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Hydrolyzed microalgae from biorefinery as a potential functional ingredient in Siberian sturgeon (*A. baerii* Brandt) aquafeed.

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Abstract

The development of sustainable and functional feed represents an opportunity and a need for the aquaculture industry, supporting beneficial physiological effects on fish that go beyond traditional feed formulations. This study aims to evaluate the potential application of microalgae, produced through a sustainable process, as a functional ingredient in practical diets for Siberian sturgeon (*Acipenser baerii*) fingerlings. For this purpose, the effects of the dietary administration of two different microalgae, *Nannochlopsis gaditana* and *Scenedesmus almeriensis,* cultivated on conventional synthetic medium (SM) or diluted pig manure (PM) and included in diets as crude or hydrolyzed biomasses, were tested.

Growth performance and oxidative *status* of the fish were evaluated and compared in relation to the different diets. Biochemical characterization revealed a higher protein and lipid content both in *N. gaditana* and *S. almeriensis* which were grown on PM. Anyway, regardless of the growth medium used, *N. gaditana* presented higher protein and lipid content than *S. almeriensis*. Microbiological analysis shows no evidence of pathogen contamination (absence of *Salmonella* spp.; *E. coli* <100 cfu/g), neither in microalgae produced on SM nor in those produced on PM. Growth performance, nutrient utilization and muscle composition in fish fed microalgae-supplemented diets were similar

to those of the control group, showing they fulfilled the fish nutrient requirements for assuring sturgeon fingerlings growth and fillet nutritional quality. However, sturgeon fed-diets containing hydrolyzed *N. gaditana* biomass, grown on PM, reached greater average final weight then the other fish groups, included the control group. These results suggest the potential application of microalgae obtained by biorefinery as a protein and lipid source in practical diets for Siberian sturgeon (*A. baerii*). In particular, *N. gaditana* was revealed to be a potential functional ingredient in aquafeed, usable to improve the sustainability of microalgae production and of the aquaculture sector, through a circular bioeconomy approach.

Keywords: Sturgeon, Aquafeed, Hydrolysis, Microalgae, Biorefinery, Circular-Economy.

1. Introduction

The global population is forecast to increase rapidly by 2050, requiring a significant increase in food production, with a special demand for high-quality protein. Under this context, it is expected aquaculture industry will grow a further 37% between 2016 and 2030, and its heavy reliance on feeds produced from wild-caught fish will be not sustainable [1]. There is a growing interest in developing functional and sustainable aquafeed from alternative sources while ensuring that farmed fish supply meets consumption demands [2, 3]. Thus, the use of microalgae as dietary ingredients and additives in aquaculture has received a lot of attention and numerous studies reported they can be used in aquafeeds as a sustainable ingredient in the replacement of fish meal and fish oil without compromising fish growth and nutrient utilization [4, 5, 6, 7]. Moreover, it has been reported that sometimes microalgae dietaries improve fish growth performance, as weight gain and protein deposition in muscle, playing positive effects on fish quality attributes [8] and fish health *status* [9, 10]. Other studies showed that microalgae inclusions in aquafeed can be beneficial to fish in terms of antioxidative properties [11, 12], lipid metabolism [13], gut functionality [14, 15, 16] and immune response and disease resistance [14, 17].

Despite numerous strengths supporting the use of microalgae in aquafeed, some critical issues were reported as limiting factors in their actual use and are mostly related to their high production cost and their cell wall structure and composition which acts like a protective barrier that reduces the bioavailability of the intracellular nutrients [18].

One of the potential solutions to reduce microalgae production costs is the development of integrated biorefineries based on the use of microalgal biotechnology to recovery and recycle nutrients using some agro-industrial by-products. Indeed, different microalgal species show good ability to grow on a complex waste stream of organic origin (digestate, pig manure wastewater, etc.) performing the remediation of nutrient pollutants, while producing biomass [19, 20, 21]. However, studies that incorporated processed microalgae from biorefineries as nutrients sources in aquafeed are limited. Previously results related to the potential inclusion of microalgal biomasses obtained from a biorefinery in aquafeed suggest their potential use as a valuable nutrients source, able to ensure both adequate growth performance and healthy gastrointestinal tracts in different fish species, such as Siberian sturgeon (*A. baerii*), Atlantic salmon (*S. salar*), common carp (*C. carpio*) and European sea bass (*D. labrax)* [22, 23, 14].

