The acidophilic microalga *Coccomyxa onubensis* and atorvastatin equally improve antihyperglycemic and antihyperlipidemic protective effects on rats fed on high-fat diets

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ABSTRACT

Coccomyxa onubensis biomass may be used as a food source for animals without collateral toxic effects. As diet supplemented the microalga has significant hypoglycemic and hypocholesterolemic effects on healthy animals.

Rats were fed for 108 days with a high-fat diet, and at the end of the experiment, they were overweight and had significantly increased serum levels of glucose (2.0-fold), total cholesterol (1.6-fold), and low-density lipoprotein (LDL)-cholesterol (7.7-fold). The supplement of *C. onubensis* powder (6.25% w/w dry weight) in the high-fat diet significantly protected the rats against cardiovascular risks by reducing the serum levels of glucose (38.47%), total cholesterol (22.65%), and LDL-cholesterol (26.70%). The protective effects of the microalga were comparable with that of 10 mg/kg body weight per day of atorvastatin.

The high-fat diet decreased both ω–3 eicosapentaenoic and docosahexaenoic acids in the brain tissue of rats; however, *C. onubensis* powder could not restrict these changes. Simultaneously, the high-fat diet increased the levels of both palmitic and arachidonic (ω–6) acids in the telencephalon tissue of rats, this was prevented when microalga biomass was used in the diet of rats.

Keywords: Atorvastatin, *Coccomyxa onubensis*, polyunsaturated fatty acids, hypercholesterolemia, hyperglycemia, rats metabolic syndrome.

INTRODUCTION

Metabolic syndrome is characterized by overweight and abdominal fat distribution, as well as by high blood pressure, pro-inflammatory state, excess of reactive oxygen species, insulin resistance, high serum levels of different parameters such as glucose and triglycerides, and low levels of high-density lipoprotein (HDL)-cholesterol. Moreover, it is defined by its susceptibility to other comorbid conditions such as cognitive deficits, nonalcoholic fatty liver, and some forms of cancers (Grundy et al. 2004; Saklagen 2018). Diabetes is a metabolic disease which when complicated with hypertension increases the risk of cardiovascular (CV) diseases (Liptak et al. 2019). Statins have been broadly used as an antihypercholesterolemic drug in animals and humans because they inhibit the HMG-CoA reductase activity, and consequently the biosynthesis of cholesterol in the liver (Chen et al. 2011). Statins also improve several hemodynamic parameters that are deteriorated because of metabolic syndrome and protect rats against CV risks. Atorvastatin, a pharmacological statin, improves the hemodynamic status (Crespo and Quidgley 2015; Liptack et al. 2019) and has neuroprotective effects on the

hippocampus of diabetic rats (Paseban et al. 2019). Furthermore, the American Diabetes Association recommends the use of statins for all patients with diabetes below 40 years of age with additional CV risk factors or with ongoing CV disease.

Microalgae biomass is a major source of nutraceuticals for functional foods, such as polysaccharides, vitamins, essential amino acids, polyunsaturated fatty acids (PUFAs), minerals, carotenoids, enzymes, and fibers (Matos et al. 2017; Gómez-Zurita et al. 2020). The bioactive compounds from microalga possess several crucial properties, such as anticoagulant and/or antithrombotic, immunomodulatory ability, antitumoral, hypolipidemic, and hypoglycemic activities, as well as antibiotic, antioxidant, and anti-inflammatory activities, which make them a promising bioactive product with a wide range of applications (Forján et al. 2015; Raposo et al. 2015). Several studies have shown the hypoglycemic and hypolipidemic effects of microalgae biomass on a healthy animal when supplied as a diet supplement (Raposo et al. 2015; Matos et al. 2017; Sathasivam et al. 2019). In addition, many types of microalgae have documented health benefits from strengthening the immune system to fighting cancer and heart diseases (Bishop and Zubeck 2012).

