Journal of Cleaner Production 374 (2022) 133978

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

Towards sustainable aquaculture systems: Biological and environmental impact of replacing fishmeal with *Arthrospira platensis* (Nordstedt) (spirulina)

Gaetana Napolitano [a,*], Paola Venditti [b,d], Claudio Agnisola [b], Saverio Quartucci [c], Gianluca Fasciolo [b], Maria Teresa Muscari Tomajoli [a], Eugenio Geremia [a], Claudio Marcello Catone [a], Sergio Ulgiati [a,e]

[a] *Dipartimento di Scienze e Tecnologie, Università Degli Studi di Napoli Parthenope, via Acton n, 38-I, 80133, Napoli, Italy*
[b] *Dipartimento di Biologia, Università di Napoli Federico II, Complesso Universitario Monte Sant'Angelo, Via Cinthia, I-80126, Napoli, Italy*
[c] *ATI Biotech, Centro Ricerche di Castel Baronia, SP 235 s.n, C.da Piani, Area PIP, 83040, Castel Baronia, Avellino, Italy*
[d] *BAT Center - Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, Portici (NA), Italy*
[e] *School of Environment, Beijing Normal University, Beijing, PR China*

ARTICLE INFO

Handling Editor: Cecilia Maria Villas Bôas de Almeida

Keywords:
Oxidative stress
Condition factor
Aquaculture
Life cycle assessment
Circular economy
Sustainability

ABSTRACT

Sustainable fish food production is crucial for aquaculture. Microalgae, such as spirulina (*Arthrospira platensis*), can supplement diet antioxidants or replace expensive fishmeal with high-quality proteins. In this study, we tested fish growth and wellbeing by feeding fish on a diet in which 5% of fishmeal was replaced by spirulina (SP5 diet). The low level of spirulina in the diet was intended as supplementation and was effective in ameliorating the redox state of a model fish species (juvenile Koi Carp, *Cyprinus carpio* L.) in a preliminary lab protocol in a six-week trial. When compared with both the control diet (no Spirulina) and a diet containing 30% spirulina replacing fishmeal (SP30 diet), SP5 was able to reduce the muscle levels of reactive oxygen species (ROS), oxidative damage, and susceptibility to oxidative stress, while increasing glutathione reductase and peroxidase activity. However, high production costs and impacts still limit the use of spirulina in fish diet. Recent studies focused on growing spirulina on urban or agro-industrial wastewater, with appropriate profiles for the alga growth. Therefore, in a circular economy context, a possibility still to be tested and exploited is feeding farmed fish with spirulina produced on output wastewater recirculated back from the same farming plant. Life Cycle Assessment (LCA) was applied to estimate the sustainability of such "circular" fish farming. The LCA ReCiPe Midpoint (H) impact assessment method was used. Firstly, the LCA environmental impacts associated with the production of spirulina grown on aquaculture wastewater as well as on the standard culture medium (Zarrouk medium) were assessed and compared by means of a "gate to gate" analysis. Then, the LCA impacts of an SP5 diet for fish, in which spirulina grown on aquaculture wastewater was used to replace 5% fishmeal ($SP5_{ww}$), were compared to the diet containing spirulina grown on a standard medium ($SP5_{st}$) and that one without spirulina (control diet). Results indicated that $SP5_{ww}$ was significantly less impacting, by avoiding the treatment and disposal of wastewater and the need for the highly impacting standard culture medium. In conclusion, the proposed approach for using spirulina in aquaculture represents a valid solution for aquaculture circular economy scenario while at the same time improving fish welfare.

1. Introduction

The demand for greater availability of food by a growing population and an inefficient redistribution has led to a strong increase in fish production thanks to the development of intensive farming and aquaculture systems (FAO, 2011; Klinger and Naylor, 2012; Sprague et al., 2016). Intensive aquaculture systems satisfy the increased demand from the growing population (FAO, 2020). However, the production practices make the intensive aquaculture unsustainable because of the necessity to preserve the welfare of farmed fish (Martos-Sitcha

* Corresponding author.
E-mail address: gaetana.napolitano@uniparthenope.it (G. Napolitano).

https://doi.org/10.1016/j.jclepro.2022.133978
Received 20 May 2022; Received in revised form 28 August 2022; Accepted 31 August 2022
Available online 6 September 2022

et al., 2020) and contain the local environmental impacts (Klinger and Naylor, 2012; Osmundsen et al., 2017). Moreover, fish feed diet production is environmentally impacting (Ytrestøyl et al., 2015) because of the use of fishmeal (Oliva-Teles et al., 2015) used for many fish feed diets for its nutritional profile (NRC, 2011). Fishmeal undergoes also periodic fluctuations in availability and pricing (Gasco et al., 2018). Several studies are focusing on fishmeal replacement using alternative sources of high-quality proteins, including microalgae (Christaki et al., 2011). Many characteristics, such as high nutritional value and growth rate, antioxidant properties, and capacity to reinforce the animals against diseases, make microalgae optimal for aquaculture feed (Roy and Ruma, 2015). Their high nutritional value is linked to the protein content that exceeds 70% of total mass, the fatty acid profile showing a high content of g-linoleic acid, as well as high levels of vitamins, minerals, and bioactive compounds such as biliproteins, chlorophylls, and carotenoids) able to protect the organisms from environmental stressors and pathogens in aquaculture systems (Ravi et al., 2010). Algal biomass supplementation may enhance mineral and vitamin content in feeds for aquaculture species (Shields, 2012) and increase the growth performance (Dernekbasi et al., 2010).

The microalga spirulina is produced for several commercial sectors (Priyadarshani and Rath, 2012). Recent studies highlighted the suitability of spirulina as a potential nutritional supplement or substitute for fishmeal because of its nutritional properties. It has been proposed that the biochemical and nutritional properties of spirulina make it a functional feed which can improve organisms' health and stressors resistance, even if administered in small amounts. Spirulina seems to improve the immune system (Watanuki et al., 2006; Ibrahem and Ibrahim, 2014), the resistance to bacteria (Adel et al., 2016) and the immune memory (Ragap et al., 2012). Spirulina can improve the antioxidant capacities of the diet (Kim et al., 2013), reduce the oxidative damage of lipids (Teimouri et al., 2015), and improve the quality of fish muscle to the benefit of human consumers health (Teimouri et al., 2015). Intensive aquaculture conditions are associated with overproducing reactive oxygen species (ROS), reactive nitrogen species and nitric oxide in farmed species (Seo et al., 2020). ROS are highly reactive molecules deriving from the incomplete reduction of molecular oxygen in the cell (Venditti et al., 2015) and can promote the oxidation of biomolecules acting as prooxidants (Lushchak, 2011). The cells are equipped with an efficient antioxidant defences system comprising enzymes and non-enzymatic compounds able to neutralize the prooxidant effect of ROS and maintain the redox balance (Napolitano et al., 2021a). When antioxidant molecules are not able to face ROS production, a condition of oxidative stress associated with oxidative damage (Lushchak, 2011) onsets. As a consequence, the incidence of disease and death increases (Iwama et al., 2011). Despite the increased interest in using spirulina for aquaculture feeding, its high production cost still limits its use in many applications. The standard medium used for the growth of spirulina, the Zarrouk's medium (Rajasekaran et al., 2015), requires expensive and impacting components while offering optimal biomass production. Recent studies focused on industrial processing wastes and by-products with appropriate nutrient profiles for the growth of spirulina (Ragaza et al., 2020). The utilization of hydrolysate of protein fish derived from fish waste has been proposed as an improvement in the spirulina production sustainability (Shanthi et al., 2021).

One possibility still to be exploited is the spirulina production using aquaculture wastewater as growth medium. The use of this medium could be a valid strategy to ameliorate the sustainability of aquaculture system for two reasons. First, it could highlight the potential role of the alga as a bioremediation agent for aquaculture wastewater, thus avoiding the costs due the treatment and disposal of wastewater. Secondly, the algal biomass obtained by wastewater could be used to replace partially or totally fishmeal in the fish diet in the view of a circular economy. In this study we evaluated the sustainability of a spirulina (*Arthrospira platensis*) culture based on fish farm wastewater with partially recirculating spirulina for feeding fish. Preliminarily, we tested the efficacy of spirulina fishmeal replacement in terms of improved antioxidant capacity of feed for farmed fish, using juvenile Koi Carp (*Cyprinus carpio* L.) as a model species. We tested two levels of spirulina (by replacing 5% of fishmeal, the SP5 diet, or 30% of fishmeal, the SP30 diet). This choice takes into account that diets containing percentages of *A. platensis* between 25% and 100% have no positive effects on the growth performance of fish (Nandeesha et al., 1998), while low levels (1%–3% in diets) improve performance and growth parameters for juvenile common carp (Ramakrishnan et al., 2008). Thus, spirulina has a supplementing role in SP5 and a fishmeal proteins replacement role in SP30. We evaluated the effects of six weeks administration of the two diets on the levels of oxidative damage to lipids and proteins, ROS content, the *in vitro* susceptibility to oxidants, and the activities of antioxidant enzymes (glutathione peroxidase and glutathione reductase). Growth and condition factor (CF) were also determined. Successively, we used the Life Cycle Assessment (LCA) approach to compare the environmental impacts associated to the production of spirulina grown on standard medium and aquaculture wastewater, and its use for fishmeal replacement in the feed diet at the most effective level resulting from the preliminary tests. LCA assesses the environmental impacts associated with a product, process, or service throughout its life-cycle by inventorying material resources, energy inputs and environmental issues, through a cradle to grave approach (Cleveland and Ayres, 2004). The results obtained in this research allow us to suggest a production model that is more environmentally friendly and sustainable, while also considering the putative beneficial effect of spirulina on fish health status.

2. Materials and methods

2.1. The investigated systems

Conventional fish farming uses commercial feed to support growth and marine or aqueduct water, depending on the farmed fish (Fig. 1-a).

Introducing Spirulina into the diet requires adding a new process to the system, namely the algal production as a diet component. Algal growth may be based on an industrial culture medium (Fig. 1-b) or the circular feedback of fish farming wastewater, thus saving on the industrial culture medium and at least partially treating the wastewater before discharge (Fig. 1-c), according to the rich literature on algal uses for water treatment (Colella et al., 2021; Geremia et al., 2021; Catone et al., 2021). In Fig. 1-c the outflowing wastewater is no longer a polluting flow to be treated but a co-product flow of relatively clean water no longer needing specific treatment. This co-product is generally named "avoided product," i.e., saving on the wastewater treatment process due to its alternative use as the source of nutrients for algae. In so doing, the algae act in the wastewater treatment process while being grown to integrate into the fish diet.

The three options are assessed in this study and compared by means of Life Cycle Assessment method. Based on the personal experience of some of the Authors (Quartucci, 2022; Geremia, E., 2019), water evaporation is approximately 4.3% in summer, 1.4% in Spring and Fall, and generally zero in Winter, when most farms are not operative. Therefore, evaporation has been disregarded in this LCA study, being below the usual cut-off rate.

