the generation of a proton gradient across the cristae membrane and ATP production, contributing to triclorfon-induced bioenergetic homeostasis.

To evaluate the possible effects of consumption of fillets from fish exposed to trichlorfon, we decided to evaluate the fatty acids profiles in fillets, because these are thought to be excellent sources of PUFAs (omega-3 and omega-6) (Mohanty et al., 2019). In the present study, the total contents of omega-3 and omega-6 were significantly reduced in fillets of silver catfish exposed to 11 mg/L trichlorfon; this can be considered a negative impact with respect to consumers of fish because PUFAs improve human health (DiNicolantonio, 2017). In particular among specific fatty acids, there was significant reduction in levels of linoleic acid and gamma-linolenic acid, two important PUFAs associated with positive effects for cardiovascular metabolism (Marangoni et al., 2020). According to these authors, linoleic acid is an essential fatty acid and is a key nutrient throughout all human life, because an overall still non-optimal linoleic acid intake may contribute to high worldwide mortality from coronary heart disease. Similar to our observations, Ewere et al. (2019) showed that exposure to 0.01–0.05 mg/L imidacloprid for 14 days significantly reduced total content of PUFAs and omega-6 levels in muscle of Sydney rock oysters, concluding that this may affect human health through the consumption of lower quality oysters. In line with this, total content of PUFAs was significantly lower in muscle of freshwater *Channa striatus* exposed to 2 mg/L of methomyl for 15 days, which also significantly reduced levels of linoleic, gamma-linolenic, and alpha-linolenic acids, representing negative impacts on consumers (Chinnamani et al., 2018). Changes in the relative proportions of fatty acids can also be a common mechanism for coping with metabolic impairment caused by environmental contaminants (Sánchez-Muros et al., 2013), because lipids are alternative energy sources, possibly explaining the alteration on lipid metabolism in pacu (*Piaractus mesopotamicus*) exposed to trichlorfon (Venturini et al., 2015). In summary, exposure to trichlorfon impairs fatty acid profiles in fish fillets, representing a concern for consumers of fish.

The inclusion of rutin in fish diet before the exposure to 11 mg/L triclorfon improved bioenergetic balance because ameliorated, although not completely, trichlorfon-induced interference with mitochondrial and cytosolic CK activity, as well as preventing the inhibition of complex IV activity in the respiratory chain; this reinforces the mitoprotective effects of rutin reported by Leffa et al. (2017) in murine models. The improvement in muscle mitochondrial and cytosolic CK activity caused by rutin addition indicates better capacity of silver catfish to cope with triclorfon-induced impairment of bioenergetics, as recently observed by Baldissera et al. (2019b) in gills of grass carp (*Ctenopharyngodon idella*) fed 300 mg grape pomace flour/kg diet (that containing 3.55 mg rutin/kg grape pomace flour) and experimentally infected with *Pseudomonas aeruginosa.* According to these authors, supplementation with this feed for 60 days prevented the inhibition on both CK fractions elicited by *P. aeruginosa* infection, reinforcing the mitoprotective effects of rutin on energetic metabolism in fish. Moreover, inclusion of rutin in fish feed prevented trichlorfon-induced inhibition of complex IV activity, in agreement to observations by Leffa et al. (2017) in brain striatum and hypothalamus of mice fed 200 mg/kg/day rutin for 13 weeks. According to these authors, rutin antioxidant effects is a pathway linked to its protective effect on bioenergetic balance, because excessive free radical production and oxidative stress impairs CK activity and complexes of respiratory chain. An *in vitro* study conducted by Enogieru et al. (2019) also found protective effects of rutin related to bioenergetics. According to these authors, 25–100 μM rutin protected against calcium dysregulation, impairment of mitochondrial membrane potential and impairment of oxidative phosphorylation in human brain cells exposed to 1-methyl-4-phenylpyridinium. Similarly, use of others natural compounds such as *Spirulina platensis* (Abdelkhalek et al., 2017) or allicin (Abdel-Daim et al., 2015) can represent interesting approaches to ameliorate diazinon- and deltamethrin-induced oxidative stress, respectively, suggesting that natural products can be used to reduce the negative impact of pesticides on fish. These findings suggested that rutin treatment reduces or prevents bioenergetic imbalances and

mitochondrial damage. In summary, addition of rutin to fish feed can reduce trichlorfon-induced muscular bioenergetic homeostasis.