Extremophilic microalgae can accumulate unique metabolites usually involved in adaptative mechanisms to resist unusual environmental or nutritional conditions. The thermoacidophilic microalga *Galdieria sulfuraria* showed that it possesses chemical and nutritional characteristics that can be used in the nutrition of animals and humans (Graciani et al. 2013). *Coccomyxa onubensis* is a eukaryotic microalga isolated from the acidic water of the Tinto river in the Province of Huelva (Spain) (Bermejo et al. 2018). Furthermore, on the basis of histological, hematological, and biochemical analyses, we found that a standard diet enriched with 6.25% (w/w) of *C. onubensis* biomass is non-toxic to healthy rats, and induce in them a significant hypoglycemic and hypolipidemic effects (Navarro et al. 2016). Actually, we are interested if *C. onubensis* powder supplementing a high-fat diet may protect rats against the induced hyperglycemic and hyperlipidemic effects. In addition, we compare the microalga with the therapeutic power of atorvastatin against these pathologic parameters. Furthermore, neuroinflammation is a hallmark feature of brain disorders, caused by an unhealthy diet, and fatty acids act as signaling molecules involved in inflammatory responses (Gimenez et al. 2018; Melo et al. 2019). Dietary intake determines the fatty acids profile of the brain tissue in mammals, where neurotransmission depends on interactions between arachidonic (20:4ω–6) and docosahexaenoic (DHA, 22:6ω–3) acids (Rappoport et al. 2007). Thus, a second goal of this study is the analysis of fatty acids profile changes in the high-fat diet fed rats brain tissue, and the effect induced by microalga in the diet.

MATERIALS AND METHODS

Microalgal biomass production.

The microalga *Coccomyxa onubensis* (SAG 2510) was grown and harvested as shown in a previous study (Navarro et al., 2016). The biomass was dried in an oven with fan-assisted circulation and converted into a powder of grain size <100 µm using a vibratory disc mill. The powder was further vacuum-packed and stored at −80 °C until use. The microalga biomass is composed by high protein (44.60% of dry weight) and dietary fiber (15.73%), together with a moderate carbohydrate content (24.80%), and a low lipid (5.40%) and nucleic acid (4.8%) content. An additional dietary advantage of this biomass is its low monosaccharide and disaccharide content (0.1%), and its high PUFAs (65% of total fatty acids), and also lutein, 7.5 $mg.gdw^{-1}$ (Navarro et al. 2016; Bermejo et al. 2018).

Experimental diet preparation

We found that a diet enriched with 6.25% (w/w) of *C. onubensis* biomass is non-toxic to healthy rats (Navarro et al. 2016). The experimental diets used in this study were based on conventional rodent chow pellets from Harlan Laboratories, Inc. (Indianapolis, IN, USA), which were ground into powder using a jaw crusher (Retsch GmbH BB200) and a vibratory disc mill until the grain size was <100 µm. The four diets were prepared as follows: Diet 1 (control) included only standard diet powder (with 14% protein); in Diet 2 (high-fat), 2% (w/w) of free cholesterol, 0.5% (w/w) of cholic acid, and 10% (w/w) of pork lard were added to Diet 1; in Diet 3 (microalga), 62.5 mg of microalgae dry powder plus 937.5 mg of Diet 2 powder were mixed; and in Diet 4 (atorvastatin), 10 mg/kg body weight per day of atorvastatin was added to Diet 2 powder. Protein in Diet 3 is 1.9% higher than in the others. In all diets, the powders were mixed homogeneously, reconstituted with distilled water in a kneader (Fimar AM1, Rimini, Italy), and then made into pellets again using an extruder Fimar pasta machine MPF4. These pellets were dried in an oven with fan-assisted circulation to obtain the same degree of humidity as the original standard rodent diet. Dried pellets were stored at −20 °C under vacuum until used.

Animal handling

All experiments were performed on four-week-old Long–Evans male rats (n = 24), weighing 145–160 g, obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA). Animals were handled in accordance with Directive 8609/CEE of the European Community Council and Spanish Legislation (RD 53/2013). The protocols used in this study were approved by the Ethics

Committee of the University of Huelva (Spain). Animals were allowed to acclimatize for five days with free access to food (Diet 1) and water under controlled conditions of temperature (22.0±1.3 °C) and a 12 h light-dark cycle before starting the experiments. Rats were then randomly distributed into four groups of six rats each, with similar mean weights. Two animals were housed in each cage provided with entertainment woods sticks (chew toys for rats and mice), and they were fed with one of the four described diets. Rats were weighed every alternate day, and biochemical analysis for all animals was performed after 108 days of feeding.

Hematological and biochemical analysis

Rats were fasted for 12 h and anesthetized with inhaled isoflurane prior to sacrifice. Following a cardiac puncture, blood was collected from the left ventricle in a 2-mL glass BD Vacutainer K3 EDTA tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and processed immediately according to the manufacturer's instructions for hematological studies. The hematological analysis was performed using a Sysmex XT–4000i automated hematology analyzer (Sysmex America Inc., Lincolnshire, Illinois, USA).