2.2. Spirulina-containing diets and fish redox state trial

2.2.1. Animals and diet administration

The diet administration has been conducted in an authorized laboratory at the start-up ATI Biotech s.r.l. (Castel Baronia, AV, Italy, www.atibiotech.com). Twenty-four juveniles of Koi carp purchased from Carmar s.r.l. (San Giorgio a Cremano, NA) were used for the experimental plan. The animals were divided into three groups, and each experimental group was kept in tanks of 95 L filled with freshwater and equipped with a mechanical-biological filtering system, a lighting

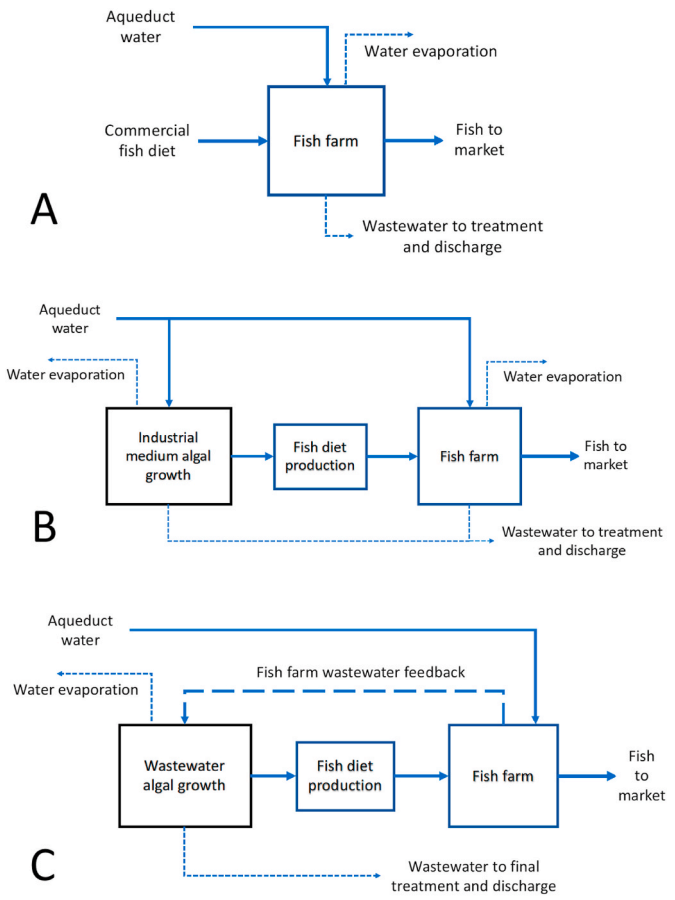

Fig. 1. A. Conventional fish farming, based on commercial diets and wastewater treatment and discharge. B. Spirulina production based on industrial medium and its use as integrator of fish diets. C. Circular wastewater treatment via algal production, fish diet integration and fish farming. Fish farm wastewater is feedback to algal production, partially cleaned by algal growth and then submitted to final treatment. Harvested algae are transferred to fish diet production sector, to integrate other components, and then supplied to the fish farm.

system (photoperiod: 12:12h day-night) and a heating system (25 °C). The supply of food was guaranteed by an automatic feeder (Grässlin Rondomatic 400). Cleaning of tanks and filters, checking of animals' health, and feeders loading were performed once a week. The quality of the water was assessed weekly by measuring water nitrate and nitrite content, hardness and pH (JBL pro aquatest kit). During the first week of adaptation, all animals were administered control diet. The control diet was prepared according to Steffens et al. (1995) with the following ingredients (Table 1): fishmeal, produced from *Clupea harengus* (ElleVi), type 0 wheat flour (Lotus Flower), dry yeast (Sophie), corn germ oil (Crudigno), vitamins and minerals supplement (Sera Fishtamin). After the adaptation period, the animals of the three tanks received different diets for six weeks: control diet (C), spirulina 5% (SP5) in which 5% of fishmeal was replaced with the alga, and spirulina 30% (SP30) in which the percentage of fishmeal replacement was of 30%. The diets were isoproteic and isocaloric (Table 1). Animals received a daily amount of food corresponding to 5% of body weight. All along the treatment period and once a week, animals were individually weighed and photographed for recognition by livery characterization and the subsequent measurement of length and hight through image analysis (ImageJ).

The treatment of animals in these experiments was in accordance with the guidelines set forth by the University's Animal Care Review Committee (Approval number 47339-2013).

Table 1
Formulation and composition of experimental diets.

	Diets		
	C	SP5	SP30
Ingredients (per kg of pelletted feed)			
Fish meal (g)	280	266	196
Wheat flour (g)	500	500	500
Dry yeast (g)	150	150	150
Corn germ oil (ml)	92	92	92
Spirulina (g)	0	14	84
Water (ml)	500	500	500
Vitamins/minerals (ml)	63	63	63
Nutrient composition (g kg^{-1} dry matter)			
Carbohydrates	450	450	470
Lipids	130	130	120
Proteins	260	270	270
Gross energy (kcal $100g^{-1}$)	390	392	397

2.2.2. Spirulina growth conditions

Arthrospira platensis (Nordstedt) was produced at ATI Biotech on modified Zarrouk medium (Rajasekaran et al., 2015), pH 10 at 24 ± 0.5 °C, under trichromatic light (blue, red, and orange LEDs 320 μmol photons m^{-2} s^{-1}) and flushed with air. Bicarbonate ($NaHCO_3$ 17 g L^{-1}) was used as inorganic carbon source. The algal growth was monitored daily by measuring the optical density (OD) at 500 nm. The cells harvested at late exponential phase (OD_{500}: 1.2, density 1.5 g L^{-1}), were separated mechanically from the culture medium and the cellular pellet was dried at 40 °C in a hot air oven for about 10 h until getting a constant weight. Subsequently, the dried cells were used as the replacement of fishmeal for Koi Carp feed.

2.2.3. Fish condition factor (CF)

Animal weight and standard length were measured to assess fish growth performance. Care was taken to minimize the manipulation stress of the animals during measurement. Body weight (W), expressed in grams, g) was measured by keeping each animal in a Becher containing a pre-weighted volume of water from maintenance tanks. The standard length (LS) (expressed in centimetres, cm) was determined using the program ImageJ on a photo (4624 × 3472, 118 pixels/cm) of the animal in a morphometry chamber. The biometric parameters were used for evaluation of the Condition Factor (CF). This one furnishes information about the health of the animals and is calculated following the Fulton equation (Eq. (1)) (Ujjania et al., 2013).

$$CF = \frac{100^{*}W}{LS^{3}} \quad (1)$$

2.2.4. Preparation of muscle tissue homogenates

At the end of treatments, the animals were euthanized by immersion in MS222 solution of 200 mg/L, muscle tissues were dissected, quickly frozen in liquid nitrogen, and stored at −80 °C after deprivation of connective tissue. Frozen tissues were successively homogenized (1:5 w/v) by using a glass Potter–Elvehjem homogenizer set at 500 rpm for 1 min in an ice-cold buffer solution (HM) containing 220 mM mannitol, 70 mM sucrose, 1 mM EDTA, 0.1% fatty acid-free albumin (BSA), 10 mM Tris, pH 7.4. Aliquots of homogenate were used for the determination of oxidative damage indexes (lipid hydroperoxide and protein-bound carbonyl, Hps and CO respectively), *in vitro* susceptibility to oxidants, ROS content and antioxidants enzyme activities (Glutathione peroxidase and Glutathione reductase, GPx and GR respectively).

2.2.5. Oxidative damage and in vitro susceptibility to oxidants

Lipid peroxidation was determined by measuring the muscle level of Hps (Venditti et al., 2014, 2016). The rate of NADPH oxidation, performed by the coupling of GPx and GR activities in the presence of 0.01 mg of tissue proteins, was followed at 340 nm using a multi-mode

microplate reader (Synergy™ HTX Multi-Mode Microplate Reader, BioTek).

Protein oxidation measured on 60 mg of tissue homogenates was assayed by measuring the absorbance at 355 nm of the hydrazones. These last represent the products of the reaction between 2,4-dinitrophenylhydrazine and tissue protein carbonyls (Venditti et al., 2014, 2016). The absorbance was measured using a DU 800 Spectrophotometer (Beckman Coulter).

The *in vitro* susceptibility to oxidants was evaluated by stressing 0.01 mg of tissue proteins with 100/1000 μM iron/ascorbate (Fe/As) for 10 min at room temperature. The change in the hydroperoxide levels after stopping the reaction by adding 0.2% 2,6-di-t-butyl-p-cresol (BHT) furnishes information on the tissue capacity to face an oxidative insult. The hydroperoxide levels were measured as previously described by using a multi-mode microplate reader (Synergy™ HTX Multi-Mode Microplate Reader, BioTek).

2.2.6. Tissue ROS content

The content of ROS was measured following the fluorescence of dichlorofluorescein after ROS-induced conversion of 2′,7′-dichlorodihydrofluorescin diacetate (non-fluorescent compound) in dichlorofluorescein (fluorescent compound) according to Driver et al. (2000) and modified by Napolitano et al. (2022). The fluorescence was measured using a multi-mode microplate reader (Synergy™ HTX Multi-Mode Microplate Reader, BioTek) with excitation and emission wave at 485 and 530 nm, respectively.

2.2.7. GPx and GR activities

GPx activity of 0.01 mg tissue homogenate proteins was assayed at 30 °C following the rate of NADPH oxidation in the presence of H2O2, GSH, and GR according to Flohé and Günzler (1984).

GR homogenate activity of 0.01 mg tissue homogenate proteins was assayed at 30 °C, measuring the rate of NADPH oxidation in the presence of GSSG (Carlberg and Mannervik, 1975).

For each procedure, the rate of NADPH oxidation was followed at 340 nm using a multi-mode microplate reader (Synergy™ HTX Multi-Mode Microplate Reader, BioTek).

2.2.8. Data analysis

The data expressed as the means ± standard error of eight different animals for each group were analyzed by the one-way or two-way analysis of variance methods ($p < 0.05$), followed by Tuckey or Dunnett's post-hoc test, as needed (Prism 9, GraphPad Software, LLC).

2.3. Life Cycle Assessment (LCA) methodology

The LCA used in this study relies on the ISO 14040 methodology (ISO, 2006). Analyses were conducted by using the SimaPro 9 LCA software (PRé Sustainability B.V., Amersfoort, The Netherlands) and the ReCiPe Midpoint (H) impact assessment method (Prè, 2016). ReCiPe 2016 is an improvement on ReCiPe 2008, and its predecessors CML 2000 and Eco-indicator 99. The method is updated regularly, to incorporate new data and new research (https://pre-sustainability.com/articles/recipe/). We used the LCA approach in two steps. First, we aimed to identify, quantify, and evaluate the environmental impacts associated with the production of spirulina grown on aquaculture wastewater compared to the industrial culture medium. The functional unit for incoming and outgoing flows was 1 kg of dry spirulina. We evaluated the environmental impacts by implementing a "gate to gate" analysis, i.e. limited to spirulina production only (Jiménez-González et al., 2000). This analysis allowed us to estimate the quantity of spirulina that can be produced on aquaculture wastewater. We considered only the SP5 diet (5% of fishmeal was replaced with the alga), which significantly improved the redox state of fish in the Lab trial (see Results). In the second step, LCA analysis was performed to assess the environmental impacts associated with the production of control and two SP5 diets. The first SP5 diet is produced using spirulina grown on the industrial medium (SP5ind), the other using spirulina grown on wastewater aquaculture (SP5ww). The functional unit adopted in this second step is 1 kg of feed produced.