On the other hand, the application of enzymatic hydrolysis treatments is a promising tool for weakening the cellulosic-rich cell wall present in some microalgae species [24]. This kind of process improves the release of intracellular components such as low molecular weight bioactive peptides and free amino acids, increasing nutrient bioavailability and functional properties in fish [25, 26, 27]. Therefore, the production of microalgae-biomasses based on the biorefinery approach and the development of hydrolysates would enable to ensure high bioavailability of the intracellular nutrients and bioactive compound provided by microalgae contextually containing the zootechnical wastewater disposal costs. Moreover, the application of this circular economy approach makes the feed and fish production chain more sustainable.

In this study, the evaluation of the effects of the dietary inclusion of two microalgae, *Nannochloropsis gaditana* and *Scenedesmus almeriensis*, cultivated on synthetic medium and pig manure, was carried out in Siberian sturgeon (*Acipenser baerii*) fingerlings.

The aim was to study the potential application of microalgae from biorefinery as a functional ingredient in practical diets intended for freshwater fingerlings, assessing different parameters related to growth performance and oxidative *status*.

2. Materials and methods

2.1 Enzymatic hydrolysis of algal biomass

N. gaditana and *S. almeriensis* biomasses were produced in the SABANA facilities at the University of Almería using two culture media: Synthetic Medium (SM), consisting of a solution of dissolved fertilizers in water and Pig Manure medium (PM), a solution of clean water plus manure (10%). This dilution has been selected on the basis of previous experiences, as it provides a similar amount of nutrients than those contained in synthetic medium prepared using fertilizers. Fertilizers used in the trials were: $NaNO_3$, $MgSO_4$ and KH_2PO_4, in order to have a concentration of 200 mg L^{-1} of N and 50 mg L^{-1} of P, approximately.

Microalgae were cultivated in open thin-layer reactors of 80 m^2 (3.0 m^3) in continuous mode at a dilution rate of 0.3 day^{-1}, under controlled dissolved oxygen and pH conditions by on-demand injection of air or CO_2. The daily average culture conditions were irradiance of 240 μmol photons m^{-2} s^{-1}, temperature 25 ± 4 °C, pH 8.0 ± 0.2 and dissolved oxygen 12 ± 5 mg/L. Microalga biomass was harvested by centrifugation (RINA centrifuge, Riera Nadeu SA, Spain), frozen, freeze-dried and milled. Then, the homogenized powder was stored in the dark at −20 °C until use. Both biomasses were subjected to an enzymatic hydrolysis treatment in order to improve the digestibility and increasing the nutrient bioavailability and functional properties compared with the untreated raw microalgal biomasses. For each enzymatic hydrolysis, wet sludge of *N. gaditana* and *S. almeriensis*

containing 300 g L^{-1} (dry weight) were transferred into a 10 L reactor. Then, microalgae biomass was incubated at 55 ºC under continuous agitation for 12 h in presence of a commercial cellulase (22178, Sigma-Aldrich, Madrid, Spain) providing a 0.05 enzyme to microalgae ([E]/[S]) ratio. In order to understand the degree of the hydrolysis process, the amount of reducing sugars released from microalgae was assessed using the dinitrosalicylic acid (DNS) method according to Miller [28]. Additionally, two batches of crude raw *N. gaditana* and *S. almeriensis* sludge, produced with synthetic medium and diluted pig manure, were freeze-dried without any previous treatment. In concluding, the following algal biomasses were produced considering the type of fertilizer and pre-treatment used: Crude *N. gaditana* grown on Synthetic Medium (C-NSM), or on Pig manure (C-NPM), Hydrolyzed *N. gaditana* grown on Synthetic Medium (H-NSM), or on Pig manure (H-NPM), Crude *S. almeriensis* grown on Synthetic Medium (C-SSM) or on Pig Manure (C-SPM), Hydrolyzed *S. almeriensis* grown on Synthetic Medium (H-SSM) or on Pig Manure (H-SPM).