Samples for serum biochemistry determinations were collected in 3-mL tubes using the Advanced BD Vacutainer SST II gel separator and suction system (Oxford, UK). Blood samples were first stored in a refrigerator and protected from light for 60 min to allow clot retraction, and serum was obtained after centrifuging at 1,500 ×g for 15 min at 4 °C. Enzyme activities were determined using a Cobas 8000 Modular Analyzer (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. Plasma total cholesterol (TC) and triglyceride concentrations were determined enzymatically using the Cobas 8000 Modular Analyzer as per the manufacturer's instructions. Moreover, the analyzer was used to analyze HDL and low-density lipoprotein (LDL) cholesterol contents following the manufacturer's instructions in each case.

Organ extraction

The different organs (liver, spleen, epididymis, kidneys, heart, and encephalon) were extracted and weighed from all four groups of animals. Postmortem organ tissues from the different groups were extracted carefully, rinsed with a cold 0.9% (w/v) NaCl solution, and weighted. Each encephalon was sectioned in four parts (telencephalon, mesencephalon, brainstem and cerebellum, and spinal cord) using a scalper and a stereomicroscope Nikon SMZ1000 (Nikon

Instruments Inc. NY, USA). Each part was immediately frozen with liquid nitrogen and stored at −80 °C until use.

Lipids determination

Total lipids were extracted from the encephalon using a modified method of Folch et al. (1957) with butyl-hydroxyl-toluene as an antioxidant. For fatty acid analysis, lipids were transmethylated using sodium methoxide in methanol (0.5%), and the resulting fatty acid methyl esters were analyzed by gas chromatography using a 5890 Series II gas chromatograph (Hewlett-Packard (Avondale, PA, USA) equipped with a flame ionization detector and a capillary silica column BPX70 SGE Analytical Science (Trajan Scientific and Medical, Victoria, Australia) of 10-m length, 0.1-mm internal diameter, and 0.2 µm inner coating film (Perona et al. 2000).

Reagents: The calcium atorvastatin used was purchased from Sandoz Farmacéutica, S.A. (Aravaca, Madrid, Spain). Cholesterol and cholic acid of >98% were obtained from Sigma-Aldrich (Steinheim, Germany). Lard was obtained from Campofrío Food Group S.A. (Madrid, Spain).

Statistical analyses

Statistical analyses were performed using the SPSS version 19 statistical analysis package. The variables don't follow the normal distribution for that reason we used test non-parametric. The data of the four dietary groups were analyzed using a non-parametric test (Kruskal-Wallis). In order to compare the two variables, Mann-Whitney's U-test was used. A p-value of <0.05 was considered significant. In the case of lipids determination, differences among groups were assessed using one-way analysis of variance, followed by Tukey's post hoc test.

RESULTS

Effect of C. onubensis biomass on rat body and organs weight

The rats from the four groups consistently had weekly weight gain, and at the end of the experiment, the weight of animals fed with Diets 1 (control), 2, 3, and 4 was 429.79±17.13 g, 458.24±17.80 g, 463.01±18.80 g, and 433.73±7.46 g, respectively. No visible behavioral changes were observed in rats during the experimental period. Liver weight was almost doubled in the rats fed on high-fat diets (25.18±2.73 g) versus control animals (13.17±0.91 g),

and a significant weight gain was observed in the spleen and epididymis, whereas no significant weight changes were observed in the kidneys, hearts, and brains of the animals (Table 1).

Structure and function of the liver

The fat was accumulated throughout the liver of rats fed with Diet 2 compared with that of the control rats (Diet 1) (Fig. 1). In addition, rats fed with Diet 2 showed a significant increase in the activity of phosphatase alkaline (43%), glutamate-oxaloacetate transaminase (93.5%), and glutamate-pyruvate transaminase (102%) compared with the control rats, which are consistent with the severe damage produced in the liver (Table 1 and Fig. 1). *C. onubensis* powder or atorvastatin could not restrict weight gain or fat deposition in the liver.

Hematological and serum profiles of rats fed with the different diets

The hematological parameters such as hemoglobin, hematocrit, erythrocytes, leukocytes, lymphocytes, and platelets were similar and compatible with healthy animals for the four experimental groups (data not shown). However, the serum biochemical profile of rats fed with Diet 2 showed the hemodynamic parameters similar to animals with metabolic syndrome, that is, altered glucose (97.4% higher than control) and total cholesterol (TC) (60.15% higher than normal). In these animals, the amount of LDL-cholesterol was significantly elevated, which was 7.7-fold higher than the control animals, whereas the levels of other plasma parameters such as albumin; creatinine; ferritin; bilirubin; and the Ca^{2+}, Na^{+}, K^{+}, Cl^{-}, and Fe^{2+} ions were similar across the different groups of rats and within the normal ranges for healthy rats (data not shown).

