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Chemical composition and apparent digestibility of a panel of dried microalgae and cyanobacteria biomasses in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Despite a growing interest in microalgae and cyanobacteria as potential sources of nutrients in aquafeeds, little information is presently available on their nutritive value for carnivorous fish species. The aim of this study was to evaluate chemical composition and nutrient digestibility of a panel of microalgae and cyanobacteria dried biomasses (MACB), using rainbow trout (*Oncorhynchus mykiss* W.) as a fish model. Nine test diets were obtained by mixing 80 parts of a reference diet, added with 20 g/kg of acid insoluble ash as an indigestible marker, to 20 parts of each of the following dried whole-cell biomass: *Arthrospira platensis*, *Nostoc sphaeroides*, two strains of *Chlorella sorokiniana*, *Nannochloropsis oceanica*, *Tisochrysis lutea*, *Phaeodactylum tricornutum*, *Porphyridium purpureum* and *Tetraselmis suecica*. The digestibility measurements were conducted with rainbow trout (52.4 ± 1.5 g) kept in six tank units each including three 60-L vessels singularly stocked with 12 fish and fitted with a settling column for faecal recovery. Per each diet, faeces were collected over three independent 10-day periods. Apparent digestibility coefficients (ADCs) of dry matter, crude protein (CP), organic matter and gross energy (GE) of single MACB were calculated by difference relative to those of the reference diet. The MACBs had heterogeneous chemical composition (CP, from 20 to 69%; Lipid, 5–27%; GE, 12.5–-22.6 MJ/kg dry matter basis) reflecting their overall biodiversity. Most of them can be considered as virtually good sources of minerals and trace elements and exhibit an essential amino acid profile comparable or even better than that of soybean meal commonly used in fish feeds with *P. purpureum* showing the best protein profile. The digestibility results obtained with rainbow trout allowed ranking the MACBs into two major groups. A first one, including *C. sorokiniana*, *N. oceanica* and *T. suecica,* resulted in markedly lower ($P < 0.05$) crude protein and energy ADC (64–73%; 51–59%, respectively) compared to a second group including *P. purpureum*, *T. lutea* and cyanobacteria (CP-ADC, 83–88%; GE-ADC, 74–90%) while *P. tricornutum* resulted in intermediate values. Overall, the present study confirms the consistently reported role of cell-wall structure/composition in affecting accessibility of nutrients to digestive enzyme. Based on the overall outcomes, only *T. lutea* and cyanobacteria actually meet the requirements for being used as protein sources in aquafeeds provided their mass production becomes more feasible and cost-effective, hence attractive for the feed-mill industry in the near future.

1. Introduction

It is generally agreed that any further sustainable growth of the aquaculture industry which relies on the use of feeds, could greatly benefit from feed ingredients and raw materials that come from lower trophic levels (Tibbetts, 2018). In this direction, cultivated microalgae and cyanobacteria deserve attention also as sources of nutrients in aquafeeds besides their roles as functional feed supplements or live feeds in mollusc, crustacean and fish hatcheries. Currently the availability and prices of most dried microalgae and cyanobacteria suitable as ingredients for aquafeeds, remain far from being economically feasible (Norsker et al., 2011; Tibbetts, 2018; Tredici et al., 2016). However,

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technological improvements in microalgae and cyanobacteria mass cultivation (e.g., reduction of energy inputs, use of recycled nutrients) could reduce production costs, making market prices more competitive with those of the most commonly used feedstuffs (Tredici et al., 2016). Moreover, MicroAlgae and Cyanobacteria dried Biomass (MACB) could have certain advantages over conventional feed raw materials or ingredients in terms of overall environmental footprint (Taelman et al., 2013; Tibbetts, 2018), even if this greatly depends on the algal species, cultivation system and location (Maiolo et al., 2020; Tredici et al., 2016).

A number of studies have appeared on nutrient composition of several MACBs, showing that some of them may actually be regarded as potential feedstuffs in diets for fish and shrimps based on their high crude protein and lipid levels, relatively well-balanced amino acid profile and, as is the case of certain marine species, n-3 LC-PUFA content (Becker, 2007; Bobin-Dubigeon et al., 1997; Niccolai et al., 2019; Shah, 2019; Tibbetts, 2018). The inclusion of protein and/or n-3 LC-PUFA-rich intact microalgae dried biomass in partial replacement of dietary fish meal and oils, has been the subject of several feeding experiments in different fish species (Ayala et al., 2020; Cardinaletti et al., 2018; Gong et al., 2018; Sarker et al., 2020; Shah et al., 2018; Sørensen et al., 2016; Vizcaíno et al., 2018; Vizcaíno et al., 2016; Vizcaíno et al., 2014).

In most of these studies the diets including MACB from 1 up to 30% resulted in similar growth response when compared to the controls but, quite often, also in variable or declining nutrient digestibility, thus making it difficult extrapolating their nutritive value.

As with any other feed ingredient, besides the biochemical composition, estimating nutrient and energy digestibility represents a basic step to evaluate the actual potential of intact MACB as feed ingredients in aquafeeds (Allan et al., 2000; Guedes and Malcata, 2012; Tibbetts et al., 2006). From the limited data available so far, it appears that there is a huge variability in nutrient and energy bioavailability of whole-cell MACB in different fish species with apparent digestibility values that sometimes compare favourably with those of commonly used plant protein-rich derivatives (Batista et al., 2017, 2020; Burr et al., 2011; Gaylord et al., 2008; Sarker et al., 2016; Sevgili et al., 2019; Teuling et al., 2017; Tibbetts, 2018). The microalgae or cyanobacteria species and, within a given species, culture and downstream processing methods/conditions of the raw ingredients, represent major factors in affecting nutrient composition and digestibility of intact cell biomass which also depends on the feeding habits and digestive morpho-physiology of the different fish species (Burr et al., 2011; Sarker et al., 2016; Sevgili et al., 2019; Teuling et al., 2017). There is strong evidence that the cell wall or membrane composition and structure of intact MACB could determine the accessibility of the intracellular nutrients to digestive enzymes, hence affecting their digestibility. Based on this, microalgae with relatively thin and less complex cell wall are expected to result in higher nutrient and energy digestibility than those with a thick, cellulosic cell wall (Batista et al., 2020; Scholz et al., 2014; Teuling et al., 2019; Teuling et al., 2017). The dominant adverse effect of a thick and complex cell wall could lead to similar low nutrient apparent digestibility coefficients in fish species differing in trophic level, digestive anatomy and ecophysiology (Teuling et al., 2017). On the other hand, there is also evidence of different nutrient digestibility of a same intact MACB in different fish species (Gong et al., 2018; Safari et al., 2016; Sarker et al., 2016; Sevgili et al., 2019). This suggests that actual nutrient bioavailability could also result from complex interactions between fish and microalgae or cyanobacteria species, strain or even batch and the need at testing single MACB in different fish species.