Both LCA steps relied on three types of data: 1) *Primary data,* collected directly in the field in collaboration with the operators of the various process units; 2) *Secondary data,* derived from literature or existing databases that are representative of the reality of the sector; 3) *Tertiary data*, derived from assumptions and calculations or other sources.

2.3.1. Inventory analysis for spirulina production

Table 2 shows the inventory for spirulina production on standard medium as implemented at ATI Biotech. In this Company, spirulina is grown as described above, and the production cycle takes place in 220 days. The concentration of spirulina biomass produced is 1.5 g/L. Specifically, spirulina suspension was filtered by a vibrating screen (20 m^3 per day). After separation, the water and the biomass that was not retained by the vibrating screen are returned to the cultivation tank, whereby there is a recirculation of water. By means of this procedure, 30 kg of wet microalgae biomass are effectively extracted per day. Subsequently, other phases such as pressing, extrusion, drawing and drying, reduce this quantity of biomass to 3 kg of dry weight per day, with an annual production of 660 kg.

Table 3 shows the inventory for spirulina production on aquaculture wastewater. The amount of aquaculture wastewater produced in 220 days (i.e., one whole working year) was estimated based on the daily value in a recirculating aquaculture system (RAS) published by Calone et al. (2019). The water discharge for this system is reported to be 460 L Day^{-1}, so that the volume of wastewater obtained in 220 days is equal to

Table 2
Main inputs and outputs for dry spirulina production on standard medium (ATI Biotech).

	Unit	Amount	Type of data	Source
Input				
Tap Water	L/yr	3.43×10^5	Primary	ATI Biotech
$NaHCO_3$	kg/yr	4.49×10^3	Primary	ATI Biotech
$NaNO_3$	kg/yr	6.37×10^2	Primary	ATI Biotech
K_2SO_4	kg/yr	9.64×10^1	Primary	ATI Biotech
NaCl	kg/yr	6.90×10^1	Primary	ATI Biotech
K_2HPO_4	kg/yr	4.77×10^1	Primary	ATI Biotech
$MgSO_4$	kg/yr	2.72×10^1	Primary	ATI Biotech
EDTA	kg/yr	2.10×10^0	Primary	ATI Biotech
Polyethylene terephthalate	kg/yr	3.89×10^1	Primary	ATI Biotech
Polyvinyl chloride	kg/yr	6.16×10^1	Primary	ATI Biotech
Polystyrene	kg/yr	2.92×10^1	Primary	ATI Biotech
Steel, low alloyed	kg/yr	5.00×10^0	Primary	ATI Biotech
Textile, woven cotton	kg/yr	2.00×10^{-3}	Primary	ATI Biotech
Electricity, local photovoltaic	kWh/yr	6.60×10^3	Primary	ATI Biotech
Electricity, low voltage from grid	kWh/yr	7.90×10^3	Primary	ATI Biotech
Heat, heat production, wood pallet	MJ	3.39×10^5	Primary	ATI Biotech
Output *(products and co-products)*				
Dry spirulina	kg/yr	6.60×10^2		

Table 3
Main inputs and outputs for dry spirulina production on aquaculture wastewater.

	Unit	Amount	Type of data	Source
Input				
Aquaculture wastewater	L/yr	1.01×10^5	Secondary	Calone et al. (2019)
Polyethylene terephthalate	kg/yr	3.89×10^1	Primary	ATI Biotech
Polyvinyl chloride	kg/yr	6.16×10^1	Primary	ATI Biotech
Polystyrene	kg/yr	2.92×10^1	Primary	ATI Biotech
Steel, low alloyed	kg/yr	5.00×10^0	Primary	ATI Biotech
Textile, woven cotton	kg/yr	2.00×10^{-3}	Primary	ATI Biotech
Electricity from grid, low voltage	kWh/yr	3.08×10^3	Primary	ATI Biotech
Output: products and co-products				
Dry spirulina	kg/yr	1.50×10^2	Tertiary	
Algae treated cleaned wastewater	L/yr	1.01×10^5	Secondary	Calone et al. (2019)

101,200 L. This quantity was entered as an input to the system, without attributing any impact value to it (zero burden approach, Santagata et al., 2017), as its use for spirulina growth avoids the impact of wastewater treatment and disposal. In fact, the algal growth acts as a wastewater treatment process and yields a relatively clean water as coproduct of the algae outflow. The amount of spirulina obtainable from cultivation on the aquaculture wastewater produced in 220 days was estimated from a spirulina biomass production of 0.34 g L^{-1} as reported by Cardoso et al. (2021). From 20 m^3 of wastewater that passes through the vibrating screen every day, 6.8 kg of wet spirulina can be obtained. This corresponds to 0.68 kg of dry spirulina per day and an annual production of 149.6 kg.

2.3.2. Inventory of control and SP5 diets

The inventory related to the production of diets was based on the Koi carp diet formulation (Steffens et al., 1995) that we used as a control diet in the fish redox state trial. Two experimental diets were considered in which one of two types of spirulina (one produced on standard medium, $SP5_{st}$, and the other on aquaculture wastewater, $SP5_{WW}$) replaced 5% of fishmeal. The inventory considers all the inputs (such as electricity and feed) necessary for the growth of the organisms, putting together primary, secondary, and tertiary data, depending on their availability. One kg of feed was chosen as the functional unit. Since corn germ oil is not included in the EcoInvent database, we replaced it with rapeseed oil, characterized by similar properties. Furthermore, unlike the control diet, in the SP5 diets we used fishmeal derived from process waste instead of from fish stocks. This approach can further reduce environmental impacts and counteract the depletion of fish stocks from a circular economy perspective. The inputs and outputs to produce the diets are reported in Table 3 (control diet) and Tables 4–5 (SP5 diets).

2.3.3. Environmental impacts assessment

For the LCA Impact Analysis we applied the ReCiPe Midpoint (H) method (Prè, 2016). This method can convert the results of the life cycle inventory into a sufficiently large number of performance indicators, each one expressing the influence of the process on a specific impact category. The classes of indicators included in the ReCiPe method are divided into 18 midpoint indicators and 3 endpoint indicators. We decided to use the class of midpoint indicators that offers more precise and detailed results in each specific impact category. Table 6 shows the selected categories and their relative acronyms and units of measure.

Table 4
Main inputs and outputs for preparation of control feed.

	Unit	Amount	Type of data	Source
Input				
Rape oil, crude	kg/yr	9.20×10^{-2}	Secondary	Steffens et al. (1995)
Protein feed, 100% crude	kg/yr	1.50×10^{-1}	Secondary	Steffens et al. (1995)
Fishmeal, 63–65% from residues	kg/yr	2.80×10^{-1}	Secondary	Steffens et al. (1995)
Tap water	L/yr	5.00×10^{-1}	Secondary	Steffens et al. (1995)
Wheat grain, organic	kg/yr	6.33×10^{-1}	Secondary	Steffens et al. (1995)
$Na_4P_2O_7$	kg/yr	4.95×10^{-3}	Secondary	Steffens et al. (1995)
Co	kg/yr	7.00×10^{-8}	Secondary	Steffens et al. (1995)
NaCl	kg/yr	1.50×10^{-3}	Secondary	Steffens et al. (1995)
$MgSO_4$	kg/yr	1.70×10^{-4}	Secondary	Steffens et al. (1995)
$ZnSO_4$	kg/yr	1.97×10^{-5}	Secondary	Steffens et al. (1995)
CaCO	kg/yr	3.97×10^{-3}	Secondary	Steffens et al. (1995)
Electricity from grid, low voltage	kWh/yr	1.27×10^{-1}	Secondary	Steffens et al. (1995)
Output: products and co-products				
Control Feed	kg	1.00×10^0		

Table 5
Main inputs and outputs for preparation of SP5 feed containing spirulina grown on standard medium ($SP5_{st}$) (Source: ATI Biotech) or on aquaculture wastewater ($SP5_{ww}$).

	Unit	Amount	Type of data	Source
Input				
Rape oil, crude	kg/yr	9.20×10^{-02}	Secondary	Steffens et al. (1995)
Protein feed, 100% crude	kg/yr	1.50×10^{-01}	Secondary	Steffens et al. (1995)
Fishmeal, 63–65% from residues	kg/yr	2.66×10^{-01}	Secondary	Steffens et al. (1995)
Tap water	L/yr	5.00×10^{-01}	Secondary	Steffens et al. (1995)
Wheat grain, organic	kg/yr	6.33×10^{-01}	Secondary	Steffens et al. (1995)
$Na_4P_2O_7$	kg/yr	4.95×10^{-03}	Secondary	Steffens et al. (1995)
Co	kg/yr	7.00×10^{-08}	Secondary	Steffens et al. (1995)
NaCl	kg/yr	1.50×10^{-03}	Secondary	Steffens et al. (1995)
$MgSO_4$	kg/yr	1.70×10^{-04}	Secondary	Steffens et al. (1995)
$ZnSO_4$	kg/yr	1.97×10^{-05}	Secondary	Steffens et al. (1995)
$CaCO_3$	kg/yr	3.97×10^{-03}	Secondary	Steffens et al. (1995)
$MnSO_4$	kg/yr	8.40×10^{-04}	Secondary	Steffens et al. (1995)
Dry-spirulina	kg/yr	1.40×10^{-02}	Primary	Local Company
Electricity from grid, low voltage	kWh/yr	1.27×10^{-01}	Secondary	Steffens et al. (1995)
Output: products and co-products				
Feed SP5	kg	1.00×10^0		

Table 6
Categories of impact method ReCiPe 2016 Midpoint (H) V1.03.