2.2 Feed formulation and production

The dried algal biomasses were analyzed to determine their nutritional composition and microbiological evaluation (Tables 2 and 3). Then, nine experimental diets were formulated to be isoproteic (51.0% dw) and isolipidic (14.0% dw). The control diet (CT) mimics the ingredient composition of the commercial microalgae-free diets used for feeding sturgeon fingerlings. The experimental diets were prepared to contain 10% of each one of the microalgal biomass detailed above by partially replacing fish meal (FM) soybean and fish oil (Table 1), and the formula was established considering the nutritional composition of the dried algal biomasses (Table 2). Diets were elaborated by Ceimar-University of Almería (Service of Experimental Diets). In brief, all the ingredients were ground and mixed in a vertical spiral-shaped mixer (Sammic BM-10, capacity 10-L, Sammic, Azpeitia, Spain) before being supplemented with fish oil and diluted choline. The ingredients were mixed for 15 min, then integrated with water to obtain a homogeneous dough that was subjected to a cold extrusion process (Miltenz 51SP, JSConwell Ltd. New Zealand) for making

pellets with 1 mm diameter and 1.5 mm length. A temperature of about 60 °C was applied for the extrusion process for preserving the potential functional properties of the microalgae. Feeds were dried in a chamber at 25 ºC (Air-Frio, Almería, Spain) for 24 hours and stored in plastic bags under vacuum packaging conditions at −20 °C until use.

2.3 Fish, feeding trial and sampling

The feeding trial was carried out at the experimental facilities of the Istituto Sperimentale Italiano Lazzaro Spallanzani (Rivolta d'Adda, Italy). Four hundred thirty-two Siberian sturgeon (*A. baerii*) fingerlings (mean body weight 12.3 ± 0.1 g) were randomly divided among 27 groups of 16 specimens each and kept in twenty-seven 120-L fiberglass tanks in a recirculating aquaculture system (daily water exchange 2%, with mechanically filtered and UV treated water). Water parameters were monitored daily and kept constant and optimal for this species (temperature 18.9 ± 0.6 °C, dissolved oxygen 9.4 ± 0.98 mg L^{-1}, pH 8.0 ± 0.1, NH_4-N <0.06 mg L^{-1}, NO_2-N <0.2 mg L^{-1}). The photoperiod used was 12 h of artificial light and 12 h of darkness. After 15 days of acclimatization, dietary treatments were randomly assigned in triplicate to the groups. Fish were fed for 40 days with experimental diets by hand, six days per week in two daily meals (9:00 am and 5:00 pm) with a feed ratio equal to 3% of body weight.

At days 0, 15, 30 and at the end of the growth trial (40 days), fish were group-weighed after 24 h fasting period, under moderate anesthesia (MS222, 50 mg L^{-1}) to assess zootechnical parameters.

Growth performance and nutrient utilization were estimated using the following parameters: survival rate (SR, %), initial body weight (IBW, g), final body weight (FBW, g), feed intake (FI), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated as shown below:

FI (g): daily feed ingested x days

SGR (%):100 x [(ln final body weight – ln initial body weight)/days]

FCR: FI (g) / weight gain (g)

Six fish per tank (18 fish per dietary treatment) were randomly selected, euthanized with a bath of tricaine (Pharmaq) at a lethal concentration, then dried on absorbent paper and subjected to individual

biometric measurements (total length, body weight). The condition index was then calculated following the Fultons K-index:

$K = WL^{-3}$, where W is the weight and L is the total length of the fish.

A pool of the respective skinned fillets from three fish per tank (9 fish per dietary treatment) was frozen and stored at -20 °C for proximate and fatty acid analysis. A pool of livers from the same fish was frozen and stored at -20 °C until superoxide dismutase (SOD) and catalase (CAT) activity were measured.

The fish handling procedures and sampling methods used in the trial followed the guidelines of the E.U directive 2010/63/EU on the protection of animals used for scientific purposes.