The present study evaluated chemical composition and in vivo macronutrient and energy apparent digestibility of a panel of whole-cell microalgae and cyanobacteria dried biomass including two strains of *Chlorella sorokiniana, Nannochloropsis oceanica, Tetraselmis suecica, Phaeodactylum tricornutum*, *Porphyridium purpureum*, *Tisochrysis lutea* (formerly, *Isochrysis galbana T-iso*), *Arthrospira platensis*, and *Nostoc sphaeroides,* using the rainbow trout (*Onchorynchus mykiss W.*) as a carnivorous fish model.

2. Material and methods

2.1. Microalgae and cyanobacteria strains, biomass origin and composition

The algal and cyanobacterial biomasses used in this experiment are listed in Table 1 and were described in a previous experiment by Niccolai et al. (2019). *Arthrospira platensis* F&M-C256, *N. sphaeroides* F&M-C117, *C. sorokiniana* F&M-M49, and IAM C-212, *T. suecica* F&M-M33, *P. purpureum* F&M-M46, *P. tricornutum* F&M-M40, *T. lutea* F&M-M36, and *N. oceanica* F&M-M24 belong to the culture collection of Fotosintetica & Microbiologica (F&M) S.r.l. (Sesto Fiorentino, Florence, Italy). All the strains were cultivated in GWP®-II photobioreactors (Tredici et al., 2016) in semi-batch mode and were produced at the facilities of F&M S.r.l. and in those of the Institute of BioEconomy of the Italian National Research Council (CNR), located in Sesto Fiorentino, Florence (Italy) with the exception of *A. platensis* F&M-C256 and *T. lutea* F&M-M36, produced at Microalghe Camporosso (Imperia, Italy).

The microalgae and cyanobacteria strains were cultivated in different media according to their requirements: *Nostoc sphaeroides* F&M-C117, *Chlorella sorokiniana* F&M-M49 and *Chlorella sorokiniana* IAM C-212 in BG11 medium (Rippka et al., 1979), marine microalgae in F medium (Guillard and Ryther, 1962), *A. platensis* in Zarrouk medium (Zarrouk, 1966), while starved *Tetraselmis suecica* in F medium deprived of the nitrogen source. *N. sphaeroides* F&M-C117, starved *T. suecica* F&M-M33 and *P. purpureum* F&M-M46 were collected in the early stationary phase, while the remaining algal biomasses in the linear growth phase.

Microalgal and cyanobacterial biomasses were all harvested by centrifugation (Westfalia, mod. SSD18, GEA Group Aktiengesellschaft, Düsseldorf, Germany). MACBs were frozen at −20 °C, freeze-dried at −40 °C and 0.10 mbar vacuum pressure. Then they were finely ground to powder by a centrifugal mill (ZM 1000, Retsch GmbH, Haan, Germany) to pass through a 500 μm screen. All the biomasses were preserved under vacuum in plastic bags, kept at −18 °C until used.

2.2. General methodology, reference and test diets description and preparation

The apparent digestibility coefficients (ADC) of dry matter (DM), organic matter (OM), gross energy (GE) and crude protein (CP) of MACBs were measured *in vivo* as outlined by National Research Council (2011). The indirect method was adopted and ADCs were calculated by difference from those of a reference diet. This latter diet, whose ingredient and proximate composition are shown in Table 2, was prepared including 20 g/kg of Celite®, (Hyflo Supelco, Merck Life Sciences Ltd., Milan, Italy) a source of acid insoluble ash as an inert indicator. From this preparation, nine test diets were obtained by mixing in a 80:20 weight ratio the reference diet and the dried biomass of *Arthrospira platensis* (ART), *Chlorella sorokiniana* (strains CLFM49 and CLIAM),

Table 1
List of tested microalgae and cyanobacteria biomasses.

Algal/cyanobacteria biomass	Culture medium
Arthrospira platensis F&M-C256	Zarrouk (Alkaline)
Nostoc sphaeroides F&M-C117	BG11 (Freshwater)
Chlorella sorokiniana F&M-M49	BG11 (Freshwater)
Chlorella sorokiniana IAM C-212	BG11 (Freshwater)
Nannochloropsis oceanica F&M-M24	F medium (Marine)
Tetraselmis suecica F&M-M33	F medium (Marine)
Porphyridium purpureum F&M-M46	F medium (Marine)
Phaeodactylum tricornutum F&M-M40	F medium (Marine)
Tisochrysis lutea (T-ISO) F&M-M36	F medium (Marine)

Table 2
Ingredient and proximate composition of the reference diet (g/kg).

Ingredient composition	(g/kg)
Chile SP Fish meal	400.0
Wheat gluten meal	200.0
Wheat meal	160.0
Soybean meal	100.0
Fish oil	50.0
Rapeseed oil	30.0
Soy lecithin	20.0
Vitamin premix[a]	10.0
Mineral premix[b]	10.0
Celite®	20.0
Proximate composition (% DM)	
Moisture	5.7
Crude protein	50.7
Crude lipid	14.5
Ash	11.5
Carbohydrate[c]	23.3
Gross energy (MJ/kg DM)	21.3

[a] Composition of the vitamin mix (% mix): Thiamine HCl, 0.16; Riboflavin, 0.39; Piridoxine HCl, 0.21; Cyanocobalamine, 0.21; Niacin, 2.12; Calcium pantotenate, 0.63; Folic Acid, 0.10; Biotin Vit H, 1.05; Choline Chloride, 83.99; Myoinositol, 3.15; Stay C® DSM, 4.51; Vit E, 3.15; Menadione Vit K3, 0.24; Vit A (2500 IU/kg diet), 0.03; Vit D3 (2400 IU/kg diet), 0.05.
[b] Composition of the mineral mix (% mix): $HPO_4{\cdot}2H_2O$, 78.9; NaCl, 17.65; MgO, 2.725; $FeCO_3$, 0.335; KI, 0.005; $ZnSO_4{\cdot}H_2O$, 0.197; $MnSO_4{\cdot}H_2O$, 0.094; $CuSO_4{\cdot}5H_2O$, 0.027; Na Selenite, 0.067.
[c] Calculated by difference.

Nannocloropsis oceanica (NAN), *Nostoc sphaeroides* (NOS), *Tisochrysis lutea* (TISO), *Phaeodactylum tricornutum* (PHAE), *Porphyridium purpureum* (POR) and N-starved *Tetraselmis suecica* (TETR). The ten dry diets were then added with water (~600 g/kg) and the doughs thus obtained were extruded into 4 mm pellets using a meat mincer provided with a knife at the die. The wet pellets were then dried overnight at 45°C and preserved at +3/5 °C until used.