Impact categories	Acronyms	Unit of measure
Global warming potential	GWP	kg CO_2 eq
Stratospheric ozone depletion	ODP	kg CFC11 eq
Ionizing radiation	IRP	kBq Co-60 eq
Ozone formation, Human health	OFHH	kg NOx eq
Fine particulate matter formation	PMFP	kg $PM_{2.5}$ eq
Ozone formation, Terrestrial ecosystems	OFTE	kg NOx eq
Terrestrial acidification	TAP	kg SO_2 eq
Freshwater eutrophication	FETP	kg P eq
Marine eutrophication	MEP	kg N eq
Terrestrial ecotoxicity	TEP	kg 1,4-DCB
Freshwater ecotoxicity	FETP	kg 1,4-DCB
Marine ecotoxicity	METP	kg 1,4-DCB
Human carcinogenic toxicity	HTPc	kg 1,4-DCB
Human non-carcinogenic toxicity	HTPcn	kg 1,4-DCB
Land use potential	LUP	m^2a crop eq
Mineral resource scarcity	SOP	kg Cu eq
Fossil resource scarcity	FFP	kg oil eq
Water consumption	WCP	m^3

After entering the input and output data into the SimaPro LCA software, we generated two types of results based on the selected impact categories: "characterized" and "normalized". The characterized results provide the quantitative determination of the contribution of the individual emissions and resource use to the related impact categories. They are expressed through an appropriate unit of measure, which unifies all the contributions of different substances (e.g. CO_2, CO, CH_4, N_2O) to the same impact category (in this case, GWP), by using appropriate equivalence factors. The environmental impact is quantified by multiplying the amount of the substance or resource involved with its characterization factor. However, characterized values cannot be compared to each other, due to their quantification through different units.

The normalization step aims to express the results of the characterization phase with equivalent numerical factors instead of specific units of measurement per category. These factors quantitatively and synthetically represent the environmental effects of the system considered with respect to an impact considered as reference, in a certain year and geographical area. The normalization procedure divides the characterized values by a reference value (or normal effect), which generally represents the average impact (per unit & per person) on a global, regional, or European scale, referring to a specific time interval. In this way the impact of the considered system is standardized with respect to the unitary impact of the reference system (SimaPro database manual methods library, 2020). In our case, we utilized global data (World, 2010 from the SimaPro library 2020) as a reference.

3. Results

3.1. Spirulina and fish redox state

Overall, the results of the evaluation of the redox state of the fish following fishmeal replacement with spirulina indicated that replacing 5% of fishmeal is highly effective in improving the redox state of animal muscle tissue, by reducing oxidative damage, susceptibility to oxidative stress, and ROS levels, and increasing the activity of the enzymes of the glutathione system. On the contrary, replacing a significant amount of fishmeal (30%) with spirulina non beneficial effects were observed. Fig. 2 reports the effect of feeding juveniles of Koi carp for 6 weeks with the SP5 and SP30 diets on the mean weight (Panel A) and CF value (Panel B). The initial average weights (±SEM) were 2.92 ± 0.58g; 2.85 ± 0.89g and 2.71 ± 0.54g, for C, SP5 and SP30, respectively. The initial average CF were 2.19 ± 0.32; 1.97 ± 0.20; 2.15 ± 0.32, for C, SP5 and SP30, respectively. While there was a similar and significant growth with control ($p < 0.01$) and Sp5 ($p < 0.05$) diets, there was no significant growth with the Sp30 diet. Accordingly, CF decreased significantly ($p < 0.05$) in the Sp30 group of animals.

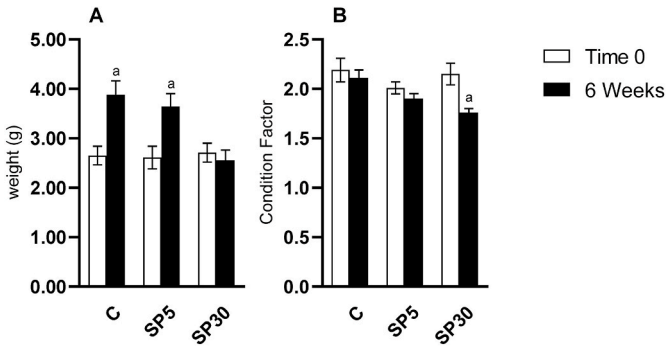

Fig. 2. Mean weight (Panel A) and Mean CF value (Panel B) of experimental groups of juvenile Koi carp at the beginning and at the end of the treatment period (6 weeks) with differentiated diets. Values are means ± SEM of eight determinations. C: animal fed with a control diet; SP5: animal fed with a diet containing 5% fishmeal replaced with spirulina; SP30: animal fed with a diet containing 30% fishmeal replaced with spirulina. [a] significant versus time 0 (two-way ANOVA with Tukey multiple comparison *post-hoc* test, $p < 0.05$).

Fig. 3 shows the effect of SP5 and SP30 diets on the indexes of oxidative damage (Panel A–B) and ROS levels of muscle homogenates. SP5 significantly reduced both the lipid hydroperoxide and protein-bound carbonyl levels, while SP30 diet did not influence the indexes of oxidative damage. The replacement of 5% of fishmeal in the diet with dry spirulina powder significantly reduced tissue ROS level, while this parameter was not influenced by the SP30 diet administration.

Fig. 4 reports the effect of SP5 and SP30 diets on GPx and GR activities (Panel A–B), and the *in vitro* susceptibility to oxidants (ΔHps, Panel C) of muscle homogenates. Feeding the animals with a 5% dry spirulina powder replaced diet significantly increased both antioxidants enzyme activity. The replacement of 30% fishmeal in the diet with dry spirulina powder did not affect enzymatic activities. The *in vitro* susceptibility to Fe2+-ascorbate-induced oxidative stress, measured as changes in the lipid-bound hydroperoxide content, was lower in SP5 group and not significantly different from the control animals in the SP30.

3.2. Life Cycle Assessment (LCA) results

3.2.1. Analysis of the impacts related to the dry spirulina production phase

Table 7 shows the comparison between the characterized impacts of spirulina production by using standard medium and aquaculture wastewater as growing medium and the functional unit of 1 kg. Spirulina production on standard medium was analyzed, based on the inventory reported in Table 2, yielding about 660 kg of dry spirulina over 220 days. Instead, production on wastewater aquaculture, according to the inventory shown in Table 3, provided about 149.6 kg of dry spirulina in the same period.

The normalized impacts related to the spirulina production process on both industrial and wastewater media are shown in Fig. 5 and Fig. 6 respectively and compared in Fig. 7.

As can be seen from Fig. 5, in the production of spirulina on standard medium there is a notable prevalence of impacts in the categories of toxicity. The inputs that mostly contribute to the toxicity categories are sodium bicarbonate (in brown in the graph) and the generation of heat using wood pellets burned in the stoves (in dark blue in the graph).

The normalized impacts of spirulina production on aquaculture wastewater (Fig. 6) shows significant differences compared to those of Fig. 5. In this case, the standard medium for cultivation and pellet stoves for heating are not used and the contribution of spirulina production towards the toxicity categories is lower than in Fig. 5. The use of aquaculture wastewater (in blue in the graph) as a substitute for the industrial medium helps to reduce impacts. Growing spirulina on wastewater, by avoiding the impact coming from water purification

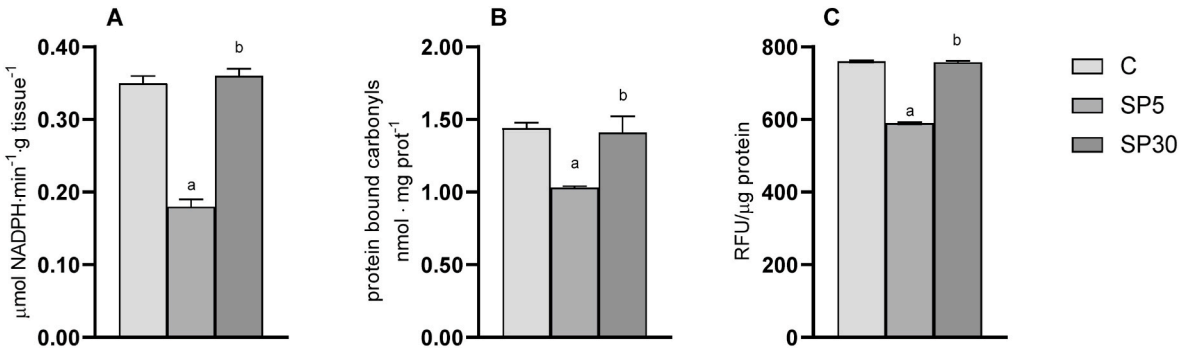

Fig. 3. Effect of SP5 and SP30 diets on indexes of oxidative damage (Panel A and B) and ROS levels in juvenile Koi Carp muscle. Hydroperoxides (HPs) are expressed in μmol NADP min^{-1} g^{-1} tissue. Protein-bound carbonyls (CO) are expressed in nmol mg^{-1} protein. ROS levels are expressed as Relative Fluorescent Units, RFU/μg tissue protein. Values are means ± SEM of eight determinations. C: animal fed with a control diet; SP5: animal fed with a diet containing 5% fishmeal replaced with spirulina; SP30: animal fed with a diet containing 30% fishmeal replaced with spirulina. [a] significant versus control group; [b] significant versus SP5 diet (One-way ANOVA, Tukey post hoc text, $p < 0.05$).

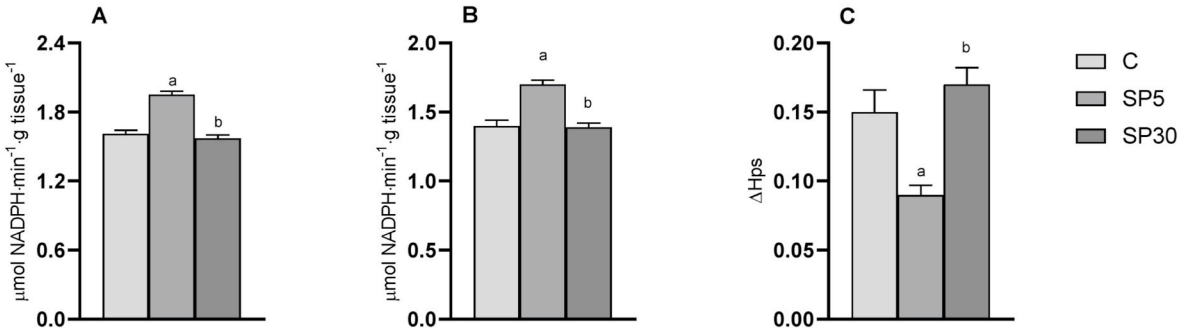

Fig. 4. Effect of SP5 and SP30 diets on glutathione peroxidase (GPx) and glutathione reductase (GR) activities (Panel A–B), and *in vitro* susceptibility to oxidants (Panel C) in juvenile Koi Carp muscle. GPx and GR activities are expressed in μmol NADPH min^{-1} g^{-1} tissue. *In vitro* susceptibility to oxidants was measured as the level changes on the lipid bound hydroperoxide, ΔHP, expressed as nmol NADPH min^{-1} per mg protein. Values are means ± SEM of eight determinations. C: animal fed with a control diet; SP5: animal fed with a 5% dry spirulina powder replaced diet; SP30: animal fed with a 30% dry spirulina powder replaced diet. [a] significant versus control group; [b] significant versus SP5 diet (One-way ANOVA, Tukey post hoc text, $p < 0.05$).

processes (wastewater treatment and disposal), also contributes to the reduction of toxicity categories. These represent avoided impacts, and for this reason the contribution of this input is negative (less than 0) in the graph. In conclusion, in this case the input responsible for the greatest contributions towards the toxicity categories is the use of electricity (in red in the graph).