The use of dietary flavonoids has been considered a reliable and suitable approach to improve meat quality (North et al., 2019a). For this reason, we decided to determine whether addition of rutin in fish feed would reduce or prevent the impairment of fatty acid profiles in fillets caused by trichlorfon. Feed enriched with 3 mg rutin/kg feed prevented all alterations on fatty acid profiles in muscle elicited by trichlorfon, which can be considered a positive effect for fish and human health. Using feed enriched with quercetin, an important flavonoid, North et al. (2019b) showed that its inclusion in rabbit feed (2 g/kg diet for 7 weeks) improved meat quality due via increased PUFAs content. It is important to highlight that it is the first evidence regarding beneficial effects of rutin on muscle fatty acid composition; and mechanism of action remains unknown; nevertheless, it may involve its antioxidant properties, as shown for quercetin (North et al., 2019b). It is compelling to emphasize that the protective effects of rutin on fatty acid profiles can be an indirect consequence of improvement of bioenergetic balance, because fatty acid metabolism is altered during metabolic imbalance, i.e., because rutin improved bioenergetic balance, there is no need for fatty acid mobilization as an alternative energy source.

We found that acute exposure (48 h) to 11 mg/L trichlorfon severely inhibited activity of muscle cytosolic and mitochondrial CK, a crucial enzyme to maintain cellular energy homeostasis, as well as activities of complexes II-III and IV of the respiratory chain, an important pathway for ATP production. This exposure impairs fatty acid profiles in muscle, negatively impacting safety of human consumers. The most important data of the present study is that dietary addition with the flavonoid rutin improves bioenergetic homeostasis and fatty acid profiles in muscle, representing a suitable alternative to reduce trichlorfon-induced muscle damage.

Ethics committee

The methodology used in the experiment was approved by the Ethical and Animal Welfare Committee of the Universidade do Estado de Santa Catarina (1339060919).

Credit author statement

Matheus Baldissera, Carine Souza: Conceptualization, sample collection, data curation and writing-reviewing; Adriana Meinhart: rutin analysis; Belisa Parmeggiani and Guilhian Leipnitz: complexes measurement; Renato Zanella, Osmar Prestes, Lucila Ribeiro and Daniela Muenchen: pesticides measurement in water; Roger Wagner and Raquel Vendrusculo: fatty acid analysis Bernardo Baldisserotto and Aleksandro da Silva: Conceptualization and review; Carla Zeppenfeld: diet formulation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.111127.

References

Abdel-Daim, M.M., Abdelkhalek, N.K.M., Hassan, A.M., 2015. Antagonistic activity of dietary allicin against deltamethrin-induced oxidative damage in freshwater Nile tilapia; *Oreochromis niloticus*. Ecotoxicol. Environ. Saf. 111, 146–152.

Abdelkhalek, N.K.M., Eissa, I.A.M., Ahmed, E., Kilany, O.E., El-Adl, M., Dawood, M.A.O., Hassan, A.H., Abdel-Daim, M.M., 2017. Protective role of dietary *Spirulina platensis* against diazinon-induced Oxidative damage in Nile tilapia; Oreochromis niloticus. Environ. Toxicol. Pharmacol. 54, 99–104.

Abdelkhalek, N.K.M., Ghazy, E.W., Abdel-Daim, M.M., 2015. Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: impact on lipid peroxidation and oxidative stress. Environ. Sci. Pollut. Res. 22, 3023–3031.