2.3. Experimental protocol and design

The *in vivo* digestibility measurements were carried out at the indoor aquaculture facilities of the Aquaculture & Wildlife division of the Di4A, University of Udine, Italy, (authorization n. 03/2018 UT of the Italian Ministry of Health according to the D.L.vo 26/2014 on the protection of animals used for scientific purposes). Measurements were performed using 6 units designed according to the original layout developed by the University of Guelph (Cho, 1992). Each unit consisted of three 60-L tanks fitted with a common drainpipe and a settling column for faecal collection. The experiment used 216 rainbow trout (*Oncorhynchus mykiss* W.) juveniles selected from a resident batch with an individual average weight of 52.4 ± 1.5 g. Each tank within a unit was randomly stocked with 12 fish.

The six units were subjected to a flow–through supply of well water ensuring a total volume renewal/h (~18–20 L·min^{-1}) and optimal water quality to trout (Temperature, 13.2 ± 0.3 °C; pH, 8.2 ± 0.2; D.O., 8.6 ± 0.2 mgL^{-1}; total ammonia-N, <0.01 mgL^{-1}; Nitrite-N, <0.02 mgL^{-1}).

Fish were left to adapt to the culture conditions over 15 days before starting measurements. To obtain three independent faecal samples per each diet, the nine MACB-including test preparations and the reference diet were turned around among the tank units in five consecutive 10-day faecal collection periods each preceded by a week adaptation to the next new diets.

Fish were fed two meals daily (7:45 and 16:00, solar time) to apparent satiety (until the first feed item was refused). After each meal, tanks and settling columns were flushed to avoid faeces from being contaminated by uneaten pellets. Faeces were recovered from the settling columns twice a day: approximately 7 h after the morning meal and 13 h after the last meal. They were immediately separated from the surrounding water by centrifugation (10,000 ×*g*; 20 min; 5°C). They were stored at −20°C until the end of each collection period, when the daily amounts of each unit (diet) were pooled and freeze-dried before analysis.

The apparent digestibility coefficients of dry matter, organic matter, crude protein and gross energy of the diets and test MACBs were computed as follows:

$$ADC_{Diet} = 1 - [(\%\text{indicator in the diet}/\%\text{indicator in faeces}) \times (\%\text{nutrient in faeces}/\%\text{nutrient in the diet})]$$

$$ADC_{test}\ MACB = ADC\ test\ diet + [(ADC\ test\ diet - ADC\ reference\ diet) \times (0.8 \times D_{ref.}/0.2\ D_{test}\ MACB)]$$

where "D_{ref}" is the level of nutrient or the gross energy content of the reference diet and "D_{test} MACB" is the level of nutrient or gross energy content of the test microalgae dried biomass.

2.4. Analytical methods

The moisture, total nitrogen and ash content of each dried MACB, test diets and faeces were determined according to AOAC (1998). In case of MACBs the analyses were performed on a pooled sample obtained by blending the 3 lots of the same batch used for producing the test diets. Total lipid content of the MACBs was determined according to (Folch et al., 1957). The acid-insoluble ash (AIA) content of the test diets and faeces was determined according to the method CEE-EU (G.U. European Community n. L.155/21. 12.7.71) and the gross energy content of test ingredients, diets and faeces was measured by an adiabatic bomb calorimeter (IKA C7000 Werke GmbH and Co., Staufen, Germany). The amino acid analysis of the dried MACBs was performed using a HPLC system provided with a LC 200 Perkin Elmer pump fitted with an ISS-100 auto sampler (20 μL loop) and a fluorimetric detector (Perkin Elmer. Norwalk. CT. USA), EX 250 nm and EM 395 nm. Separation was achieved by using a AccQ,Tag Aminoacid Analysis column (Waters Corporation. Milford. MA. USA) and a Waters pre-column filter. The column was thermostated at 31 °C and the flow rate was 0.8 mL/min (Liu et al., 1995). Mobile phase A consisted of acetate–phosphate aqueous buffer, and mobile phase B was acetonitrile 100%. Acid hydrolysis with HCl 6 M at 115–120 °C for 22–24 h was used for all amino acids except cysteine (Cys) and methionine (Met), for which performic acid oxidation followed by acid hydrolysis was used. Tryptophan was not determined. Based on the amino acid composition, the chemical score (CS) values of MACBs were calculated by dividing the proportion of each EAA in the test MACBs protein by the corresponding proportion of the same EAA in white fish meal as the reference protein. The lowest value of this ratio indicates the first limiting essential amino acid in the MACB protein, and therefore its CS value. The elemental compositions of MACBs (except that of *C. sorokiniana* IAM C-212 strain which had not been analysed) were measured by inductively coupled argon plasma optical emission spectrometry (ICPOES) according to SW-846 Method 6010C (EPA, 2012).

2.5. Data analysis

The nutrient and energy apparent digestibility coefficients (ADCs) of the test diets and freeze-dried MACBs were subjected to ANOVA using a mixed, two-factor model including the diet/MACB as a fixed effect and the three-tank unit as a random one. Where appropriate, ADC mean values were subjected to Duncan's multiple comparison test, for $P<0.05$. Before ANOVA, data were tested for normality and homogeneity (Shapiro Wilk and Levene tests). Data were processed and analysed using R (R Core Team, 2020).

3. Results

3.1. Proximate composition and gross energy content of MACBs

The proximate composition and gross energy content of MACBs are shown in Table 3.

Levels of total nitrogen in the species studied ranged from 3.2 to 11% DM corresponding to conventional crude protein (N x 6.25) values varying from 20 to 69% DM.

T. suecica (TETR) grown under nitrogen starvation and *P. purpureum* (POR) were low in CP whereas *N. sphaeroides* (NOS) and *A. platensis* (ART) exhibited the highest levels. Intermediate values were observed in the remaining microalgae. The lipid level was also quite different among the species here investigated. *T. lutea* (TISO) resulted the highest in lipid (26.9% DM) followed by *P. tricornutum* (PHAE) and *N. oceanica* (NAN) (17.2 and 16.2% DM, respectively). Similar lipid levels were found in *C. sorokiniana* IAM C-212 (CLIAM) and *N. sphaeroides* (13.8 and 12.5% DM, respectively) while *P. purpureum* (POR), *A. platensis* and N-starved *T. suecica* (TETR) showed lower lipid contents with values varying from 5.2 up to 7.6% DM. Very high values of carbohydrate were observed in N-starved *T. suecica* and, on the opposite, the lowest amount in *T. lutea*.

N. sphaeroides dried biomass was the lowest in ash (4.5% DM) followed by *A. platensis* and the two *Chlorella* strains. Most of the remaining species had very similar ash content (14.6–16.4%DM) except *P. purpureum* which was very high in ash (35.9% DM). The gross energy content also differed appreciably among the different MACBs, ranging from 12.52 MJ/kg in *P. purpureum* up to 22.60 MJ/kg in *N. sphaeroides*.

3.2. Amino acid profile and mineral composition

Table 4 shows the amino acid profile of the different MACBs relative to that of an ideal reference dietary protein source for fish like white fish meal (FM) or compared to that of a plant protein-rich ingredient largely used in fish feeds, such as the defatted and dehulled, soybean meal (SBM).