The results show that the most impacting categories are those related to toxicity: terrestrial ecotoxicity (TEP), freshwater ecotoxicity (FETP), marine ecotoxicity (METP), human carcinogenic toxicity (HTPc) and human non-carcinogenic toxicity (HTPcn) (Figs. 5 and 6). The use of aquaculture wastewater (Fig. 6) erases the contribute to the toxicity categories of the main component of standard medium, such as magnesium sulfate, sodium bicarbonate and sodium nitrate, while energy represents the most impacting input in both cases (as heat plus grid and PV electricity in Fig. 5, while being only PV electricity in Fig. 6). The direct comparison reported in the Fig. 7 shows that spirulina grown on wastewater is less impacting in all toxicity categories. Furthermore, as shown in Fig. 7, the contribution of spirulina production on aquaculture wastewater towards the FETP and HTCPcn categories is negative (less than 0). The reason is linked to the negative contribution (less than 0) of the avoided impacts of aquaculture wastewater is greater than the positive one given by the sum of the other inputs, as shown in Fig. 6.

3.2.2. *Analysis of the impact related to the control and SP5 diets*

The analysis of the impacts related to the production of the feed diets were performed by using the inventories shown in Tables 3–5 for Control feed, SP5 feed containing spirulina derived from standard medium (ATI Biotech) ($SP5_{st}$), and SP5 feed containing spirulina derived from aquaculture wastewater ($SP5_{ww}$), respectively. The inventories refer to the production of 1 kg of feed for each of the diets. Table 8 shows the characterized impacts of the production of control and SP5 feeds.

The normalized impacts related to control feed, $SP5_{st}$, $SP5_{ww}$, and their comparison were also assessed. Figs. 8–10 show the contribution of the different inputs to the toxicity categories, while Fig. 11 shows the comparison among the investigated cases.

As shown in Fig. 8, the most impacting categories in the control feed production are marine ecotoxicity (METP), with fishmeal as the highest component, and human non-carcinogenic toxicity (HTPcn), mainly due to wheat grain. The comparison of normalized impacts between control, $SP5_{st}$ and $SP5_{ww}$ feed reported in the Fig. 11, shows that $SP5_{ww}$ is overall the less environmentally impacting, particularly in METP and HTPcn categories. Instead, $SP5_{st}$ is more impacting than the control diet because of the components needed for the industrial production of the growing medium (Fig. 9). Growing spirulina on aquaculture wastewater clearly reduces the impact of the SP5 diet (Fig. 10). Although the impact of $SP5_{WW}$ is similar to that of control diet, the combination with the healthy effect on animals and the reduced impact of treatment and disposal of aquaculture wastewaters, make it advantageous when spirulina is used to feed the same animals from which those wastewaters are produced.

4. Discussion

By combining experimental data on the health status of fish with process performance data from LCA approach, our results support a model of circular economy and the possibility of improving the

Table 7
Characterized impacts related to the spirulina production process on standard medium and wastewater aquaculture with functional unit set to 1 kg of dry spirulina.

Impact category	Unit	Standard medium	Aquaculture wastewater
Global warming	kg CO_2 eq	2.92×10^{01}	9.82×10^{00}
Stratospheric ozone depletion	kg CFC11 eq	1.02×10^{-04}	6.07×10^{-06}
Ionizing radiation	kBq Co-60 eq	1.95×10^{-01}	9.75×10^{-02}
Ozone formation, Human health	kg NOx eq	1.14×10^{-01}	1.93×10^{-02}
Fine particulate matter formation	kg PM2.5 eq	9.48×10^{-02}	1.39×10^{-02}
Ozone formation, Terrestrial ecosystems	kg NOx eq	1.16×10^{-01}	1.97×10^{-02}
Terrestrial acidification	kg SO_2 eq	1.76×10^{-01}	4.31×10^{-02}
Freshwater eutrophication	kg P eq	2.90×10^{-03}	-1.00×10^{-04}
Marine eutrophication	kg N eq	4.43×10^{-03}	-3.82×10^{-03}
Terrestrial ecotoxicity	kg 1,4-DCB	1.79×10^{02}	2.14×10^{01}
Freshwater ecotoxicity	kg 1,4-DCB	2.16×10^{-02}	-3.51×10^{-03}
Marine ecotoxicity	kg 1,4-DCB	1.28×10^{-01}	7.53×10^{-03}
Human carcinogenic toxicity	kg 1,4-DCB	3.06×10^{-01}	6.19×10^{-02}
Human non-carcinogenic toxicity	kg 1,4-DCB	9.65×10^{00}	-1.04×10^{00}
Land use	m^2a crop eq	1.99×10^{01}	3.87×10^{-01}
Mineral resource scarcity	kg Cu eq	1.31×10^{-01}	1.76×10^{-02}
Fossil resource scarcity	kg oil eq	6.90×10^{00}	3.20×10^{00}
Water consumption	m^3	1.08×10^{00}	7.95×10^{-01}

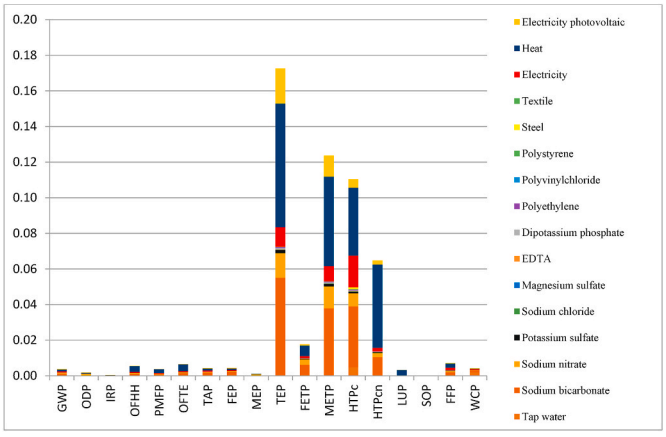

Fig. 5. Normalized impacts related to the spirulina production on standard medium referred to a functional unit of 1 kg of dry spirulina.

sustainability of the aquaculture systems while ameliorating fish welfare.

The experiments on the redox state of juvenile Koi Carp fed with diets in which dry spirulina replaced part of fishmeal indicate that a low (5%, SP5) but not a high (30%, SP30) alga diet inclusion improves the health status of the fish. The alga offers antioxidative protection acting by scavenging free radicals or increasing the antioxidant enzyme activities. In fact, SP5 reduces the oxidative stress status (reduced ROS levels and oxidative damage to lipids and proteins) while increasing the antioxidant defence system (increased GPx and GR activity and reduced *in vitro* susceptibility to oxidants) in fish muscle. These findings agree with previous reports on spirulina stimulating antioxidant systems in fish (Abdelkhalek et al., 2015; Napolitano et al., 2020) and mammals (Vázquez-Velasco et al., 2014). The capacity of SP5 to reduce oxidative

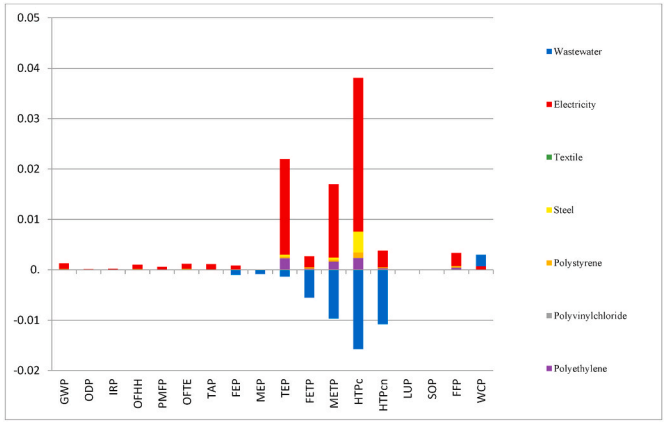

Fig. 6. Normalized impacts related to the Spirulina production on aquaculture wastewater referred to a functional unit of 1 kg of dry spirulina.

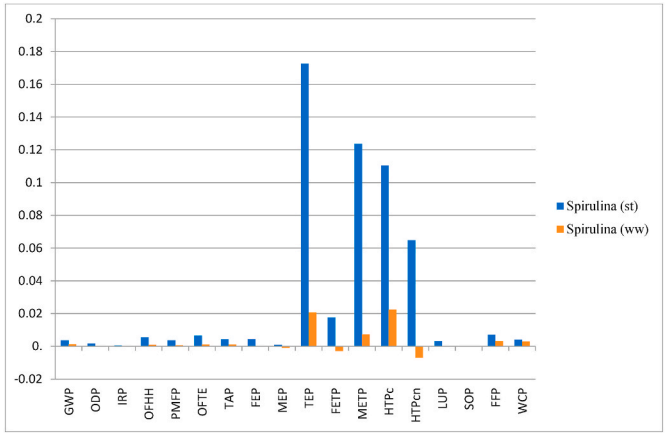

Fig. 7. Comparison between normalized impacts related to the Spirulina production on industrial and wastewater medium with functional unit set to 1 kg of dry Spirulina.

stress when administered at 5% can be important since in intensive aquaculture systems often fish face metabolic hypoxia due to the high rate of production of nitrogenous waste compounds (Napolitano et al., 2021b). Hypoxia is known to affect growth (Abdel-Tawwab et al., 2019), reproduction (Mallya, 2007) and oxidative tissue damage of animals (Napolitano et al., 2019b). Likely, the protection offered by low concentrations of spirulina in the diet against oxidative stress is due to its content of some specific bioactive compounds, mainly phycocyanin and carotenoids. Phycocyanin can scavenge ROS (Carfagna et al., 2015; Romay et al., 1998; Riss et al., 2007) and decreases ROS production (Riss et al., 2007), possibly by reducing superoxide formation at mitochondrial level (Panday et al., 2015; Napolitano et al., 2019a). Carotenoids, in particular β-carotene, can scavenge ROS, and reduce nitrite levels and nitric oxide synthase activity (Romay et al., 2003). C-phycocyanin also exhibits immunomodulatory activity and decreases LPS-induced ROS production (Chen et al., 2014; Mittal et al., 2014). The antioxidant and anti-inflammatory properties of spirulina are related to the capacity of the alga to restrain ROS production.

The administration of SP30 diet did not improve the redox state of fish and it affected fish growth during the six weeks of observation and reduced the condition factor (CF), a well-known index of fish physiological status and wellness (Lima-Junior et al., 2002). The reduction in CF is of particular importance, as heavier fish for a given length (higher CF) are healthier because extra weight means extra energy reserve. In contrast, lighter fish lacks energy reserves and tend to be more susceptible to environmental stressors (Morado et al., 2017). Similar results on

Table 8
Characterized impacts related to the diets with functional unit set to 1 kg of dry spirulina.