Baldissera, M.D., Souza, C.F., Zeppenfeld, C.C., Velho, M.C., Klein, B., Abbad, L.B., Ourique, A.F., Wagner, R., Da Silva, A.S., Baldisserotto, B., 2020. Dietary supplementation with nerolidol nanospheres improves growth, antioxidant status and fillet fatty acid profiles in Nile tilapia: benefits of nanotechnology for fish health and meat quality. Aquaculture 516, 734635.

Baldissera, M.D., Souza, C.F., Descovi, S.N., Zanella, R., Prestes, O.D., de Matos, A.F.I.M., da Silva, A.S., Baldisserotto, B., Gris, A., Mendes, R.E., 2019a. Disturbance of energetic homeostasis and oxidative damage provoked by trichlorfon as relevant toxicological mechanisms using silver catfish as experimental model. Chem. Biol. Interact. 299, 94–100.

Baldissera, M.D., Souza, C.F., Descovi, S.N., Verdi, C.M., Zeppenfeld, C.C., da Silva, A.S., Santos, R.C.V., Baldisserotto, B., 2019b. Grape pomace flour ameliorates *Pseudomonas aeruginosa*-induced bioenergetic dysfunction in gills of grass carp. Aquaculture 506, 359–366.

Baldissera, M.D., Souza, C.F., Descovi, S.N., Zanella, R., Stefani, L.M., da Silva, A.S., Baldisserotto, B., 2018a. Purinergic signalling as a potential pathway for trichlorfon induced inflammation and impairment of the immune response using freshwater. Aquaculture 497, 91–96.

Baldissera, M.D., Souza, C.F., Golombieski, J.I., Seben, D., Sippert, L.R., Salbego, J., Zanella, R., Baldisserotto, B., 2018b. Thiamethoxam induced hepatic energy changes in silver catfish via impairment of the phosphoryl transfer network pathway: toxicological effects on energetics homeostasis. Environ. Toxicol. Pharmacol. 60, 1–4.

Bhattacharjee, P., Borah, A., Das, S., 2020. Quercetin-induced amelioration of deltamethrin stress in freshwater teleost, *Channa punctata*: multiple biomarker analysis. Comp. Biochem. Physiol., C 227, 108626.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Chang, X., Wang, X., Feng, J., Su, X., Liang, J., Li, H., Zhang, J., 2020. Impact of chronic exposure to trichlorfon on intestinal barrier, oxidative stress, inflammatory response and intestinal microbiome in common carp (*Cyprinus carpio* L.). Environ. Pollut. 259, 113846.

Chinnamani, S., Mageswari, M., Sathya, C., Murugaian, P., Sivasuriyan, S., 2018. Sub-lethal effect of methomyl-based pesticide on chemical compositions and fatty acid profiles of fresh water fish *Channa striatus*. Int. J. Biol. Res. 3, 363–371.

Cunha, M.A., Zeppenfeld, C.C., Garcia, L.O., Loro, V.L., Fonseca, M.B., Emanuelli, T., Veeck, A.P.L., Copatti, C.E., Baldisserotto, B., 2010. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet. Ciência Rural. 40, 2107–2114.

Da Rosa, V.M., Ariotti, K., Bressan, C.A., da Silva, E.G., Dallaporta, M., Júnior, G.B., da Costa, S.T., de Vargas, A.C., Baldisserotto, B., Finamor, I.A., Pavanato, M.A., 2019. Dietary addition of rutin impairs inflammatory response and protects muscle of silver catfish (*Rhamdia quelen*) from apoptosis and oxidative stress in *Aeromonas hydrophila*-induced infection. Comp. Biochem. Physiol., A C226, 108611.

Deng, Y., Qiu, M., Wang, Y., Wang, R., Lu, P., Sun, L., Li, X., Gooneratne, R., 2019. Protective effect of antioxidant-enriched diets on T-2-toxin-induced damage in tilapia (*Oreochromis niloticus*). Aquaculture 506, 341–349.