The EAA profile of all MACBs showed exceeding or similar proportions of arginine, leucine, aromatic amino acid (Phe + Tyr) and threonine when compared to those of FM and SBM. Based on chemical score, calculated using the essential amino acid profile of FM as a reference protein (excluding tryptophan that was not determined), lysine was first limiting in *A. platensis* and *N. sphaeroides*, but just marginally limiting in *N. oceanica, T. suecica, P. purpureum* and *P. tricornutum.* Like in SBM, sulphur AA (Met+Cys) were most limiting in *C. sorokiniana* F&M-M49, while the branched-chain isoleucine and valine were first limiting, although marginally, in *T. lutea* and *C. sorokiniana* IAM C-212.

The proportion of single non-essential amino acids (NEAA) showed relatively moderate variation among the different MACBs with the major exception of proline, much higher in *N. oceanica* compared to the other species. Apart from higher incidence of serine and proline, the profile of most NEAA overlapped that of FM while alanine and glycine were higher and, concurrently, those of aspartic and glutamic acid were lower in MACBs when compared to SBM.

As shown in Table 5, the biomass of marine species resulted in higher levels of certain macro-elements like Na and K compared to their freshwater counterparts. *P. purpureum* was particularly rich in Na and *T. suecica* had the highest calcium content. Trace elements levels differed markedly among MACBs with *P. tricornutum* being the richest source of Cu, Fe and Mn while *A. platensis* was the poorest.

3.3. In vivo digestibility of the test diets and MACBs

The apparent digestibility coefficients (ADCs) of the reference and test diets with 20% inclusion of the selected MACBs are summarised in Table 6. The reference diet and those including *P. purpureum*, *T. lutea*, and *A. platensis* were similar in DM and OM digestibility ($P > 0.05$) which were higher relative to those measured with the other diets ($P < 0.05$). All diets including MACBs resulted in slightly reduced N (crude protein) ADC when compared to the reference diet ($P < 0.05$). A similar pattern was observed also for the ADC of gross energy except in the case of the diets including *P. purpureum* and *A. platensis* which did not differ from the value measured with the reference diet.

The second part of Table 6 shows marked differences in the ADCs of the different MACBs here investigated. *P. purpureum*, *T. lutea*, *N. sphaeroides* and *A. platensis* resulted in notably higher ADC for DM, OM, N (CP) and GE ($P < 0.05$) when compared to the corresponding values calculated for *T. suecica, N. oceanica* and both strains of *C. sorokiniana* which did not differ from each other ($P > 0.05$) apart from a slightly but significantly improved N apparent digestibility in *T. suecica* and *N. oceanica* compared to *Chlorella*. *P. tricornutum* resulted in intermediate ADC values which were similar or slightly better ($P < 0.05$) than those of the latter group of MACB for DM whereas it did not differ significantly from the former group in GE and OM ADC values.

4. Discussion

The present study evaluated chemical composition, *in vivo* macronutrient and gross energy apparent digestibility of a panel of microalgae and cyanobacteria cultured in photobioreactors. Although the chemical composition of most of the species here evaluated had been already described (Becker, 2007; Brown, 1991; Brown et al., 1997; Cardinaletti et al., 2018; Gong et al., 2018; Guil-Guerrero et al., 2004; Niccolai et al., 2019; Radhakrishnan et al., 2016; Rebolloso Fuentes et al., 2000; Sevgili et al., 2019; Tibaldi et al., 2015; Tibbetts et al., 2015a; Tulli et al., 2012) some data presented here on amino acid profiles and mineral composition, despite batch-specific, are of great value and new for certain MACBs. Besides, the present investigation generated data on *in vivo* digestibility of certain MACBs in fish for which little or no information is currently available.

It is well known that the biochemical composition of MACB could be extremely variable being influenced by many factors among which, the species/strain, culture conditions, growth phase, physiological status and post-cultivation processing are widely recognised as major determinants (Hu, 2013). Moreover, analytical methods for common feedstuffs are far from being standardized and are often inadequate for microalgae (Laurens et al., 2012; Tibbetts et al., 2015a). The above

Table 3
Proximate composition (% DM) and gross energy content (MJ/kg DM) of test MACBs.

	ART	NOS	CLFM49	CLIAM	NAN	TETR	POR	PHAE	TISO
Moisture %	4.1	8.3	8.0	11.9	3.3	6.6	4.5	9.1	6.4
N x 6.25 (CP)	64.1	69.1	53.5	51.6	43.7	20.1	28.2	40.7	43.5
Total lipid	6.4	12.5	9.4	13.8	16.2	7.6	5.2	17.2	26.9
Ash	7.0	4.5	7.1	10.6	14.6	16.4	35.9	16.3	14.7
Carbohydrate[a]	22.5	13.9	30.0	24.0	25.5	55.9	30.6	25.8	14.9
Gross energy	20.84	22.60	21.49	21.80	21.91	17.05	12.52	19.22	22.36

ART, *Arthrosphira platensis;* NOS, *Nostoc sphaeroides*; NAN, *Nannochloropsis oceanica*; CLFM49, *Chlorella sorokiniana* F&M-M49; CLIAM, *Chlorella sorokiniana* IAM C-212; TETR, *Tetraselmis suecica*; POR, *Porphyridium purpureum*; PHAE, *Phaeodactylum tricornutum*; TISO, *Tisochrysis lutea*.

[a] Calculated by difference.

Table 4
Amino acid profile (% total AA) and chemical score of the tested MACBs.

	ART	NOS	CLFM49	CLIAM	NAN	TETR	POR	PHAE	TISO	FM[a]	SBM[a]
EAA											
Arg	8.6	8.9	8.7	6.9	7.2	6.5	8.8	8.0	7.4	6.4	7.8
His	2.5	2.2	2.6	1.9	2.4	1.9[c]	2.2	1.9	2.0	2.0	2.6
Ile	5.9	3.7	4.0	3.5	3.9	3.9	5.1	4.1	3.2[c]	3.7	4.7
Leu	9.6	9.1	9.6	8.2	8.6	9.3	9.0	7.8	7.7	6.5	7.8
Lys	3.7[c]	4.3[c]	7.4	9.3	6.0[c]	5.6[c]	6.1[c]	6.0[c]	8.3	6.9	6.3
Met+Cys	2.8	4.0	3.0[c]	4.4	3.9	3.5	4.6	3.5	4.6	3.5	3.1[c]
Phe + Tyr	12.2	11.0	10.1	8.3	9.6	7.6	9.6	7.8	7.7	5.9	8.9
Thr	6.1	5.8	6.2	4.8	5.4	5.4	5.4	5.2	5.0	3.9	4.2
Val	6.0	3.3[c]	5.1	3.8[c]	4.0	4.5	5.5	4.1	4.2	4.5	4.9
NEAA											
Ala	6.7	8.7	7.6	9.3	6.9	8.6	7.1	7.2	9.3	6.3	4.7
Asp	7.1	8.6	7.2	9.0	7.9	9.9	8.6	9.9	10.0	9.1	11.7
Glu	11.6	10.9	10.1	10.5	10.2	12.4	10.4	13.1	10.6	12.8	18.5
Gly	6.4	7.8	7.2	8.2	7.1	6.9	5.4	6.2	7.7	6.0	4.4
Pro	5.6	5.8	7.1	8.1	12.3	8.6	5.9	9.7	6.6	4.2	5.7
Ser	5.9	6.0	5.2	5.4	5.5	5.5	6.6	5.7	5.6	3.8	4.7
CS[b]	53	62	85	85	87	81	88	87	87	100	89