Impact category	Unit	Control	$SP5_{st}$	$SP5_{ww}$
Global warming	kg CO_2 eq	8.63×10^{-01}	1.26×10^{00}	9.88×10^{-01}
Stratospheric ozone depletion	kg CFC11 eq	7.43×10^{-06}	8.86×10^{-06}	7.51×10^{-06}
Ionizing radiation	kBq Co-60 eq	3.80×10^{-03}	6.47×10^{-03}	5.11×10^{-03}
Ozone formation, Human health	kg NOx eq	3.40×10^{-03}	4.91×10^{-03}	3.59×10^{-03}
Fine particulate matter formation	kg PM2.5 eq	2.65×10^{-03}	3.95×10^{-03}	2.81×10^{-03}
Ozone formation, Terrestrial ecosystems	kg NOx eq	3.44×10^{-03}	4.99×10^{-03}	3.64×10^{-03}
Terrestrial acidification	kg SO_2 eq	1.34×10^{-02}	1.57×10^{-02}	1.39×10^{-02}
Freshwater eutrophication	kg P eq	1.20×10^{-04}	1.60×10^{-04}	1.18×10^{-04}
Marine eutrophication	kg N eq	4.11×10^{-03}	4.17×10^{-03}	4.05×10^{-03}
Terrestrial ecotoxicity	kg 1,4-DCB	2.12×10^{00}	4.58×10^{00}	2.37×10^{00}
Freshwater ecotoxicity	kg 1,4-DCB	2.73×10^{-03}	3.03×10^{-03}	2.68×10^{-03}
Marine ecotoxicity	kg 1,4-DCB	8.43×10^{-03}	9.98×10^{-03}	8.29×10^{-03}
Human carcinogenic toxicity	kg 1,4-DCB	5.47×10^{-03}	9.70×10^{-03}	6.28×10^{-03}
Human non-carcinogenic toxicity	kg 1,4-DCB	4.30×10^{00}	4.43×10^{00}	4.28×10^{00}
Land use	m^2a crop eq	1.41×10^{00}	1.69×10^{00}	1.42×10^{00}
Mineral resource scarcity	kg Cu eq	2.22×10^{-03}	4.04×10^{-03}	2.46×10^{-03}
Fossil resource scarcity	kg oil eq	1.63×10^{-01}	2.56×10^{-01}	2.04×10^{-01}
Water consumption	m^3	3.78×10^{-02}	5.29×10^{-02}	4.88×10^{-02}

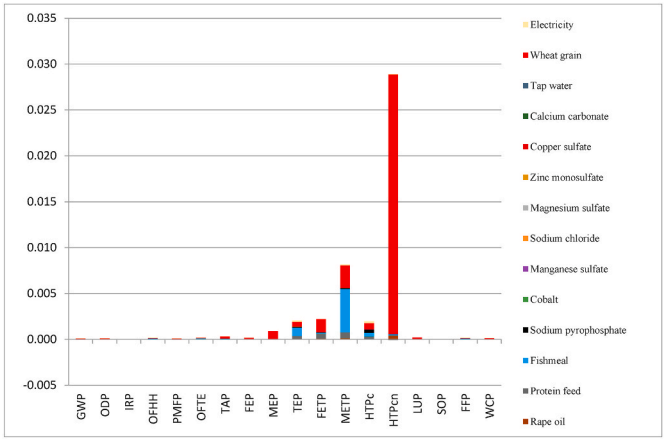

Fig. 8. Normalized impacts related to control feed production process (functional unit set to 1 kg of control feed).

the antioxidant capacity of a diet with high levels of fishmeal replacement with spirulina (50%) have been found in studies on *Macrobrachium rosenbergii* cultures (Raji et al., 2018). In contrast, other authors reported that high levels of spirulina in the diet ameliorates iron-induced oxidative stress in Indian knife fish *Notopterus notopterus*, suggesting that the alga can be used as a dietary supplement for fishes cultured in water bodies with iron overload (Mohanty and Samanta, 2018). Moreover, several studies have indicated the antioxidant or prooxidant effects of individual foods, herbs, dietary supplements, polyphenolic compounds, and specific phytochemicals, depending on the administration dose

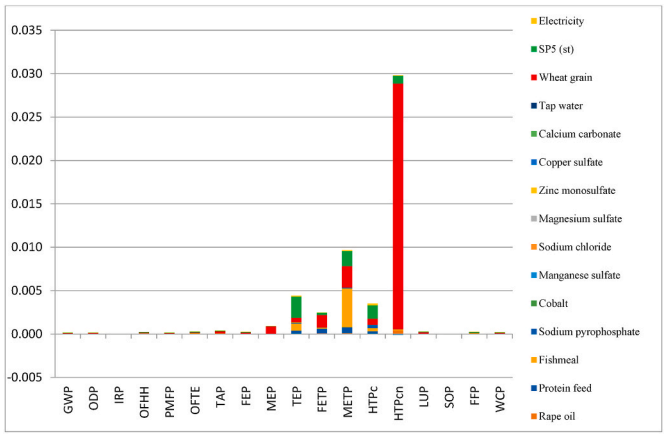

Fig. 9. Normalized impacts related to SP5 feed production process in which the 5% of fishmeal is replaced with spirulina grown on standard medium ($SP5_{st}$) (functional unit set to 1 kg of SP5 feed).

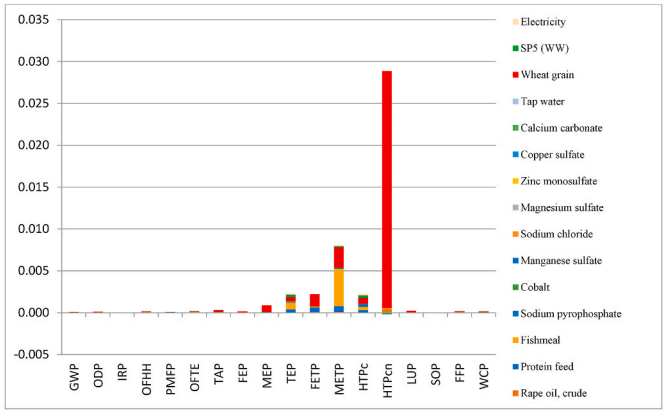

Fig. 10. Normalized impacts related to SP5 feed production process in which the 5% of fishmeal is replaced with spirulina grown on wastewater aquaculture ($SP5_{ww}$) (functional unit set to 1 kg of SP5 feed).

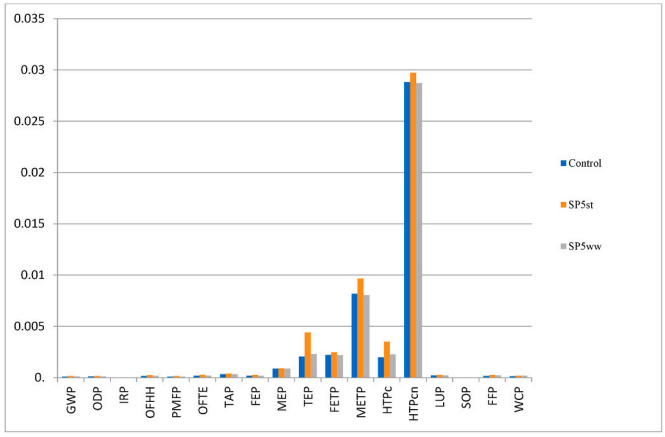

Fig. 11. Normalized impacts related to the production phase of the control feed, as well as SP5 diets in which the 5% of fishmeal is replaced with spirulina grown on standard medium ($SP5_{st}$), and SP5 diets in which the 5% of fishmeal is replaced with spirulina grown on wastewater aquaculture ($SP5_{ww}$) (functional unit set at 1 kg).

("biphasic dose response" or "Hormesis") (Skaperda et al., 2021). These results raise caution on using spirulina as a source of protein replacement for fishmeal proteins and that it is not always appropriate to administer large doses of spirulina.

Based on the above considerations, we analyzed the sustainability of the spirulina production for diet supplementation (spirulina replacing 5% of fishmeal) in an aquaculture system by means of the LCA approach, to ascertain if the nutritional properties identified are also associated to a decrease of environmental impacts. It is known that spirulina production incurs higher costs due to the expensive components needed for concocting the medium (Ragaza et al., 2020) and the high energy cost of maintaining the production (Catone et al., 2021; Geremia et al., 2021). In an LCA study, Smetana et al. (2017) demonstrated that fish food proteins derived from microalgae grown on standard medium are more impactful than the ones derived from both animal and vegetable sources. In particular, the impact categories identified by the authors are Global warming potential, Ozone depletion, Acidification, Eutrophication, Energy demand, Freshwater depletion and Land use. Similar results were found by Maiolo et al. (2020), which evaluated the sustainability of three partial substitutes for fishmeal: dried microalgae biomass, insect meal and poultry by-product meal. The performance-based ranking indicated the insect and poultry scenarios as the most sustainable options. The impact categories involved in the impact of microalgae production by Maiolo et al. were Global warming, Acidification, Cumulative energy demand and Water use. According to these studies, the main drivers of the environmental impacts of protein production from microalgae are the supply of nutrients (fertilizers and carbon) and energy consumption for heating in the winter months and ensuring the mix of fluid within the system. Smetana et al. (2017) suggest that cultivation in environments with more favourable climatic conditions could reduce impacts by up to 25% due to the lower need for energy use for heating. On the other hand, this could increase water consumption due to the greater evaporation (environmental shifted burden) (Bohnes et Laurent, 2019).

Because of the high impact of spirulina production on standard medium, we compared the alga production on standard medium with the production on aquaculture wastewater. As suggested by comparing the characterized impacts, growing spirulina on aquaculture wastewater is less impacting on the environment. The analysis of the normalized effects highlights that the toxicity categories affected by spirulina production are less impacting when spirulina is grown on aquaculture wastewater. The last, in fact, avoids the impacts due to the treatment and disposal of wastewater and those linked to the use of the standard culture medium.

The subsequent analysis comparing the control diet with two SP5 diets, one using spirulina grown on standard medium and the other using spirulina grown on aquaculture wastewater, highlighted the advantage to use spirulina grown from aquaculture wastewater. Fishmeal is one of the main contributors to the control diet's toxicity categories, and even a small substitution of fishmeal with spirulina reduces this impact. The best result is given when spirulina grown on the aquaculture wastewater is used to supplement the diet of the same fish culture from which the wastewater is derived. In such a fish production chain, the standard medium's environmental and economic costs are abolished, as well as those linked to the disposal of aquaculture wastewater.

5. Conclusions and perspectives

Our results are very relevant in a circular economy scenario. Applied, for example, to our model species, Koi carp, we can conclude that the quantity of spirulina obtained from aquaculture wastewater can easily cover the replacement of 5% fishmeal. In fact, assuming: i) an optimum Koi carp stocking density of 2.1 kg m^{-3} in aquaponics (Nuwansi et al., 2021) with approximatively 213 kg of fish in 101′200 L of water; and ii) a daily amount of diet corresponding to 5% of the body weight, a sufficient 149.6 Kg of spirulina could be produced and reutilized. The proposed model takes advantage from different issues. It could reduce the environmental impacts by reducing water consumption and the treatment and disposal of aquaculture wastewater. Further, it could also avoid the costs linked to the use of the commercial medium for spirulina growth. Moreover, it could partially reduce the use of fishmeal as a protein source for the formulation of feed for aquaculture, that is responsible for the depletion of fish stocks. However, other two questions must be solved. The first one concerns the quality of spirulina produced on aquaculture wastewater. Further studies are required to test its composition. The second one is related to the very large contribution of wheat production to the toxicity categories, as reported in the graphs of the normalized impacts of feed. Agricultural crops are responsible for high environmental impacts such as climate change, acidification, eutrophication, etc. (Brentrup et al., 2004). Up to now, in fact, the development of agricultural crops has been oriented only to economic profit by neglecting the social and environmental balance that are not sufficiently considered (Baum and Bieńkowski, 2020).