Depaoli, M.R., Kasrten, F., Madreiter-Sokolowski, C.T., Klec, C., Gottschalk, B., Bischof, H., Eroglu, E., Waldeck-Weiermair, M.W., Simmen, T., Graier, W.F., 2018. Real-time imaging of mitochondrial ATP dynamics reveals the metabolic setting of single cells. Cell Rep. 25, 501–512.

DiNicolantonio, J.J., 2017. Good fats versus bad fats: a comparison of fatty acids in the promotion of insulin resistance, inflammation, and obesity. Mo. Med. 114, 303–307.

Duncan, W.P., Idalino, J.J.S., da Silva, A.G., Moda, R.F., da Silva, H.C.M., Matoso, D.A., Gomes, A.L.S., 2020. Acute toxicity of the pesticide trichlorfon and inhibition of acetylcholinesterase in *Colossoma macropomum* (Characiformes: serrasalmidae). Aquacult. Int. 28, 815–830.

Dzeja, P.P., Terzic, A., Wieringa, Be, 2004. Phosphotransfer dynamics in skeletal muscle from creatine kinase gene-deleted mice. Mol. Cell. Biochem. 256, 13–27.

Dzeja, P.P., Vitkevicius, K.T., Redfield, M.M., Burnett, J.C., Terzic, A., 1999. Adenylate kinase-catalyzed phosphotransfer in the myocardium: increased contribution in heart failure. Circ. Res. 84, 1137–1143.

Enogieru, A.B., Haylett, W.L., Miller, H.C., van der Westhuizen, F.H., Hiss, D.C., Ekpo, O. E., 2019. Attenuation of endoplasmic reticulum stress, impaired calcium homeostasis, and altered bioenergetic functions in MPP^{+}-exposed SH-SY5Y cells pretreated with rutin. Neurotox. Res. 36, 764–776.

Ewere, E.E., Reichelt-Brushett, A.R., Benkendorff, K., 2019. Imidacloprid and formulated product impacts the fatty acids and enzymatic activities in tissues of Sydney rock oysters, *Saccostrea glomerata*. Mar. Environ. Res. 151, 104765.

Fisher, J.C., Ruitenbeek, W., Berden, J.A., Trijbels, J.M.F., Veerkamp, J.H., Stadhouders, A.M., Sengers, R.C.A., Janssen, A.J.M., 1985. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin. Chim. Acta 153, 23–36.

Grings, M., Parmeggiani, B., Moura, A.P., Alvorcem, L.M., Wyse, A.T.S., Wajner, M., Leipnitz, G., 2018. Evidence that thiosulfate inhibits creatine kinase activity in rat striatum via thiol group oxidation. Neurotox. Res. 34, 693–705.

Hartman, L., Lago, R.C., 1973. Rapid preparation of fatty acid methyl esters from lipids. Lab. Pract. 22, 475–476.