[a] FM: Danish white fish meal; SBM: defatted, dehulled, toasted soybean meal (composition from author's laboratory data).
[b] CS: Chemical score (values do not include tryptophan).
[c] First limiting EAA based on chemical score, i.e. the ratio between EAAi in MACBi or SBM/EAAi in FM.

Table 5
Mineral (%) and trace elements (mg/kg) composition of the test MACBs.

	ART	NOS	CLFM49	NAN	TETR	POR	PHAE	TISO	FM[a]	SBM[a]
Minerals										
Calcium	0.48	0.34	1.07	0.84	2.88	1.04	0.16	0.36	6.65	0.34
Magnesium	0.26	0.53	0.26	0.66	0.51	1.31	0.43	0.43	0.18	0.30
Phosphorous	1.42	1.62	1.32	0.18	0.86	1.39	1.04	1.59	3.59	0.69
Potassium	1.05	0.57	1.24	1.79	1.04	1.29	2.85	1.46	0.85	2.14
Sodium	0.74	0.04	0.11	2.83	2.40	6.75	1.92	2.97	0.78	0.02
Trace elements										
Copper	n.d.	46	21	4	14	11	238	2	5–10	20
Iron	324	777	533	676	747	595	967	319	110–545	176
Manganese	29	88	52	90	29	21	132	43	4–37	36
Zinc	20	172	83	84	200	56	143	118	90–210	55

n.d. not detected.
[a] FM: Danish white fish meal; SBM: defatted, dehulled, toasted soybean meal (range composition from National Research Council, 2011).

points could make it difficult to compare nutrient composition of the same or similar MACB among experiments. As frequently questioned (Tibbetts et al., 2015a) comparing protein levels of MACB with literature values is often biased by the method used to determine the protein content and by the different N-to-Protein factors applied for conversion of total N to crude protein (Mariotti et al., 2008). In this study we adopted the conventional N-to Protein conversion factor for feedstuffs (6.25) even if, according to Tibbetts et al. (2015a), CP values obtained by applying the factor 4.78 more closely fitted protein levels calculated as the sum of all amino acids. Irrespective of the conversion factor used, crude protein values of the different MACBs here studied easily fit the wide range of CP values (between 6 and 71%) reported in comprehensive reviews on chemical composition of microalgae (Becker, 2007; Chacón-Lee and González-Mariño, 2010). As expected, *T. suecica* grown under nitrogen starvation was the lowest in CP followed by *P. purpureum* which resulted in a low-medium protein content, not much different from that reported for this species by other authors, ranging from 20 to 40% DW depending on the N concentration in the culture media (Kavitha et al., 2016; Li et al., 2019; Rebolloso Fuentes et al., 2000). All the other MACBs resulted in medium to high CP contents with values similar to those reported for the same species in previous studies (Cardinaletti et al., 2018; Guil-Guerrero et al., 2004; Niccolai et al., 2019; Radhakrishnan et al., 2016; Tibaldi et al., 2015; Tibbetts et al., 2015a). Hence, based on CP levels most MACBs apparently possess a good potential as sources of dietary protein to fish. This seems somewhat corroborated by the amino acid profiles which reveal common features of the panel of MACBs here investigated with values consistently reported for the same species in previous studies (Acquah et al., 2020; Brown, 1991; Tibbetts, 2018; Tibbetts et al., 2015a; Wild et al., 2018). In fact, they were all characterized by high proportions of arginine, threonine, leucine and aromatic AA, relative to a good quality white fish meal. On the other hand, lysine was particularly limiting in the EAA profile of the two species higher in crude protein (*A. platensis* and *N. sphaeroides*), hence adversely affecting the potential biological value of their protein fraction. For *A. platensis* this seems consistent with EAA profile data found in the literature (Volkman and Brown, 2005). Among the different MACBs, *P. purpureum* displayed an EAA pattern without major limitations and close to that of white FM. It overlaps that reported by Kavitha et al. (2016) with the only exception of a higher proportion of sulphur AA found in the present study. A related species, *P. aerugineum*, was also found to be equal or superior in terms of essential amino acid index to an animal protein source of high biological value such as egg albumin (Tibbetts et al., 2015a). As already noted (Tibbetts et al., 2015a) the overall amino acid composition of the various MACBs here investigated reveals also close similarities or even better profiles when compared to those of conventional plant protein-rich ingredients commonly included in aquafeeds, like SBM, making most of them virtually suitable dietary amino acid supplements in fish diets, should their actual amino acid bioavailability be demonstrated. It should be noted however that essential amino acid profiles and chemical score of

Table 6
Apparent digestibility coefficients (ADCs) of dry matter, crude protein, gross energy and organic matter of the test diets and individual MACBs. Data are mean values of triplicate measurements.

	ADC (%)			
	DM[1]	N (CP)[2]	GE[3]	OM[4]
Diets				
Reference	79.0^{a}	94.9^{a}	86.5^{ab}	84.7^{a}
C. sorokiniana F&M-M49	76.5^{bc}	91.1^{f}	83.3^{de}	81.6^{d}
C. sorokiniana IAM C-212	75.4^{c}	90.9^{f}	83.3^{de}	80.6^{d}
N. oceanica	76.1^{c}	92.4^{e}	82.4^{e}	80.7^{d}
T. suecica	75.8^{c}	92.8^{e}	83.8^{d}	81.8^{cd}
P. tricornutum	77.6^{b}	93.8^{c}	85.4^{bc}	83.7^{ab}
A. platensis	79.2^{a}	93.4^{d}	85.8^{abc}	84.7^{a}
N. sphaeroides	78.7^{a}	93.7^{cd}	85.3^{c}	83.1^{bc}
P. purpureum	79.0^{a}	94.3^{b}	86.9^{a}	84.5^{a}
T. lutea	79.4^{a}	94.2^{b}	85.2^{c}	84.7^{a}
pooled s.e.[5]	0.28	0.23	0.28	0.31
MACBs				
C. sorokiniana F&M-M49	55.9^{bc}	64.1^{d}	58.5^{c}	57.1^{c}
C. sorokiniana IAM C-212	48.9^{c}	63.8^{d}	58.8^{c}	50.8^{c}
N. oceanica	57.0^{bc}	73.4^{c}	51.0^{c}	53.1^{c}
T. suecica	50.7^{c}	70.0^{c}	57.0^{c}	56.1^{c}
P. tricornutum	65.4^{b}	83.2^{b}	74.8^{b}	73.4^{b}
A. platensis	78.9^{a}	83.5^{b}	79.0^{b}	82.3^{ab}
N. sphaeroides	78.2^{a}	88.0^{a}	75.1^{b}	74.0^{ab}
P. purpureum	80.1^{a}	88.4^{a}	89.9^{a}	85.5^{a}
T. lutea	81.0^{a}	87.5^{a}	74.4^{b}	81.9^{ab}
pooled s.e.[5]	2.63	1.91	2.53	2.78

Column means not sharing the same letters differ significantly (a, b, c, d, e, f; $P < 0.05$).
[1] DM: dry matter.
[2] N, nitrogen; CP: crude protein.
[3] GE: gross energy.
[4] OM: organic matter.
[5] s.e.: standard error.