CRediT authorship contribution statement

Gaetana Napolitano: Conceptualization, Methodology, Samples analysis, Formal analysis, Graph creation, Writing – original draft, Writing – review & editing. **Paola Venditti:** Conceptualization, Methodology, Samples analysis, Formal analysis, Graph creation, Writing – original draft, Writing – review & editing. **Claudio Agnisola:** Conceptualization, Methodology, Samples analysis, Formal analysis, Graph creation, Writing – original draft, Writing – review & editing. **Saverio Quartucci:** Conceptualization, Data curation, Supervision, Writing – review & editing, Funding acquisition. **Gianluca Fasciolo:** Investigation, Software, Writing – original draft. **Maria Teresa Muscari Tomajoli:** Investigation, Software, Writing – original draft. **Eugenio Geremia:** Investigation, Software, Writing – original draft. **Claudio Marcello Catone:** Investigation, Software, Writing – original draft. **Sergio Ulgiati:** Conceptualization, Data curation, Supervision, Writing – review & editing, Funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgements

The Authors Napolitano, Geremia, Catone and Ulgiati gratefully acknowledge the support from the project "Promoting Circular Economy in the Food Supply Chain (ProCEedS)", funded by the H2020-MSCA-RISE-2018 programme (Grant Number: 823967). Geremia, Catone and Ulgiati also acknowledge the support from the Italian Ministry of Foreign Affairs and International Cooperation (Ministero degli Affari Esteri e della Cooperazione Internazionale-Direzione Generale per la Promozione del Sistema Paese; Grant number: PGR05278, China-Italy High Relevance project "Urban Circular Economy").

References

Abdel-Tawwab, M., Monier, M.N., Hoseinifar, S.H., et al., 2019. Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. Fish Physiol. Biochem. 45, 997–1013. https://doi.org/10.1007/s10695-019-00614-9.

Abdelkhalek, N.K., 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. Int. 22 (4), 3023–3031. https://doi.org/10.1007/s11356-014-3578-0.
Adel, M., Yeganeh, S., Dadar, M., Sakai, M., Dawood, M.A., 2016. Effects of dietary Spirulina platensis on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (Huso huso Linnaeus, 1754). Fish Shellfish Immunol. 56, 436–444. https://doi.org/10.1016/j.fsi.2016.08.003.
Baum, R., Bieńkowski, J., 2020. Eco-efficiency in measuring the sustainable production of agricultural crops. Sustainability 12 (4), 1418. https://doi.org/10.3390/su12041418.
Bohnes, F.A., Laurent, A., 2019. LCA of aquaculture systems: methodological issues and potential improvements. Int. J. Life Cycle Assess. 24 (2), 324–337.
Brentrup, F., Jürgen, K., Hermann, K., Joachim, L., 2004. Environmental impact assessment of agricultural production systems using the life cycle assessment methodology: I. Theoretical concept of a LCA method tailored to crop production. Eur. J. Agron. 20, 247–264.
Calone, R., Pennisi, G., Morgenstern, R., Sanyé-Mengual, E., Lorleberg, W., Dapprich, P., Winkler, P., Orsini, F., Gianquinto, G., 2019. Improving water management in European catfish recirculating aquaculture systems through catfish-lettuce aquaponics. Sci. Total Environ. 687, 759–767. https://doi.org/10.1016/j.scitotenv.2019.06.167.
Cardoso, L.G., Duarte, J.H., Costa, J.A.V., et al., 2021. Spirulina sp. as a bioremediation agent for aquaculture wastewater: production of high added value compounds and estimation of theoretical biodiesel. Bioenergy Res. 14, 254–264. https://doi.org/10.1007/s12155-020-10153-4.
Carfagna, S., Napolitano, G., Barone, D., Pinto, G., Pollio, A., et al., 2015. Dietary supplementation with the microalga Galdieria sulphuraria (Rhodophyta) reduces prolonged exercise-induced oxidative stress in rat tissues. Oxid. Med. Cell. Longev. 732090 https://doi.org/10.1155/2015/732090.
Carlberg, I., Mannervik, B., 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J. Biol. Chem. 250, 5475–5480.
Catone, C.M., Ripa, M., Geremia, E., Ulgiati, S., 2021. Bio-products from algae-based biorefinery on wastewater: a review. J. Environ. Manag. 293, 112792 https://doi.org/10.1016/j.jenvman.2021.112792. ISSN 0301-4797.
Chen, H.W., Yang, T.S., Chen, M.J., et al., 2014. Purification and immunomodulating activity of C-phycocyanin from Spirulina platensis cultured using power plant flue gas. Process Biochem. 49, 1337–1344.
Christaki, E., Florou-Paneri, P., Bonos, E., 2011. Microalgae: a novel ingredient in nutrition. Int. J. Food Sci. 62 (8), 794–799. https://doi.org/10.3109/09637486.2011.582460.
Cleveland, C.J., Ayres, R.U., 2004. Encyclopedia of Energy. Elsevier Academic Press, Amsterdam.
Colella, M., Ripa, M., Cocozza, A., Panfilo, C., Ulgiati, S., 2021. Challenges and opportunities from circular wastewater management. The case of Campania Region, Italy. Journal of Environmental Management 297, 1–15, 113171.
Dernekbasi, S., Unal, H., Karayucel, I., Aral, O., 2010. Effect of dietary supplementation of different rates of Spirulina (Spirulina platensis) on growth and feed conversion in Guppy (Poecilia reticulata Peters, 1860). J. Anim. Vet. Adv. 9, 1395–1399. https://doi.org/10.3923/javaa.2010.1395.1399.
Driver, A.S., Kodavanti, P.R.S., Mundy, W.R., 2000. Age-related changes in reactive oxygen species production in rat brain homogenates. Neurotoxicol. Teratol. 22, 175–181. https://doi.org/10.1016/s0892-0362(99)00069-0.
FAO, 2011. Global food losses and food waste—Extent, causes and prevention. Rome, Italy.
FAO, 2020. The State of World Fisheries and Aquaculture 2020. (Sustainability in Action. Rome.
Flohé, L., Günzler, W.A., 1984. Assays of glutathione peroxidase. Methods Enzymol. 105, 114–120. https://doi.org/10.1016/S0076-6879(84)05015-1.
Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., Caruso, G., 2018. Book chapter: Fishmeal Alternative Protein Sources for Aquaculture Feeds. Feeds for the Aquaculture Sector, 978-3-319-77940-9. https://doi.org/10.1007/978-3-319-77941-6_1.
Geremia, E., 2019. Environmental Impact of the Production of Arthrospira Platensis for Food Purposes. An Application of Life Cycle Analysis. Master Thesis. Environmental Sciences, Parthenope University of Napoli, Italy.
Geremia, E., Ripa, M., Catone, C.M., Ulgiati, S., 2021. A review about microalgae wastewater treatment for bioremediation and biomass production—a new challenge for Europe. Environments 8 (12), 136. https://doi.org/10.3390/environments8120136.
Ibrahem, M.D., Ibrahim, M.A., 2014. The potential effects of Spirulina platensis (*Arthrospira platensis*) on tissue protection of Nile tilapia (*Oreochromis niloticus*) through estimation of P53 level. J. Adv. Res. 5, 133–136. https://doi.org/10.1016/j.jare.2013.03.009.
ISO, 2006. ISO 14040:2006: Environmental Management - Life Cycle Assessment - Principles and Framework. https://www.iso.org/standard/37456.html.
Iwama, G.K., Pickering, A., Sumpter, J., Schreck, C., 2011. Fish Stress and Health in Aquaculture. Cambridge University Press, Cambridge, UK.
Jiménez-González, C., Kim, S., Overcash, M.R., 2000. Methodology for developing gate-to-gate Life cycle inventory information. Int. J. LCA 5, 153–159. https://doi.org/10.1007/BF02978615.
Kim, S.S., Rahimnejad, S., Kim, K.W., Lee, K.J., 2013. Partial replacement of fish meal with Spirulina pacifica in diets for parrot fish (Oplegnathus fasciatus). TrJFAS 13 (2). https://doi.org/10.4194/1303-2712-v13_2_01.
Klinger, D., Naylor, R., 2012. Searching for solutions in aquaculture: charting a sustainable course. Annu. Rev. Environ. Resour. 37 (1), 247–276. https://doi.org/10.1146/annurev-environ-021111-161531.
Lima-Junior, S.E., Cardone, I.B., Goitein, R., 2002. Determination of a method for calculation of allometric condition factor of fish. Acta Sci. Biol Sci. 24 (2), 397–400. https://doi.org/10.4025/actascibiolsci.v24i0.2311.
Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101 (1), 13–30. https://doi.org/10.1016/j. aquatox.2010.10.006. Jan 17.
Maiolo, S., Parisi, G., Biondi, N., et al., 2020. Fishmeal partial substitution within aquafeed formulations: life cycle assessment of four alternative protein sources. Int. J. Life Cycle Assess. 25, 1455–1471. https://doi.org/10.1007/s11367-020-01759-z.
Mallya, Y.J., 2007. The Effects of Dissolved Oxygen on Fish Growth in Aquaculture. United Nations University, Fisheries Training Programme, UNU-FTP, p. 30.
Martos-Sitcha, J.A., Mancera, J.M., Prunet, P., Magnoni, L.J., 2020. Editorial: welfare and stressors in fish: challenges facing aquaculture. Front Physiol. 11–162. https://doi.org/10.3389/fphys.2020.00162. Feb 25.
Mittal, M., Siddiqui, M.R., Tran, K., Reddy, S.P., Malik, A.B., 2014. Reactive oxygen species in inflammation and tissue injury. Antioxidants Redox Signal. 20 (7), 1126–1167. https://doi.org/10.1089/ars.2012.5149, 10.1089/ars.2012.5149.
Mohanty, D., Samanta, L., 2018. Dietary supplementation of Spirulina ameliorates iron-induced oxidative stress in Indian knife fish Notopterus Notopterus. Environ. Toxicol. Pharmacol. 61, 71–78. https://doi.org/10.1016/j.etap.2018.05.007.
Morado, C.N., Araújo, F.G., Gomes, I.D., 2017. The use of biomarkers for assessing effects of pollutant stress on fish species from a tropical river in Southeastern Brazil. Acta Sci. Biol Sci. 39 (4), 431–439.
Nandeesha, M.C., Gangadhar, B., Varghese, T.J., Keshavanath, P., 1998. Effect of feeding Spirulina platensis on the growth, proximate composition and organoleptic quality of common carp, Cyprinus carpio L. Aquacult. Res. 29 (5), 305–312.
Napolitano, G., Fasciolo, G., Di Meo, S., Venditti, P., 2019a. Vitamin E supplementation and mitochondria in experimental and functional hyperthyroidism: a mini-review. Nutrients 11 (12), 2900. https://doi.org/10.3390/nu11122900.
Napolitano, G., Venditti, P., Fasciolo, G., Esposito, D., Uliano, E., et al., 2019b. Acute hypoxia/reoxygenation affects muscle mitochondrial respiration and redox state as well as swimming endurance in zebrafish. J. Comp. Physiol. B 189 (1), 97–108. https://doi.org/10.1007/s00360-018-1198-6.
Napolitano, G., Fasciolo, G., Salbitani, G., Venditti, P., 2020. *Chlorella sorokiniana* dietary supplementation increases antioxidant capacities and reduces Ros Release in mitochondria of hyperthyroid rat liver. Antioxidants 9 (9), 883. https://doi.org/10.3390/antiox9090883.
Napolitano, G., Fasciolo, G., Venditti, P., 2021a. Mitochondrial management of reactive oxygen species. Antioxidants 10 (11), 1824. https://doi.org/10.3390/antiox10111824. PMID: 34829696; PMCID: PMC8614740.
Napolitano, G., Fasciolo, G., Agnisola, C., Venditti, P., 2021b. Urea excretion and arginase activity as new biomarkers for nitrite stress in freshwater aquatic animals. Water 13, 3521. https://doi.org/10.3390/w13243521.
Napolitano, G., Fasciolo, G., Magnacca, N., Goglia, F., Lombardi, A., Venditti, P., 2022. Oxidative damage and mitochondrial functionality in hearts from KO UCP3 mice housed at thermoneutrality. J. Physiol. Biochem. https://10.1007/s13105-022-00882-9.
NRC (National Research Council), 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington, DC, p. 376.
Nuwansi, K.K.T., Verma, A.K., Chandrakant, M.H., Prabhath, G.P.W.A., Peter, R.M., 2021. Optimization of stocking density of koi carp (*Cyprinus carpio* var. koi) with gotukola (*Centella asiatica*) in an aquaponic system using phytoremediated aquaculture wastewater. Aquaculture 532, 735993. https://doi.org/10.1016/j.aquaculture.2020.735993.
Oliva-Teles, A., Enes, P., Peres, H., 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Davis, D.A. (Ed.), Feed and Feeding Practice in Aquaculture. Woodhead Publishing, Cambridge, pp. 203–233. https://doi.org/10.1016/B978-0-08-100506-4.00008-8.
Osmundsen, T.C., Almklov, P., Tveterås, R., 2017. Fish farmers and regulators coping with the wickedness of aquaculture. Aquacult. Econ. Manag. 21, 1–21. https://doi.org/10.1080/13657305.2017.1262476.
Panday, A., Sahoo, M.K., Osorio, D., Batra, S., 2015. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cell. Mol. Immunol. 12 (1), 5–23. https://doi.org/10.1038/cmi.2014.89, 10.1038/cmi.2014.89.
Prè, 2016. https://pre-sustainability.com/articles/recipe/.
Priyadarshani, I., Rath, B., 2012. Commercial and industrial applications of micro algae. A review. J. Algal Biomass. 3, 89–100. ISSN: 2229- 6905.
Quartucci, S., 2022. Personal Communication from Enterprise Management (ATI Biotech s.r.L. www.atibiotech.com.
Ragap, H.M., Khalil, R.H., Mutawie, H.H., 2012. Immunostimulant effects of dietary Spirulina platensis on tilapia Oreochromis niloticus. J. Appl. Pharmaceut. Sci. 2, 26–31.
Ragaza, J.A., Hossain, M.S., Meiler, K.A., Velasquez, S.F., Kumar, V., 2020. A review on Spirulina: alternative media for cultivation and nutritive value as an aquafeed. Aquaculture 12, 2371–2395. https://doi.org/10.1111/raq.12439.
Rajasekaran, C., Ajeesjh, C.P.M., Balaji, S., Shalini, M., Siva, R., et al., 2015. Effect of modified Zarrouk's medium on growth of different spirulina strains. Walailak J. Sci. Technol. 13 (1), 67–75.
Raji, A.A., Alaba, P.A., Yusuf, H., Abu Bakar, N.H., Mohd Taufek, N., et al., 2018. Fishmeal replacement with Spirulina Platensis and Chlorella vulgaris in African catfish (Clarias gariepinus) diet: effect on antioxidant enzyme activities and haematological parameters. Res. Vet. Sci. 119, 67–75. https://doi.org/10.1016/j.rvsc.2018.05.013.
Ramakrishnan, C.M., Haniffa, M.A., Manohar, M., Dhanaraj, M., Arockiaraj, A.J., Arunsingh, S.V., 2008. Effects of probiotics and spirulina on survival and growth of