Effect of C. onubensis biomass or atorvastatin on serum glucose and C-reactive protein levels of rats fed with a high-fat diet

The rats fed with a high-fat diet showed a significantly increased glucose level (twice) compared with animals fed with Diet 1, because the high-fat diet induces obesity in the animals, probably by resisting the insulin action. The glycemic status of animals fed with Diet 1, 3, or 4 did not show significant differences at $p < 0.05$. These data indicate that *C. onubensis* powder has a protective effect, similar to that of atorvastatin, against hyperglycemic development in rats (Fig. 2A). C-reactive protein, common biomarker of inflammation, increase significantly in obese animals fed with diets 2, 3, or 4 (Fig 2B).

Effect of C. onubensis biomass or atorvastatin on serum lipid profile of rats fed with a high-fat diet

The circulating triglyceride levels in the serum did not change significantly in rats fed with Diets 1–3; however, atorvastatin had a significant antihypertriglyceridemic effect compared with both the control group (Diet 1) and the high-fat diet-fed animals (Diet 2) (Fig. 3A). Moreover, animals fed with Diet 2 showed a significant increase in the serum TC level compared with the control group, and both *C. onubensis* powder or atorvastatin considerably protected animals against this change by significantly decreasing the serum TC content, which reached 22.65% compared with Diet 2 animals (Fig 3B).

LDL-cholesterol level in the serum of animals fed with a fat-rich diet (Diet 2) was 7.7-fold higher than that of the control group, indicating that rats proceed with cholesterol mainly through LDL-cholesterol. The presence of *C. onubensis* powder (Diet 3) or atorvastatin (Diet 4) in the diet of rats did not protect against this tendency (Fig. 3C). No significant differences were found in the level of HDL-cholesterol of the four groups at the end of the experiment (Fig. 3D).

Effect of C. onubensis biomass or atorvastatin on the fatty acids profile of brain tissue from rats fed with a rich-fat diet

The analysis of the fatty acids profile in different brain tissues of the rats is shown in Table 2. A high-fat diet significantly increases the palmitic and arachidonic acids in the telencephalon and decreased the oleic acid in the mesencephalon compared with that of the control animals (Diet 1). These changes were reverted in rats when *C. onubensis* powder or atorvastatin was included in the diet. In addition, a high-fat diet significantly decreased the eicosapentaenoic acid (EPA) content in the brainstem and cerebellum and the docosahexaenoic acid (DHA) content in the spinal bulb, telencephalon, and mesencephalon of rats, which is harmful to quality and functionality of the membranes. These changes were not prevented by adding microalga powder or atorvastatin in the diet. Finally, the presence of microalga powder (Diet 3) showed a significant decrease of both palmitic and arachidonic acids in the mesencephalon and palmitic acid in the spinal bulb, which is beneficial for the quality of biological membranes.

DISCUSSION

Metabolic syndrome is related to some diseases including diabetes, atherosclerosis, and other CV risks in animals. These diseases can be induced in laboratory animals by feeding them with a high-fat diet (Dvir et al. 2009; Li et al. 2018; Mayer et al. 2019), a sugar-rich diet (Ryan et al.

2009; Kumar et al. 2015), or drugs-containing diet (Nasirian et al. 2019), resulting in hyperglycemic and hyperlipidemic effects. **Rats fed with a high-fat diet show a typical fatty liver (Fig. 1), however, insulin resistance and abdominal fat pad measurements are not available and will be resolved in future studies.** Feeding laboratory animals with hypercaloric diets led to increased anxiety, reduced locomotion (Kohsaka et al. 2007), and impaired learning and memory retention (Winocur and Greengood 2005; Freeman et al. 2014), as a consequence of the altered metabolic and nutritional factors. In several studies, microalgae powder (or extract)-supplemented diet prevents or ameliorates these problems (Kumar et al. 2015; Yang et al. 2017; Liu et al. 2017; Hua et al. 2018; Pan et al. 2018; Wan et al. 2019; Gómez-Zorita et al. 2020). Interestingly, ***Nannochloropsis*** sp. biomass-supplemented diet had several health benefits in diabetic rats (Nasirian et al. 2019) and humans (Werman et al. 2003). *Spirulina platensis* is generally considered safe for human consumption because of the long history of its use as a food source and safe profile in animal studies (Deng and Chow, 2010). However, no clear clinical studies in humans have been reported (Nazih and Bard 2018). In addition, a high-fat diet induces obesity in rats (Table 1) and inflammatory-related diseases, as shown by the high level of C-reactive protein found in the blood of animals (Fig. 2B). When *C. onubensis* was present in the high-fat diet of rats, no improve was observed in these parameters, which is consistent with the idea that functional foods may regulate the hyperglycemia and hyperlipidemia by acting on the corresponding metabolic pathway.