2.4 Chemical analysis

Dried microalgal biomasses, experimental diets and fillet muscle tissues were analyzed for dry matter, crude protein, total lipids and ash according to AOAC methods [29] after grinding and homogenization. Total lipids were performed according to the Folch method [30] and then an aliquot of lipids (about 20 mg) was employed for fatty acid profile determination according to Christie [31]. Chromatographic conditions were set according to Bongiorno *et al.* [22]; fatty acids were identified by comparison of retention times with standard 37 fatty acids methyl esters (FAME) mixture in dichloromethane and standard Menhaden fish oil, obtained from Supelco (Supelco, Bellafonte, PA, USA) and expressed as a percentage of total fatty acids.

The amino acid profile of microalgae biomass was determined after acidic hydrolyzation (100 mg of sample were hydrolyzed in 10 mL of 6 N HCl under vacuum at 110 °C for 24 h) and then filtrate hydrolysate aliquot (1 mL) was taken and evaporated to dryness under nitrogen at 40 °C, and the dry residue was redissolved in 2 mL of distilled water. Amino acids were determined according to Graser *et al.* [32] using precolumn derivatization with o-phethaldialdehyde (OPA)/3-mercaptopropionic acid (MPA), employing Norvaline as internal standard. Derivatization and chromatographic conditions were set according to Bongiorno *et al.* [22].

The total antioxidant capacity of algae was tested on freeze-dried algae samples. A PAO Total Antioxidant Capacity Kit (#KPA-050) was purchased by JaICA (Nikken, SEIL Co., Ltd). Briefly, the measurement was based on the reduction of Cu_2^+ to Cu^+ by mean of both the hydrophilic and hydrophobic antioxidant compounds, in the presence of a chromatic reagent (Bathocuproine). The Cu+ produced by the reduction step was detected by absorbance at the wavelength of 490 nm using an Infinite® F500 (Tecan Trading AG, Switzerland) spectrometer. Extraction was performed using a mixture of ethanol: water (3:1) in order to collect both polar and apolar compounds. After this step, extracts were centrifuged at 6,000 rpm for 5 min (Megafuge 1.0R, Heraeus Instruments, Hanau, Germany) and an aliquot of supernatant (10 µL) was employed in the total antioxidant capacity assay. Total antioxidant capacity was expressed as cupric ion reducing power (µmol L^{-1}), corresponding to the uric acid concentration multiplied by 2189 (1 mM of uric acid = 2189 µmol L^{-1}). Each sample was tested in triplicate, with the exception of the antioxidant analysis which was performed in duplicate.

2.5 Microbiological analysis

The dried microalgae biomass and test diets were analyzed before their use in the feeding trial. For bacterial enumeration, 10 g of each sample was aseptically crushed with a mortar, mixed with 90 mL of sterile peptone water (bacteriological peptone 1 g L^{-1}, Thermo Scientific Oxoid, Thermofisher, Rodano, Italy) in a sterile filter stomacher bag and homogenized (Seward Stomacher 400 Circulator) for 1 min at normal speed.

Ten-fold dilutions of homogenate were prepared in the same diluent and aliquots (1 mL) were pour-plated in specific agar media. Total mesophilic bacteria were enumerated in Plate Count Agar (Oxoid) at 30 °C for 72 h [33]. *Enterobacteriaceae* were determined on Violet Red Bile Glucose agar (Oxoid) at 37 °C for 24 h [34]. *Escherichia coli* were determined on ChromID Coli agar (Biomerieux, Bagno a Ripoli, Italy) at 44 °C for 24 h [35]. For enumeration of sulphite-reducing Clostridia spores, sample homogenates were treated in a water bath at 80 °C for 10 min and cooled in iced water. Dilutions were pour-plated in Tryptose Sulphite Cycloserine agar (Merck, Darmstadt, Germany) and incubated

at 37 °C for 24-48 h under anaerobic conditions [36]. Presumptive *Clostridium perfringens* colonies on TSC were confirmed by acid phosphatase test (Sifin Diagnostics, Berlin, Germany) according to manufacturer's instructions [37]. The presence of *Salmonella* spp. was qualitatively determined in 25 g of sample in accordance with ISO standard method [38]. All microbiological analyses were performed in triplicate. The results of the microbial counts were expressed as means of log colony-forming units (CFU) per gram of sample ± standard deviations (SD).