MACBs in this study considered 9 of the 10 essential amino acids (tryptophan was missing). It is conceivable that tryptophan could be limiting in some of these ingredients for a carnivorous fish like rainbow trout and this fact may not be overlooked when comparing different protein-rich ingredients.

The crude lipid content of the different MACBs here investigated (5 to 27%) also easily fits the wide range of possible values suggested for microalgae and cyanobacteria (i.e., 1–38% DW, Becker, 2013). In comparing lipid levels of MACBs from different sources, besides different cultivation conditions also different solvents and methods of lipid extraction and analysis must be considered, since they could result in various degree of pigment co-extraction or incomplete lipid extraction due to recalcitrant cell walls providing biased lipid values. This latter issue could probably explain lower values for lipid content in nearly all the species in this study compared to those reported by Niccolai et al. (2019) for the same panel of microalgae species and strains, since the latter authors used a different technique to analyse crude lipids. In the present study lipid analysis was performed according to Folch et al. (1957), which is a gravimetric method, whereas Niccolai et al. (2019) used Marsh and Weinstein (1966) method which is based on a carbonization step followed by spectrophotometric reading. Both methods used the same extraction procedure (chloroform/methanol 2:1). Moreover, all MACBs analysed by the Marsh & Weinstein method were pre-treated with a cell wall mechanical disruption step which may have improved the extraction of the lipid component. Comparing crude lipid contents actually found in this study with those reported for the same or related microalgae species in other studies reveals strong similarities or just slightly lower values (Cardinaletti et al., 2018; Kavitha et al., 2016; Rebolloso Fuentes et al., 2000; Rosales-Loaiza et al., 2017; Tibaldi et al., 2015; Tibbetts et al., 2015a).

The ash content of most of the different MACBs here investigated ranged from 4.5% in *N. sphaeroides* to 16.4% DW in N-starved *T. suecica*, which consistently falls within the range of ash content found in previous studies on freshwater and marine microalgae species (Barone et al., 2018; Cardinaletti et al., 2018; Guil-Guerrero et al., 2004; Tibbetts et al., 2015a; Volkman and Brown, 2005). The only exception was *P. purpureum* with an unusually high ash content of 35.9% DW. Ash levels reported for this species normally ranged between 18 and 22% DW (Kavitha et al., 2016; Li et al., 2019; Niccolai et al., 2019; Rebolloso Fuentes et al., 2000). The mineral analysis here performed revealed that high ash content was associated to a very high level of Na in this batch of *P. purpureum* (6.5% DW) which is nearly four times higher that (1.8% DW) reported by Kavitha et al. (2016) for this species. This probably resulted from residual salt in the marine medium (mainly NaCl) which elevated the Na and overall ash content of the biomass. Reasonably, a through washing step could desalinate the biomass, which would result in lower ash and higher CP content. Hence, this particular sample likely does not well represent the actual nutritional potential of this marine strain. In general the data obtained in this study are in the range reported for a wide array of freshwater and marine microalgae and cyanobacteria biomasses (Campanella et al., 1998; Fabregas and Herrero, 1986; Levasseur and Tremblay, 2006; Miller et al., 1971; Tibaldi et al., 2015; Volkman and Brown, 2005). The levels of macro (% DW) and trace elements (mg/kg) ranged widely between the following values: Ca, 0.04–2.99; Mg, 0.08–1.10; P, 0.70–3.00; K, 0.13–2.40; Na, 0.2–2.7; Cu, 12–650; Fe, 1000-7000; Mn, <20–592 and Zn, 50–3700. All MACBs here investigated could be considered good dietary sources of trace elements to fish with levels comparable or exceeding those supplied by a wide range of fish meals (National Research Council, 2011).

The energy content of several microalgae and cyanobacteria dried biomass has been reported in previous studies. Gross energy values of the panel of MACBs here investigated varied between 12.5 and 22.6 MJ/kg DW and lie in the wide range (6–30.4 MJ/kg DW) of those found in microalgae and cyanobacteria biomasses used for aquaculture and/or for biofuels (Barone et al., 2018; Gong et al., 2018; McGinn et al., 2011; Tibbetts et al., 2015a; Tibbetts et al., 2015b; Tibbetts et al., 2015c; Tibbetts et al., 2015d; Tibbetts et al., 2017a, 2017b; Tibbetts, 2018; Tibbetts et al., 2020; Whyte, 1987; Wild et al., 2018).

Despite a large number of studies dedicated to the chemical composition of microalgae, little information has been generated on their digestibility in fish and more generally in monogastric animals (Acquah et al., 2020; Neumann et al., 2018; Skrede et al., 2011; Wang et al., 2020). Under this respect, the present study is the first one providing information on the *in vivo* crude protein and energy apparent digestibility of a relatively wide panel of whole-cell microalgae and cyanobacteria dried biomass as a basic step to estimate their actual nutritive value to carnivorous fish species.