The protective effects of *C. onubensis* in rats were observed at 6.25% (w/w of dry weight), which is lower than or similar to other microalgae such as *Gelidium amansii* (5% w/w) (Liu et al. 2017), *Odontella aurita* (12% w/w) (Amine et al. 2019), and *Phaeodactylum tricornutum* (12% w/w) (Mayer et al. 2019).

Several approaches have been used to identify the bioactive compounds involved in the microalgae-dependent protective effects against the induced hyperglycemic and hyperlipidemic effects in rodents. Feeding animals with 95% ethanolic (Pan et al. 2018; Wan et al. 2019) or hot water (Yang et al. 2017) extracts was found to be an effective treatment. In addition, PUFAs from *S. platensis* have hypolipidemic effects and change the intestinal flora of animals by increasing the abundance of specific beneficial bacteria including *Prevotella* and short-chain fatty acids producers (Li et al. 2018). Moreover, they have antidiabetic activity (Wan et al. 2019). Fish oil and microalgae ω–3 fatty acids when added in the dietary supplements of rats have also shown a significant curative effect against the CV risks induced by saturated fatty acids-rich diets (Haimeur et al. 2016; Mayer et al. 2019). Furthermore, algal polysaccharides are therapeutic agents for atherosclerosis (Patil et al. 2018). In this context, *C.*

onubensis is a rich supplier of lutein, PUFAs, and antioxidant enzymes (Bermejo et al. 2018), which allows this microalga to serve alone, or by working synergistically with others, as a protecting agent against CV diseases (Kumar et al. 2015; Mayer et al. 2019; Wan et al. 2019).

The ω–3 and ω–6 fatty acids influence neurotransmission and prostaglandins formation, respectively, which are vital in the maintenance of the normal brain functioning in animals (Haag 2003). Thus, it is warranted to study the effect of diet on the fatty acids profile in the brain tissue of rats. Animals fed with a high-fat diet showed a significant decrease in the EPA content in the brainstem and cerebellum, whereas the DHA content decreases in the other tissues studied. Neither *C. onubensis* powder nor atorvastatin in the diet can reverse these tendencies, which could be associated with the cognitive and emotional dysfunctions observed in obese rats (Kanoski and Davidson 2011), as an adequate dietary intake of ω–3 PUFAs improves learning and behavioral tasks (Fedorova and Salem 2006) and increasing dietary ω–3 and monounsaturated fatty acids are associated with improved cognitive performance in humans (Spencer et al. 2017).

Both palmitic and arachidonic acid contents increased in the telencephalon of rats fed with a high-fat diet (Table 2), which may be associated with pro-inflammatory conditions. However, the addition of palmitic acid (in a dose-dependent manner) to the diet rapidly reduces mouse locomotor activity (Moon et al. 2014), which is also observed in animals with metabolic syndrome. The presence of microalgae powder in the diet decreases the palmitic acid content in both the mesencephalon and spinal cord tissues, thus resulting in significant protection of the animals against neuroinflammatory related diseases. The oleic acid level decreases in the mesencephalon of rats fed with a high-fat diet and as oleic acid protects the tissues against oxidative stress, the animals lose robustness against diseases (Valentini et al. 2018).

Microalgal food supplements are becoming increasingly popular because of their promising biological effects and prebiotics content of high nutritional value; however, some products of this type have raised controversies concerning their safety (Rzymski and Jaskiewicz 2017). This study, as well as a previous study (Navarro et al. 2016), indicated that the use of *C. onubensis* powder is safe and not deleterious for animals and present some intrinsic properties similar to the atorvastatin pharmacological treatment, which improves the conditions of rats with metabolic syndrome and also ameliorates the fatty acids profile of the animal's brain.

CONCLUSIONS

C. onubensis powder (6.25% in the diet) showed a protective effect against CV risks on rats fed with high-fat diet, which is probably due to the high level of nutraceuticals (lutein, ω–3 fatty acids, and antioxidant enzymes) present in the microalga biomass. Pharmacological treatment with atorvastatin (10 mg/kg of body weight per day) also exhibit this protective effect. Furthermore, the presence of microalgae powder in the diet decreases the palmitic acid content in both the mesencephalon and spinal cord tissues, thus resulting in significant protection of the animals against neuroinflammatory related diseases.