2.6 Superoxide Dismutase (SOD) and Catalase (CAT) analysis

An aliquot of sturgeon livers from the pool made with fish coming from the same tank (about 50-100 mg from each fish) was rinsed with phosphate-buffered saline (PBS) solution, pH 7.4, to remove any red blood cells and clots, then homogenized on ice in 10 mL g^{-1} tissue of cold buffer (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA for CAT assay and 20 mM HEPES, pH 7.2, 1 mM EGTA, 210 mM mannitol and 70 mM sucrose for SOD assay). Tissues homogenized with respective buffers were centrifuged at 10,000 g for 15 min, at 4 °C and the supernatant was stored at −80 °C until the assays were performed. Enzymatic assays were performed by means of specific kit purchased by Cayman Chemical (Ann Arbor, Michigan, Catalase Assay Kit Item No. 707002 and Superoxide Dismutase Assay Kit item No 706002). The response for each enzymatic activity was determined by the absorbance showed at 540 nm for CAT and 450 nm for SOD, using an Infinite® F500 (Tecan Trading AG, Switzerland) spectrometer. CAT activity was expressed as the amount of enzyme that caused the formation of 1.0 nmol of formaldehyde (oxidation product) per min at 25 °C (nmol min^{-1} mL^{-1}). Total SOD activity (cytosolic and mitochondrial) was expressed as unit of enzyme that exhibits 50% dismutation of the superoxide radical (U mL^{-1}). Each sample was tested in triplicate.

2.7 Statistical analysis

Data are expressed as mean ± standard deviation. Prior to statistical analysis, all the data were evaluated for normality distribution, except for antioxidant properties which were analyzed in duplicate. Differences between treatments were analyzed by one-way analysis of variance (ANOVA)

and, if adequate, means were compared using Duncan's test, set for $P < 0.05$. All the analyses were carried out using the SPSS-PC release 17.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

3.1 Chemical and microbiological characterization of the microalgae biomasses

The chemical composition, total antioxidant capacity and microbiological traits of the freeze-dried biomass of *N. gaditana* grown on Synthetic Medium (NSM) and Pig Manure (NPM) and *S. almeriensis* grown on Synthetic Medium (SSM) and Pig Manure (SPM) are shown in Tables 2 and 3. The data relating to the microalgae biochemical characterization show that *N. gaditana* biomasses had a higher content in protein, lipid and ash compared to *S. almeriensis.* regardless of the growth medium used. Both *N. gaditana and S. almeriensis* microalgae, grown on PM, had a higher protein and lipid content than those grown on SM. *N. gaditana* grown on SM had the highest ash content. The higher protein content in algae grown on diluted pig manure medium could be largely influenced by the culture medium or operation and growth conditions. Also, the higher presence of bacteria (which usually have high protein content) in biomass grown on pig manure medium could contribute to the differences in composition between the two different biomasses grown on the different media (PM *vs* SM). Because operation conditions were the same, probably differences in protein content could be attributed to culture medium composition or differences in bacteria content. Higher nitrogen availability found when using pig manure can increase the protein content of the final biomass produced. In this study, the presence of bacteria was evaluated in all the dry biomasses tested and not large differences being observed (see table 3). In order to dispel any doubts, further microbiological investigation will be conducted also on fresh biomasses.

The amino acid composition, specifically the content in essential amino acids, is considered a quality criterion to determine the quality of microalgae. Overall, results appointed an amino acids

composition of the microalgae used in this study similar to that previously observed in those microalgae strains, with an adequate level of essential amino acids.

Fatty acid composition of freeze-dried microalgae (Table 2) shows that, in general, for both microalgal species, the Saturated Fatty Acids (SFA) were higher in those grown on SM, while Monounsaturated Fatty Acid (MUFA) were higher in the microalgae grown on PM. Concerning Polyunsaturated Fatty Acids (PUFA), similar values were found for each microalgae species grown on SM and PM. However, PUFA content in *S.almeriensis* was much lower than those observed in *N. gaditana*. In particular, PUFA were the predominant fraction in *N. gaditana* grown on both SM and PM (62.4% and 63.2%, respectively), followed by SFA (35.7% and 33.4%, respectively), and MUFA (1.9% and 3.5%, respectively). Instead, the SFA were the predominant lipid fraction in *S. almeriensis* grown both on SM and PM (reaching values of 47.0 and 40.2% of the total fatty acids, respectively), followed by PUFA (36.8% - 36.2%, respectively) and by MUFA (16.2% and 23.6%, respectively). Biomass antioxidant analysis showed that, regardless of the microalgal growth medium used, *N. gaditana* presented greater antioxidant capacity than *S. almeriensis*. However, the microalgae seemed to lightly implement their antioxidant properties when grown on PM (Table 2).