juvenile common carp (Cyprinus carpio). Isr. J. Aquacult. Bamidgeh 60 (2), 128–133.
Ravi, M., Lata, S., Azharuddin, D.S., Paul, S., 2010. The beneficial effects of Spirulina focusing on its immunomodulatory and antioxidant properties. J. Diet. Suppl. 2, 73–83. https://doi.org/10.2147/NDS.S9838.
Riss, J., Décordé, K., Sutra, T., Delage, M., Baccou, J.C., et al., 2007. Phycobiliprotein C-phycocyanin from Spirulina platensis is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. J. Agric. Food Chem. 55 (19), 7962–7967. https://doi.org/10.1021/jf070529g.
Romay, C.H., Armesto, J., Remirez, D., Gonzalez, R., Ledon, N., et al., 1998. Antioxidant and anti-inflammatory properties of Cphycocyanin from blue-green algae. J. Inflamm. Res. 47, 36–41. https://doi.org/10.1007/s000110050256.
Romay, C.H., Gonzalez, R., Ledon, N., Remirez, D., Rimbau, V., 2003. C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Curr. Protein Pept. Sci. 4, 207–216. https://doi.org/10.2174/1389203033487216.
Roy, S., Ruma, P., 2015. Microalgae in aquaculture: a review with special references to nutritional value and fish dietetics. Proc. Zool. Soc. 68, 1–8. https://doi.org/10.1007/s12595-013-0089-9.
Santagata, R., Ripa, M., Ulgiati, S., 2017. An environmental assessment of electricity production from slaughterhouse residues. Linking urban, industrial and waste management systems. Appl. Energy 186, 175–188. https://doi.org/10.1016/j.apenergy.2016.07.073.
Seo, J.S., Haque, M.N., Nam, S.E., Kim, B.M., Rhee, J.S., 2020. Inorganic nitrogen compounds reduce immunity and induce oxidative stress in red seabream. Fish Shellfish Immunol. 104, 237–244. https://doi.org/10.1016/j.fsi.2020.05.072. PMID: 32497726.
Shanthi, G., Premalatha, M., Anantharaman, N., 2021. Potential utilization of fish waste for the sustainable production of microalgae rich in renewable protein and phycocyanin-Arthrospira platensis/Spirulina. J. Clean. Prod. 294, 126106 https://doi.org/10.1016/j.jclepro.2021.126106.
Shields, R., 2012. Algae for Aquaculture and Animal Feeds. Technikfolgenabschätzung – Theorie und Praxis 21. Jg., Heft 1.
SimaPro database manual methods library, 2020.
Skaperda, Z., Tekos, F., Vardakas, P., Nepka, C., Kouretas, D., 2021. Reconceptualization of hormetic responses in the frame of redox toxicology. Int. J. Mol. Sci. 23 (1), 49. https://doi.org/10.3390/ijms23010049.
Smetana, S., Sandmann, M., Rohn, S., Pleissner, D., Heinz, V., 2017. Autotrophic and heterotrophic microalgae and cyanobacteria cultivation for food and feed: life cycle assessment. Bioresour. Technol. 245, 162–170. https://doi.org/10.1016/j.biortech.2017.08.113.
Sprague, M., Dick, J.R., Tocher, D.R., 2016. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. Sci. Rep. (6) https://doi.org/10.1038/srep21892, 21892, Feb 22.
Steffens, W., Wirth, M., Rennert, B., 1995. Effects of adding various oils to the diet on growth, feed conversion and chemical composition of carp (Cyprinus carpio). Arch. Tierernahr 47 (4), 381–389. https://doi.org/10.1080/17450399509381821. PMID: 7668994.
Teimouri, M., Yeganeh, S., Amirkolaie, A.K., 2015. The effects of Spirulina platensis meal on proximate composition, fatty acid profile and lipid peroxidation of rainbow trout (Oncorhynchus mykiss) muscle. Aquacult. Nutr. 22, 559–566. https://doi.org/10.1111/anu.12281.
Ujjania, N.C., Sharma, L.L., Balai, V.K., 2013. Length-weight relationship and condition factor of Indian major carp (Labeo rohita Ham., 1822) from southern Rajasthan, India. Appl. Biol. Res. 15 (2), 104–108.
Vázquez-Velasco, M., González-Torres, L., López-Gasco, P., Bastida, S., Benedí, J., et al., 2014. Liver oxidation and inflammation in Fa/Fa rats fed glucomannan/Spirulina-surimi. Food Chem. 159, 215–221. https://doi.org/10.1016/j.foodchem.2014.03.015.
Venditti, P., Napolitano, G., Barone, D., Di Meo, S., 2014. Vitamin E supplementation modifies adaptive responses to training in rat skeletal muscle. Free Radic. Res. 48 (10), 1179–1189. https://doi.org/10.3109/10715762.2014.937341.
Venditti, P., Napolitano, G., Di Meo, S., 2015. Role of mitochondria and other ROS sources in hyperthyroidism-linked oxidative stress. Immunol. Endocr. Metab. Agents Med. Chem. 15 (1), 5–36. https://doi.org/10.2174/1871522215011507101249 51.
Venditti, P., Napolitano, G., Barone, D., Pervito, E., Di Meo, S., 2016. Vitamin E-enriched diet reduces adaptive responses to training determining respiratory capacity and redox homeostasis in rat heart. Free Radic. Res. 50 (1), 56–67. https://doi.org/10.3109/10715762.2015.1106530.
Watanuki, H., Kazuki, O., Asmi Citra Malina, A.R., Tassakka Toshimitsu, K., Masahiro, S., 2006. Immunostimulant effects of dietary spirulina platensis on carp, Cyprinus Carpio. Aquaculture 258 (1), 157–163. https://doi.org/10.1016/j.aquaculture.2006.05.003.
Ytrestøyl, T., Aas, T.S., Åsgård, T., T, 2015. Utilisation of feed resources in production of Atlantic salmon (Salmo salar) in Norway. Aquaculture 448, 365–374. https://doi.org/10.1016/j.aqrep.2019.100216.