CONFLICT OF INTEREST AND FUNDING

The authors declare they have no conflict of interest.

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FIGURE LEGENDS

Fig. 1. **Macroscopic appearance of rat livers corresponding to Diet 1 (left) and Diet 2 (right).** Animals were fed for 108 days with the indicated diets and then sacrificed, and their liver was extracted as indicated in Materials and Methods.

Fig. 2. **Effect of *C. onubensis* biomass or atorvastatin on serum glucose level induced by a high-fat diet in rats.** Data shown are the average of individuals (n = 6) of each group of animals (±standard deviation [SD]). A. Shows the glucose level, and B. The C-reactive protein level. * indicates significant differences with respect to control rats (Diet 1) at $p < 0.05$. $ indicates significant differences with respect to rats fed with a high-fat diet (Diet 2) at $p < 0.05$. Other experimental details were as indicated in Materials and Methods.

Fig. 3. **Effect of *C. onubensis* biomass or atorvastatin on the serum lipid profile of rats.** Data show the average of individuals (n = 6) of each group of animals (±SD). (A) Triglyceride levels in the different groups of animals. (B) TC levels. (C) LDL-cholesterol levels. (D) HDL-cholesterol levels. * indicates significant differences with respect to control (Diet 1) at $p < 0.05$. $ indicates significant differences with respect to high-fat diet-fed animals (Diet 2) at $p < 0.05$. Other experimental details were as indicated in Materials and Methods.

Table 1. Physiological data for rats fed with standard Diet 1 (control) or fed with a high-fat diet fed animals (Diet 2), in the presence of *C. onubensis* (Diet 3) or atorvastatin (Diet 4) supplements.

Animals/Organs weight (g)	Diet 1	Diet 2	Diet 3	Diet 4
Initial experiments	151.9 ± 1.30	150.5 ± 7.5	153.7 ± 6.8	155.5 ± 8.9
Final of experiments	429.79 ± 17.13	458.74 ± 17.80	463.01 ± 16.80	433.73 ± 7.46
Livers	13.17 ± 0.91	25.18 ± 2.73[a]	26.41 ± 1.50[a]	22.65 ± 1.03[a]
Kidneys	1.39 ± 0.12	1.23 ± 0.12	1.45 ± 0.06	1.22 ± 0.11
Epididymis	1.81 ± 0.50	2.54 ± 0.40[a]	2.22 ± 0.86[a]	1.86 ± 0.23[a]
Spleens	0.74 ± 0.06	1.00 ± 0.15[a]	1.03 ± 0.09[a]	0.93 ± 0.08[a]
Hearts	1.19 ± 0.06	1.27 ± 0.07	1.27 ± 0.07	1.17 ± 0.09
Brains	2.04 ± 0.09	2.11 ± 0.05	2.03 ± 0.08	2.07 ± 0.06

Rats were fed with the indicated diets during 108 days, then the animals were sacrificed and the organs studied. Each value is expressed as the average ± SD (n=6) in all groups. Results were statistically analyzed with Kruskal-Wallis test. [a]Significant differences were observed (at $p < 0.05$) for the liver, spleens and epididymis in the different groups with respect to control group (Diet 1).

Table 2. Fatty acids profile in different fractions of the brain as a result of different diets

Brain fraction	Palmitic A	Oleic A	AA	E
Cerebellum				
Diet 1	18.58 ± 0.36	17.39 ± 0.54	8.24 ± 0.35	3.52
Diet 2	18.02 ± 1.28	17.75 ± 1.51	8.30 ± 0.50	1.94 ±
Diet 3	18.13 ± 1.00	17.69 ± 1.41	7.91 ± 0.42	2.15 ±
Diet 4	19.00 ±1.38	18.21 ± 0.80	8.27 ± 0.46	2.28 ±
Telencephalon				
Diet 1	20.19 ± 0.92	21.14 ± 0.53	11.43 ± 0.18	3.38
Diet 2	21.80 ± 0.16[a]↑	21.26 ± 0.24	12.66 ± 0.05[a]↑	3.44
Diet 3	20.84 ± 0.06[b]	21.07 ± 0.31	11.41 ± 0.11[b]	3.49
Diet 4	20.35 ± 0.12[b]	20.66 ± 1.17	11.36 ± 0.54[b]	4.18
Mesencephalon				
Diet 1	18.49 ± 0.40	21.30 ± 0.27	10.83 ± 0.10	3.29
Diet 2	18.13 ± 0.37	19.77 ± 0.63[a]↓	10.31 ± 0.42	3.43
Diet 3	16.95 ± 0.79[a]↓	20.40 ± 0.32[b]	9.37 ± 0.22[a]↓	3.33
Diet 4	17.86 ± 0.51	20.93 ± 0.13[b]	10.54 ± 0.15	3.52
Medulla				
Diet 1	14.31 ± 0.32	19.61 ± 0.27	7.89 ± 0.68	3.31
Diet 2	14.83 ± 0.26	19.21 ± 0.18	7.48 ± 0.16	3.28
Diet 3	13.61 ± 0.05[a]↓	19.82 ± 0.05	6.92 ± 0.10	3.16
Diet 4	13.53 ± 0.40[a]↓	18.49 ± 0.47[a]↓	6.83 ± 0.42	3.37