The panel includes *N. sphaeroides* and *P. purpureum* for which no information on digestibility in fish has been found in the literature. The apparent digestibility values obtained in the present study confirm the consistently reported major role of cell-wall structure and composition of different whole-cell microalgae biomass in affecting the accessibility of nutrients to digestive enzymes, hence their digestibility (Teuling et al., 2019; Tibbetts et al., 2017a). In fact, we observed here that the apparent digestibility of dry matter, organic matter and energy were significantly and substantially depressed in all microalgae species with a thick cell wall or a solid cell membrane (theca) when compared to those measured in cyanobacteria and other species with a thin and/or more simple cell envelop. In the chlorophyte *C. sorokiniana,* cell wall is rigid and mainly composed of cellulose, hemicelluloses, pectic compounds and glycoproteins (Domozych et al., 2012; Gerken et al., 2013), while in the eustimatophyte *N. oceanica,* the cell wall is composed by fibrous glycoproteins with a bilayer structure consisting of a cellulosic inner wall protected by an outer hydrophobic algaenan layer (Lora-Vilchis and Maeda-Martinez, 1997; Martínez-Fernández et al., 2004; Payne and Rippingale, 2000). In the Chlorodendrophyceae class, the genus *Tetraselmis* possesses a relatively thin but solid membrane (theca) built up of scales composed by acidic polysaccharides (Arora, 2016; Fernández-

Reiriz et al., 2015). In cyanobacteria cell walls are more fragile than in green microalgae and are mainly composed of a peptidoglycan layer with a proteic and lipopolysaccharidic outer membrane (Lee, 2008; Lu et al., 2006). In contrast with other diatoms, the cell wall of *P. tricornutum* is poor in silicon and mainly composed of organic molecules, notably, a sulphated glucuronomannan (Le Costaouëc et al., 2017). *Tisochrysis lutea* (Prymnesiophyceae) is characterized by the absence of a cell wall, presenting only a membrane covered with body scales and *P. purpureum* does not have a true cell wall but a layer of sulfated polysaccharides (Arad et al., 1985; Geresh et al., 2002; Geresh and Arad, 1991; Sobczuk et al., 2006).

The results here obtained suggest that a cell wall making nutrients poorly accessible to enzymes has a dominant adverse impact mostly on DM and energy apparent digestibility while ADC of N (crude protein) is not always concurrently much depressed. In fact, in this study the apparent N (CP) digestibility of *N. oceanica* and *T. suecica* were nearly ten points higher than in both strains of *C. sorokiniana* while DM and energy digestibility coefficients were equally low in all species. Apart from differences in protein accessibility caused by different cell wall matrices, this could be due to differences in the intrinsic properties of the proteins or a different proportion of soluble N-compounds in different microalgae and cyanobacteria biomass. Safi et al. (2013) have shown that the proportion of the hydro-soluble protein fraction in total protein varies widely (from 27 up to 80%) among different whole-cell microalgae and apparently, it correlates with their cell wall characteristics. In *N. oculata* this proportion was found to be 10 points higher than in *Chlorella* (43 vs. 33%). Intriguingly, in the same study the incidence of hydrosoluble protein in total protein was very high in *A. platensis* and *P. purpureum* (70 and 80%) which resulted in high or very high N digestibility values in the present study (83.5 and 88.4% respectively). A deeper insight in the proportions of cell wall-related nitrogen content to total N, could provide clues to better understand relatively high N (crude protein) apparent bioavailability associated to low DM and energy digestibility in certain whole-cell microalgae biomass as noted in the present, but also in other studies (Batista et al., 2020; Hart et al., 2021; Teuling et al., 2017; Tibbetts et al., 2017a).

The *in vivo* digestibility of the dry biomass of algae of the genus *Nannochloropsis* and *Chlorella* has been studied in different fish species, but comparing digestibility values from various experiments is not always consistent. In fact, beyond fish species, digestibility coefficients for individual feed ingredients are influenced by several factors among which fish culture conditions, faeces collection method, reference diet composition, ingredient origin and processing, level of inclusion of the test ingredient in the diet, and equation to calculate the digestibility coefficients, are the most relevant (National Research Council, 2011). The apparent digestibility coefficients of DM and CP of *N. oceanica* in this study (57 and 73.4%) are similar to those reported for *Nannochloropsis* sp. in rainbow trout by Sarker et al. (2020) (i.e., 56.7 and 69.3%) but differ in the Atlantic salmon (*S. salar*) (i.e. 48 and 73% respectively) as reported by Gong et al. (2018). Moreover, for *N. oceanica,* Sevgili et al. (2019) in rainbow trout (*O. mykiss*) found higher ADC values for CP (79.6%) and energy (69.3 vs. 51%) and lower OM digestibility (38.7 vs 53.1%) compared to those observed here. It should be noted however that the trial reported by Sevgili et al. (2019) was carried out under quite different conditions of water temperature (16.2 vs. 13.2 °C) and reference diet composition that could partly justify the different outcomes between the two experiments. In a recent study of Batista et al. (2020) in European sea bass (*Dicentrarchus labrax*), the apparent digestibility of whole cell *N. oceanica* biomass resulted in lower ADC of DM (32 vs. 57%) but in substantially higher crude protein and energy apparent digestibility values than those observed in the present study (81.6 and 76.2% vs. 73.4 and 51%, respectively). Beyond different fish species and water quality conditions, there is not a ready explanation for these so divergent results between experiments, though there were only marginal differences in the trial layout and reference diet composition. On the other hand, the chemical composition of the dried biomass of *N. oceanica* in the two studies were markedly different in terms of crude protein and total lipid contents, so even this factor could partly justify divergent ADC between experiments.

Differently to *C. vulgaris, C. sorokiniana* has been little studied as a possible feed ingredient in fish diets. The apparent protein digestibility (APD) of intact or mechanically disrupted *C. sorokiniana* freeze-dried biomass has recently been studied in rat by Wang et al. (2020). Intact cell biomass was found in an APD value lower than that here observed in trout (54.8 vs 64.1%) but markedly improved by mechanical cell wall disruption (70.5%). In the present experiment the dried biomass of intact *C. sorokiniana* appeared differently digested by rainbow trout when compared to the results obtained on *C. vulgaris* whole-cell meal in Atlantic salmon (Tibbetts et al., 2017a) and sea bass (Batista et al., 2020). In the trial on salmon no data were shown for dry matter digestibility, but crude protein ADC was more than 10 points higher (76.5% vs. 64%) while energy ADC were very similar to those measured in the present study (57.6 vs. 58%). Surprisingly, increasing levels of microalgae from 6 up to 30% replacing corresponding proportions of the reference diet in salmon had just minor and statistically irrelevant effects on ingredient digestibility values. In sea bass, *C. vulgaris* resulted in lower DM digestibility whereas crude protein and energy apparent digestibility were higher when compared to the results obtained in salmon and here with trout. Beyond the different fish species and culture conditions, also major differences in the chemical composition of the *C. sorokiniana* and *C. vulgaris* dried biomasses in the different studies may justify the inconsistency in certain digestibility values between experiments. Higher apparent digestibility coefficients than those observed in the present trial, have been reported for *Nannochloropsis* sp. and *Chlorella* sp. in herbivorous and omnivorous fish species like Nile tilapia and African catfish (Agboola et al., 2019; Barone et al., 2018; Teuling et al., 2017). This is not surprising since, based on carbohydrase activity, fish from lower trophic levels are expected to be capable to utilize microalgae with a cellulosic cell wall whereas carnivorous fish species do not to the same extent (Stone, 2003). In the study of Teuling et al. (2017), where Nile tilapia and African catfish were compared and differently from the outcome of the present experiment, *Chlorella* meal was found more digestible than *Nannochloropsis* but the digestibility of nutrients did not change between fish species suggesting that limitations in nutrient accessibility were dominant over those in digestive capability between herbivorous and omnivorous fish. Moreover, differences in nutrient accessibility were not related to the hardness of the algae cell wall.