About the microalgae microbiological quality (Table 3), it is possible to note that all the biomasses studied showed a total bacterial content between 5.0 and 6.0 log CFU g^{-1}, regardless of the grown medium (SM or PM) used. Hygienic indicator parameters such as *Enterobacteriaceae* and *E. coli* were found at low levels in both *N. gaditana* and *S. almeriensis* grown in SM. Sulfite-reducing Clostridia spores were found at higher levels (> 4.0 log CFU g^{-1}) in microalgae biomasses cultivated on PM. The presence of *C. perfringens* was confirmed in all samples by acid positive reaction to phosphatase assay of characteristic colonies isolated from TSC agar plates. No *Salmonella* spp. was found, neither in *N. gaditana* nor in *S. almeriensis* dried biomasses grown on both SM and PM.

3.2 Enzymatic hydrolysis of algal biomass

Fig. 1 shows the amount of reducing sugars released during the enzymatic hydrolysis of microalgae. Quantification of reducing sugars showed a significant increase in glucose concentration over the

enzymatic reaction reaching values of 8.7 g and 9.1 g free glucose equivalent per 100 g of microalgae biomass in *N. gaditana* and *S. almeriensis,* respectively. In both microalgae species, the biomass grown in PM (H-NPM and H-SPM) showed significantly lower values of releasing sugars than those observed in microalgae grown in SM, especially in *S. almeriensis* (Fig. 1B). This phenomenon might be related to the microbial composition of PM, in which there is a high content of endogenous bacteria (10^7 cells mL^{-1}) with short generation times [39]. These manure endogenous bacteria are mainly Gram-positive fermenting bacteria such as *Eubacterium*, *Bacillus*, *Lactobacillus* and *Streptococcus*, which could lead to the sugar transformation in by-products such as acetic acid due to fermentative action [40]. On the other hand, this reduction was not observed in *N. gaditana*. As marine microalga, the salinity required in the culture medium may limit the growth of all kinds of microorganisms present in algal biomass [41]. Overall, results obtained evidenced the effectiveness of previous enzymatic hydrolysis for weakening microalgae cell wall able to increase the nutritional value raising the bioavailability of intracellular components.

3.3 Chemical composition and microbiological characterization of experimental diets

The chemical composition of the experimental diets is shown in Table 4. The dry matter, crude protein, total lipids, ash contents were similar between the diets and adequate to fulfill the nutritional and energy requirements of Siberian sturgeon fingerlings.

No relevant differences were observed in the fatty acid profile of experimental diets. The amount of saturated fatty acids (SFA) ranged from 25.6% (CT) to 27.8% (C-NPM) in the diets. Monounsaturated fatty acids (MUFA) were mainly represented by the oleic acid C18:1n-9, with values between 29.3% (C-NPM) and 31.6% (CT, H-SPM). Polyunsaturated fatty acids (PUFA), with values of 42.3% (H-SSM, H-SPM) and 43.9% (C-NSM), represented the most abundant category of fatty acids and mainly represented by linoleic acid (18:2n-6) with values between 20.3% (H-SSM) and 21.9% (H-NSM).

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content were similar in all the experimental diets with values ranging from 6.97 to 7.42%, of EPA and 7.97 to 8.7% of DHA.