AA: Arachidonic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid. Units: Percent of dry we
respect to the control (Diet 1) at $p<0.05$. (b) Significantly different with respect to Diet 2 at $p<0.05$. Arrows hig
decrease.

Table 2 Fatty acids profile in different fractions of the brain as a result of different diets

Brain fraction	Palmitic A	Oleic A	AA	EPA	DHA
Cerebellum					
Diet 1	18.58 ± 0.36	17.39 ± 0.54	8.24 ± 0.35	3.52 ± 0.53	14.54 ± 1.30
Diet 2	18.02 ± 1.28	17.75 ± 1.51	8.30 ± 0.50	1.94 ± 0.24$^{a}\downarrow$	13.54 ± 1.85
Diet 3	18.13 ± 1.00	17.69 ± 1.41	7.91 ± 0.42	2.15 ± 0.15$^{a}\downarrow$	14.91 ± 1.80
Diet 4	19.00 ± 1.38	18.21 ± 0.80	8.27 ± 0.46	2.28 ± 0.48$^{a}\downarrow$	13.81 ± 1.29
Telencephalon					
Diet 1	20.19 ± 0.92	21.14 ± 0.53	11.43 ± 0.18	3.38 ± 0.01	16.08 ± 1.19
Diet 2	21.80 ± 0.16$^{a}\uparrow$	21.26 ± 0.24	12.66 ± 0.05$^{a}\uparrow$	3.44 ± 0.39	13.66 ± 0.55$^{a}\downarrow$
Diet 3	20.84 ± 0.06^{b}	21.07 ± 0.31	11.41 ± 0.11^{b}	3.49 ± 0.28	14.55 ± 0.18$^{a}\downarrow$
Diet 4	20.35 ± 0.12^{b}	20.66 ± 1.17	11.36 ± 0.54^{b}	4.18 ± 0.57	14.49 ± 0.61$^{a}\downarrow$
Mesencephalon					
Diet 1	18.49 ± 0.40	21.30 ± 0.27	10.83 ± 0.10	3.29 ± 0.03	13.89 ± 1.02
Diet 2	18.13 ± 0.37	19.77 ± 0.63$^{a}\downarrow$	10.31 ± 0.42	3.43 ± 0.14	12.59 ± 0.77$^{a}\downarrow$
Diet 3	16.95 ± 0.79$^{a}\downarrow$	20.40 ± 0.32^{b}	9.37 ± 0.22$^{a}\downarrow$	3.33 ± 0.56	11.99 ± 0.54$^{a}\downarrow$
Diet 4	17.86 ± 0.51	20.93 ± 0.13^{b}	10.54 ± 0.15	3.52 ± 0.25	11.83 ± 1.61$^{a}\downarrow$
Medulla					
Diet 1	14.31 ± 0.32	19.61 ± 0.27	7.89 ± 0.68	3.31 ± 0.10	9.31 ± 0.42
Diet 2	14.83 ± 0.26	19.21 ± 0.18	7.48 ± 0.16	3.28 ± 0.36	7.77 ± 0.86$^{a}\downarrow$
Diet 3	13.61 ± 0.05$^{a}\downarrow$	19.82 ± 0.05	6.92 ± 0.10	3.16 ± 0.32	7.79 ± 0.32$^{a}\downarrow$
Diet 4	13.53 ± 0.40$^{a}\downarrow$	18.49 ± 0.47$^{a}\downarrow$	6.83 ± 0.42	3.37 ± 0.05	7.23 ± 0.18$^{a}\downarrow$

AA arachidonic acid, EPA eicosapentaenoic acid, DHA docosahexaenoic acid. Units, percent of dry weight. aSignificantly different with respect to the control (diet 1) at $p < 0.05$. bSignificantly different with respect to diet 2 at $p < 0.05$. Arrows highlight a significant increase or decrease

Figure 1

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Fig. 1 Macroscopic appearance of the rat liver of Diet 1 (left) and Diet2 (right).