In the present study, N-starved *T. suecica* was the lowest in terms of DM ADC (50.7%) and resulted in similarly low gross energy (57%) and organic matter ADC (56.1%) like in the other green algae. The poor digestibility here observed could explain the progressive decline of nutrient digestibility observed in previous experiments with E. sea bass fed diets including increasing levels of whole cell *T. suecica* alone or combined with *T. lutea* (Cardinaletti et al., 2018; Tulli et al., 2012). Whole cell *Tetraselmis* sp. biomass has recently been studied as a feed ingredient in the E. sea bass (Batista et al., 2020) and, despite a relatively thin cell wall, it has been shown to offer a strong resistance to a mechanical stress and, putatively, to the action of digestive enzymes. In fact, in the study above, *Tetraselmis* sp. biomass was found in negative DM and lipid ADC and in low GE-ADC (48,9%) but in N (CP) apparent digestibility overlapping the value observed in the present study with trout (69.7 vs. 70%). In the sea bass, physical rupture of cell wall resulted in markedly improved DM and energy apparent bioavailability stressing the dominant role of its integrity in limiting accessibility of nutrients for digestion in this microalga.

The two cyanobacteria biomasses here investigated, i.e. *A. platensis* and *N. sphaeroides*, had high and very similar dry matter (>78%) and gross energy ADC (>75%) while *N. sphaeroides* resulted in slightly but significantly higher nitrogen (CP) digestibility compared to *A. platensis* (88 vs. 83.5%). Very similar apparent digestibility coefficients of DM, (78–82%), CP (82–85%) and gross energy (82–83%) are reported for a

commercial *Arthrospira* meal in other salmonids like Atlantic salmon and Arctic char (Burr et al., 2011). *A. platensis* dry biomass resulted in a similar range of ADC values also in Nile tilapia and African catfish (Sarker et al., 2016; Teuling et al., 2017). No data are apparently available for the digestibility of *Nostoc* in fish, but *in vitro* (pepsin) protein digestibility of *Nostoc commune* has been studied by Hori et al. (1990), and it was found not so good (40–48%). On the contrary, in the present study with trout, *Nostoc* biomass, likely due to a cell wall less recalcitrant to enzyme digestion, resulted in high protein and energy bioavailability. These conflicting outcomes suggest caution in drawing conclusions from *in vitro* data to estimate the nutrient value of microalgae and cyanobacteria biomass in fish.

In the present study, the dry biomass of the diatom *P. tricornutum* resulted in ADC which were similar to those of the two cyanobacteria species for CP (83.2%), energy (74.8%) and OM (73.4%), but intermediate between those of cyanobacteria and green algae for dry matter (65.4%) which possibly also reflects the nature of its cell wall. Despite being poorer in silicon compared to other diatoms, the cell wall of *P. tricornutum* is mostly made of organic components and it also weakly links a magnesium hydroxide layer (brucite) which formation as well as that of extracellular polysaccharides, depends on culture conditions and pH and results lowered in acidic milieu (Le Costaouëc et al., 2017). It is tempting to speculate that this type of mineralized layer could only partly be dissolved by hydrochloric acid secretion in trout stomach, thus resulting in incomplete cell wall digestion and overall digestive enzyme accessibility, which could justify intermediate digestibility values between those of green algae and cyanobacteria. The only direct study investigating the digestibility of *P. tricornutum* dry biomass has been recently performed in rainbow trout by Sevgili et al. (2019) who compared starved dry biomasses cultured in 250 and 1500 L volumes. It did not report DM digestibility estimates, but the ADC of CP and energy were unaffected by the microalgal culture volume and resulted in values which appear much consistent with those observed in the present investigation (CP-ADC, 81 vs. 83%; GE-ADC, 77.1 vs 74.9%).

P. purpureum and *T. lutea* were among the most highly digested microalgae biomasses in the present study and this could be easily regarded as the result of the absence of a true cell wall in these two species. It should be noted that the simplest is the cell envelop structure, the highest is the apparent digestibility of nutrients. In fact, in *P. purpureum,* where cell membrane is made by a simple layer of sulphated polysaccharides, CP and GE-ADCs were slightly or even significantly higher (as in case of GE), compared to those of *T. lutea*, which outer cell wall layer includes also scales. No literature data on *in vivo* digestibility are currently available for *P. purpureum* as for *T. lutea*, while for the similar genus *Isochrysis* sp. Sarker et al. (2020) have recently reported the results of a study with rainbow trout where the apparent digestibility coefficients of the ingredient were very close to those obtained in the present investigation (DM- ADC, 77.1 vs. 81%; CP-ADC, 86.5 vs. 87.5%; GE ADC, 72.6 vs. 74.4%).

5. Conclusions

The present study evaluated chemical composition of a panel of microalgae and cyanobacteria and their macronutrient and gross energy apparent digestibility in rainbow trout. Despite similar cultivating system, the biomasses studied here differed in nutrient composition reflecting their biodiversity. Based on crude protein level and essential amino acid profile most of the whole cell microalgae meals studied here are comparable or even better than other vegetable protein sources commonly used in fish feeds like dehulled oil-extracted soybean meal, with *P. purpureum* resulting in the best protein profile. However, a necessary next step must be investigating the essential amino acid ADCs of these novel protein-rich products before they can be confidently incorporated into complete feeds. This study also provided new data on the mineral composition of certain microalgae species such as *N. sphaeroides* and *P. purpureum* and confirms that the studied biomasses could be considered virtually good source of macro and trace elements to fish whenever their effective bioavailability is ascertained. Besides, the present investigation generated data on digestibility of MACBs in fish for which little or no information is currently available.

The apparent digestibility values of the different whole-cell microalgae and cyanobacteria dried biomass obtained in the present study confirm the consistently reported major role of cell-wall structure and composition in affecting the accessibility of nutrients to digestive enzymes, hence their digestibility. Despite being attractive in terms of gross nutrient composition, the biomass of the whole-cell microalgae like *C. sorokiniana*, *N. oceanica* and N-starved *T. suecica* resulted in protein and energy apparent digestibility which make them unsuitable to fulfil the requirements for being used as regular feed ingredients in aquafeeds, without prior subjecting them to a proper technological process to make their nutrient content more accessible to digestion. On the opposite, the two cyanobacteria (*A. platensis* and *N. sphaeroides*), as well as the biomass of *T. lutea* and *P. purpureum* and to a lesser extent *P. tricornutum*, resulted in apparent digestibility values of macronutrients and energy which compare favourably with those of most conventional protein-rich aquafeed ingredients (National Research Council, 2011). Based on the results obtained with the latter group of MACBs examined here, the nutritional value does not seem to set a limit to their potential use in fish feeds provided their mass production becomes more feasible, cost-effective hence attractive for the feed mill industry in the near future.

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Declaration of Competing Interest

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

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