Hughes, B.P., 1962. A method for estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathological sera. Clin. Chim. Acta 7, 597–603.
Janssen, E., Dzeja, P.P., Oerlemans, F., Simonetti, A.W., Heerschap, A., de Haan, A., Rush, P.S., Terjung, R.R., Wieringa, B., Terzic, A., 2000. Adenylate kinase 1 gene deletion disrupts muscle energetic economy despite metabolic rearrangement. EMBO J. 19, 6371–6381.
Kang, H.S., Know, N.J., Jeong, J., Lee, K., Lee, H., 2019. Web-based Korean maximum residue limit evaluation tools: an applied example of maximum residue limit evaluation for trichlorfon in fishery products. Environ. Sci. Pollut. Control Ser. 26, 7284–7299.
Ko, E., Choi, M., Shin, S., 2020. Bottom-line mechanism of organochlorine pesticides on mitochondria dysfunction linked with type 2 diabetes. J. Hazard Mater. 393, 122400.
Leffa, D.D., Rezin, G.T., Daumann, F., Longaretti, L.M., Dajori, A.L.F., Gomes, L.M., Silva, M.C., Streck, E.L., de Andrade, V.M., 2017. Effects of acerola (*Malpighia emarginata* DC.) juice intake on brain energy metabolism of mice fed a cafeteria diet. Mol. Neurobiol. 54, 954–963.
Leong, S.F., Lai, J.C., Lim, L., Clark, J.B., 1981. Energy-metabolizing enzymes in brain regions of adult and aging rats. J. Neurochem. 37, 1548–1556.
López-Pacheco, I.Y., Silva-Núñez, A., Salinas-Salazar, C., Arévalo-Gallegos, A., Lizarazo-Holguin, L.A., Barceló, D., Iqbal, H.M.N., Parra-Saldívar, R., 2019. Anthropogenic contaminants of high concern: existence in water resources and their adverse effects. Sci. Total Environ. 690, 1068–1088.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Marangoni, F., Agostoni, C., Borghi, C., Catapano, A.L., Cena, H., Ghiselli, A., La Vecchia, G., Manzato, E., Pirillo, A., Riccardi, G., Risé, P., Visioli, F., Poli, A., 2020. Dietary linoleic acid and human health: focus on cardiovascular and cardiometabolic effects. Atherosclerosis 292, 90–98.
Meinhart, A.D., Damin, F.M., Caldeirão, L., da Silveira, T.F.F., Filho, J.T., Godoy, H.T., 2017. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. Food Res. Int. 99, 522–530.
Mohanty, B.P., Mahanty, A., Ganguly, S., Mitra, T., Karunakaran, D., Anandan, R., 2019. Nutritional composition of food fishes and their importance in providing food and nutritional security. Food Chem. 293, 561–570.
Naiel, M.A.E., Shehata, A.M., Negm, S.S., El-Hack, M.E.A., Amer, M.S., Khafaga, A.F., Bin-Jumah, M., Allam, A.A., 2020. The new aspects of using some safe feed additives on alleviated imidacloprid toxicity in farmed fish: a review. Rev. Aquacult. https://doi.org/10.1111/raq.12432.
Nazeri, S., Farhangi, M., Modarres, S., 2017. The effect of different dietary inclusion levels of rutin (a flavonoid) on some liver enzyme activities and oxidative stress indices in rainbow trout, *Oncorhynchus mykiss* (Walbaum) exposed to Oxytetracycline. Aquacult. Res. 48, 4356–4362.
North, M.K., Zotte, A.D., Hoffman, L.C., 2019b. The effects of dietary quercetin supplementation and sex on the fatty acidprofile of rabbit meat, dissectible fat and caecotrophes. Meat Sci. 157, 107888.
North, M.K., Zotte, A.D., Hoffman, L.C., 2019a. The use of dietaryflavonoids in meat production: a review. Anim. Feed Sci. Technol. 257, 114291.
Oboh, G., Adebayo, A.A., Ademosun, A.O., Olowokere, O.G., 2020. Rutin restores neurobehavioral deficits via alterations in cadmium bioavailability in the brain of rats exposed to cadmium. Neurotoxicology 77, 12–19.
Osmond, A.T.Y., Colombo, S.M., 2019. The future of genetic engineering to provide essential dietary nutrients and improve growth performance in aquaculture: advantages and challenges. JWAS (J. World Aquacult. Soc.) 50, 490–509.