Microbiological traits of the test diets are shown in Table 5. Overall, the experimental diet showed a moderate microbial load. The total bacterial content was slightly higher when microalgae biomasses were grown on PM, where included crude in the diets. Concerning the hygienic quality indicators, *Enterobacteriaceae* and *E. coli* (<2.0 log CFU g^{-1}) and *Salmonella* spp. (absent in 25 g) were not detected. In the experimental diets containing microalgae biomasses grown on PM and included crude in aquafeed, a low number of *C. perfringens* spore were detected among sulfite-reducing Clostridia colonies, grown on TSC agar, This result agrees with previous studies reported the same findings when an untreated blend of microalgae, *Scenedesmus-Chroococcus*, cultivated on digestate from biorefinery was included in aquafeed formulated for *A. baerii* [22]. However, as in that previous study, no clinical signs were observed in fish fed with feed containing a low presence of *C. perfringens* spores. Furthermore, all the fish presented adequate health status at the end of the feeding trial, regardless of feed treatment applied.

3.4 Growth performance, nutrient utilization and muscle chemical composition

The growth of *A. baerii* fed on the experimental diet throughout the 40-day trial is shown in Fig. 2. Overall, all the diets were palatable and similarly ingested by fish. No illness symptoms were observed during the entire trial among all the treatments, and low mortality was recorded in all the experimental groups and the control (2.5% on average), which was not attributable to dietary treatments.

IBW on average was 12.74±0.39 g and, at the end of the feeding period, all experimental group tripled their initial weight. After 40 days of feeding experimental diets, experimental groups fed on *N. gaditana*-supplemented diets achieved better results in terms of growth and nutrient utilization parameters (FBW, SGR, FCR) than those observed in fish fed with *S. almeriensis*. However, no significant differences were found in comparison with the CT, except for the H-NPM group which achieved the best results (Table 6).

All the groups fed on *S. almeriensis*-supplement diets showed growth parameters values slightly worse than the CT group but still similar to those expected for this species.

On the other hand, no significant differences between experimental groups were found in K-factor (Table 6).

Scarce research has been done aiming to study the integration of microalgae meal in partial replacement of fish meal (FM) in diets for acipenserids. The only studies conducted showed that supplementation with 40-50% *Arthrospira* sp. [42, 43], and with 10% of a blend of *Scenedesmus-Chroococcus* [22], can be valid alternative to fishmeal and fish oil, in both white (*A. transmontanus*) and Siberian sturgeon (*A. baerii*). In the present study, the results obtained related to growth parameters are similar to those reported in other feeding trials with the same species during the same growing phase [22, 42, 43, 44, 45] where novel protein ingredients were evaluated. Recent studies, like Galafat *et al.* [25], who evaluated the effect of the low dietary inclusion of *A. platensis* hydrolysate (2 and 4%) in gilthead seabream (*S. aurata*) juveniles, underline the potential application of microalgae hydrolysates as an additive in aquafeed improving both intestinal functionality and antioxidant capacity. However, there are no previous works that pointed the effect of dietary inclusion of hydrolyzed *S. almeriensis* and *N. gaditana* from biorefinery. According to Sáez *et al.* [46] and Vizcaíno *et al.* [47], the use of *N. gaditana* as a feed additive (5%) for feeding *S. aurata* juveniles has an effect on protein utilization and antioxidant activity and increases the level of activity in several digestive enzymes. Moreover, in the case of fish fed on microalgae hydrolysates, it was found as additional effect a significant increase in the apical area of enterocytes, which might reflect a higher mucosal absorptive capacity. Bongiorno et al. [22] conducted a feeding trial, reporting no detrimental effects on growth performance and nutrient utilization, attributable to 10% dietary inclusion of a blend of microalgae for replacing fishmeal and fish oil in this species. To the best of our knowledge, the effect of microalgae hydrolysate supplementation has not been assessed before, and the level was set at 10% dietary inclusion considering the previous findings of those authors.