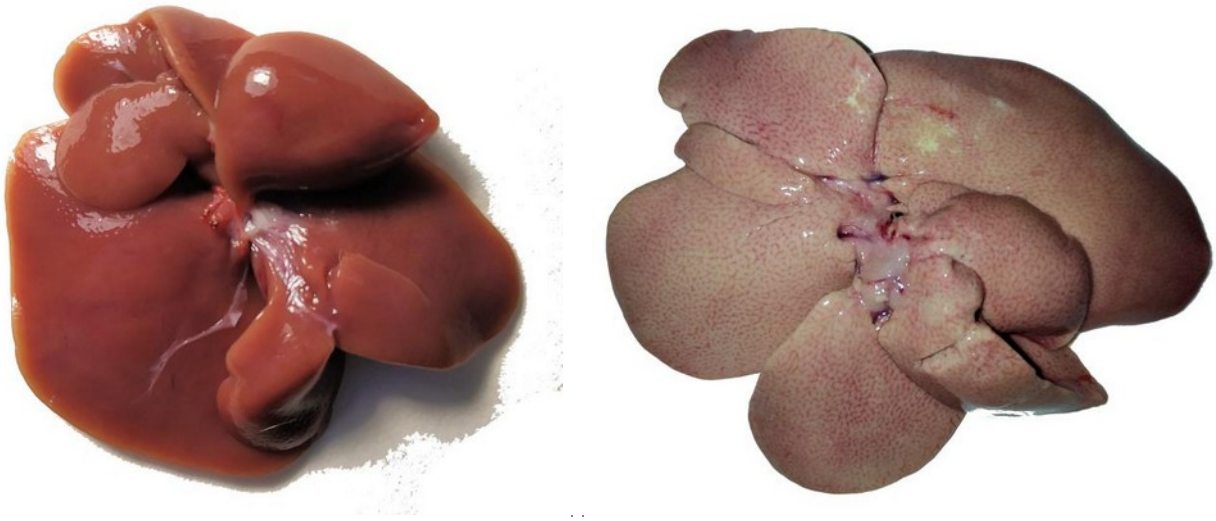

Figure 2 Modif

Figure 2

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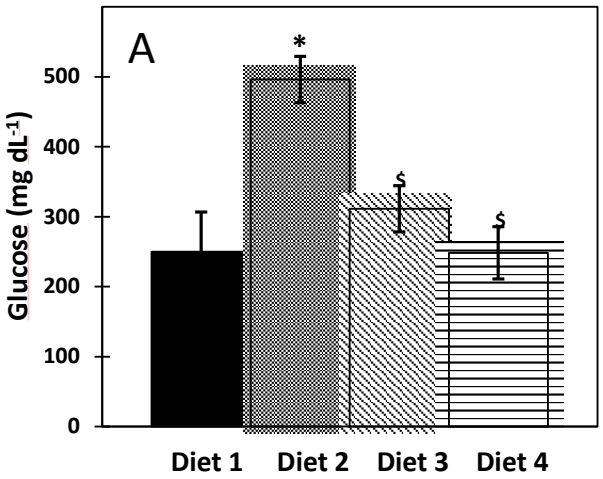

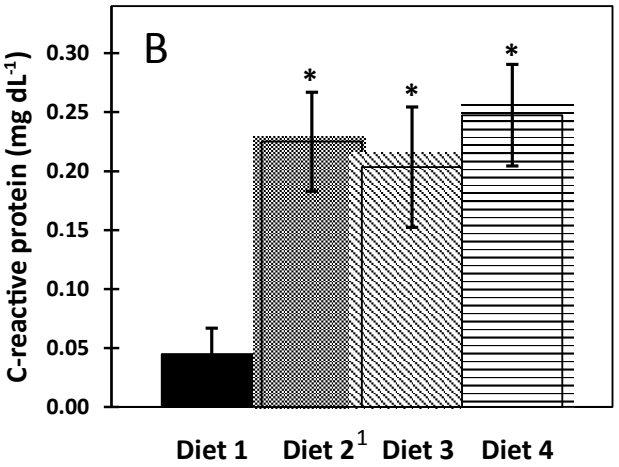

Figure 3.