Pereira, A.G., Jaramillo, M.L., Remor, A.P., Latini, A., Davico, C.E., Da Silva, M.L., Muller, Y.M.R., Ammar, D., Nazari, E.M., 2018. Low-concentration exposure to glyphosate-based herbicide modulates the complexes of the mitochondrial respiratory chain and induces mitochondrial hyperpolarization in the *Danio rerio* brain. Chemosphere 209, 353–362.
Pês, T.S., Saccol, E.M., Ourique, G.M., Londero, E.P., Gressler, L.T., Finamor, I.A., Rotili, D.A., Golombieski, J.I., Glanzner, W.G., Llesuy, S.F., Gonçalves, P.B., Radunz Neto, J., Baldisserotto, B., Pavanato, M.A., 2016. Effect of diets enriched with rutin on blood parameters, oxidative biomarkers and pituitary hormone expression in silver catfish (*Rhamdia quelen*). Fish Physiol. Biochem. 42, 321–333.
Quinlan, C.L., Orr, A.L., Perevoshchikova, I.V., Treberg, J.R., Ackrell, B.A., Brand, M.D., 2012. Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. J. Biol. Chem. 287, 27255–27264.
Rustin, P., Chretien, D., Bourgeron, T., Gerard, B., Rotig, A., Saudubray, J.M., Munnich, A., 1994. Biochemical and molecular investigations in respiratory chain deficiencies. Clin. Chim. Acta 228, 35–51.
Sánchez-Muros, M.J., Villacreces, S., De La Lama, G.M., De Haro, C., García-Barroso, F., 2013. Effects of chemical and handling exposure on fatty acids, oxidative stress and morphological welfare indicators in gilt-head sea bream (*Sparus aurata*). Fish Physiol. Biochem. 39, 581–591.
Schlattner, U., Kay, L., Tokarska-Schlattner, M., 2018. Mitochondrial proteolipid complexes of creatine kinase. Membrane Protein Complexes: Struct. Funct. 87, 365–408.
Sundberg, C.W., Fitts, R.H., 2019. Bioenergetics basis of skeletal muscle fatigue. Curr. Opin. Physiol. 10, 118–127.
Thompson, M., Ellison, S.L.R., Wood, R., 2002. Harmonized guidelines for single-laboratory validation of methods of analysis. Pure Appl. Chem. 74, 835–855.
Trujillo-González, A., Becker, J.A., Vaughan, D.B., Hutson, K.S., 2018. Monogenean parasites infect ornamental fish imported to Australia. Parasitol. Res. 117, 995–1011.
Venturini, F.P., Moraes, F.D., Cortella, L.R.X., Rossi, P.A., Cruz, C., Moraes, G., 2015. Metabolic effects of trichlorfon (Masoten®) on the neotropical freshwater fish pacu (*Piaractus mesopotamicus*). Fish Physiol. Biochem. 41, 299–309.
Verdouw, H., Vanechteld, C.J.A., Deckkers, E.M.J., 1978. Ammonia determinations based on indophenol formation with sodium salicylate. Water Res. 12, 399–402.
Visentainer, J.V., 2012. Aspectos analíticos da resposta do detector de ionização em chama para ésteres de ácidos graxos em biodiesel e alimentos. Quim. Nova 19, 380–385.
Woo, S.J., Chung, J.K., 2020. Effects of trichlorfon on oxidative stress, neurotoxicity, and cortisol levels in common carp, *Cyprinus carpio* L., at different temperatures. Comp. Biochem. Physiol., C 229, 108698.
Yonar, S.M., Yonar, M.E., Pala, A., Saglam, N., Sakin, F., 2020. Effect of trichlorfon on some haematological and biochemical changes in *Cyprinus carpio*: the ameliorative effect of lycopene. Aquacult. Rep. 16, 100246.
Zeppenfeld, C.C., Saccol, E.M.H., Pês, T.S., Salbego, J., dos Santos, A.C., Heinzmann, B. M., da Cunha, M.A., Barcellos, L.J.G., Caron, B.O., Baldisserotto, B., 2017. *Aloysia triphylla* essential oil as food additive for *Rhamdia quelen* – stress and antioxidant parameters. Aquacult. Nutr. 23, 1362–1367.
Zhang, K., Fu, Q., Ge, F., Jin, X., Guo, R., Qiao, X., Zhao, X., Zheng, X., 2019. The correlation study between fatty acids and organochlorine pesticides or δ15N values in fish tissues from Dongting Lake, China. Ecotoxicol. Environ. Saf. 182, 109358.