In the present study, sturgeons fed with diets including *N. gaditana* showed better growth performance than sturgeons fed with *S. almeriensis* and in particular, sturgeon fed with *N. gaditana*

grown on PM and hydrolyzed showed better growth than control-fed fish. Probably, enzymatic treatment may increase the bioavailability of potentially bioactive metabolites and improve intestinal functionality and feed nutrients assimilation, which lead positive effects on growth. Regarding muscle composition, the inclusion of different types of microalgae did not affect protein, lipid and ash content (Table 7). Administered experimental diets and muscle fatty acid profile showed a predominance of PUFA, followed by MUFA and SFA. In particular, the ratio of saturated to unsaturated fatty acids in the muscle, ranging from 0.31 to 0.37, reflects the ratio found in dietary treatment, where values similarly ranging from 0.34 to 0.39 (Table 4 and 7). Palmitic acid, oleic acid, linoleic acid and docosahexaenoic acid were the most abundant fatty acid found in the different fish group. Concerning the singular fatty acid, slight differences observed in sturgeon might be attributable to the effect of the diet but without a particular trend (Table 7). However, these differences never affected the percentages of long-chain n-3 series fatty acids (such as EPA and DHA), considered as nutritionally relevant FA associated to positive physiological functions, neither the n3/n6 ratio, that ranged between 1.11 and 1.23, always reaching values >1.

3.5 Liver antioxidant SOD, CAT activity

CAT and SOD are enzymatic species known to act as a protective barrier against the activity of toxic oxygen reactive species (ROS) that can be found in different species tissues [48, 49]. It is widely known that the activity of antioxidant protective systems can be modified by several environmental [50] and physiological [51, 52] factors. Particularly, CAT and SOD activity in the liver of fish can be influenced by the dietary pattern followed by the fish, especially by its lipid and starch content. Actually, higher dietary lipids content has been previously been associated to an increase in the enzymatic activity, acting against oxidation processes [53]. The liver enzymatic assays performed in the present study did not show any significant differences among groups of fish fed with different diets, as detailed in Table 8. The absence of significant differences in the hepatic CAT and SOD activity among the experimental groups suggests that supplemented diets did not induce any variation

in the oxidative *status* of *A. baerii* fingerlings, despite the relevant difference in antioxidant capacity among the 2 microalgal species. This might be imputed to the too low percentage of algae included in the diets or to the duration of the feeding trial, not long enough for reaching an observable movement of the antioxidative parameters related to dietary microalgae supplementation. On the other hand, these results agree to the fact that all the dietary formulations tested in this study represented a similar lipid content, ranging from 13.25% and 14.60%.

4. Conclusions

Scarce information is available on the use of biomass from biorefineries in aquafeed and this study contribute with new knowledge on this topic. The results obtained confirm the potential use of *S. almeriensis,* and especially of *N. gaditana* biomasses as protein and lipid source to partially substitute fishmeal and fish oil in aquafeeds for Siberian sturgeon fingerlings. In particular, both microalgae species, obtained from biorefinery based on nutrient recovery from pig manure, did not affect the proximate composition and growth performance of fish. The application of enzymatic hydrolysis pre-treatment to microalgae biomass revealed useful for improving the nutrient bioavailability and furthermore, appears to be effective for preserving the microbiological quality of both ingredients and feeds. Moreover, data observed suggested the potential application of hydrolyzed *N. gaditana* as functional ingredient in aquafeeds for improving growth performance in acipenserids and make more sustainable and feasible both wastewater treatment and microalgae production. Turning the zootechnical wastewater into high-value products minimizes waste, hence in line with the circular economy concept. Further studies will be carried out to verify the gut health *status* of fish fed on these experimental diets and a detailed economic analysis will be performed when the process will be performed at large scale.

Founding

This work has received funding from the European Union HORIZON 2020 Research and Innovation Program under the project SABANA (Grant Agreement No. 727874). We thank Agricola Italiana Alimentare (AIA srl), partner of the project, for supporting the study.

Statement of informed consent, human/animal right

No conflicts, informed consent, or human or animal rights are applicable to this study.

CRediT Author Contributions Statement

"Conceptualization, K Parati, F J Alarcon López, G Acien Fernández; Data curation, K Parati, F J Alarcón López, T Bongiorno, D Carminati, V M Moretti ; Formal analysis, Annalaura Lopez, M Vasconi, A J Vizcaíno, F J Alarcon López, T Bongiorno; Funding acquisition, G Acien Fernández, Katia Parati; Investigation, Tiziana Bongiorno, Luciano Foglio, Lorenzo Proietti; Methodology, K Parati, Tiziana Bongiorno, F J Alarcon López; Project administration, G Acien, K Parati ; Resources, Tiziana Bongiorno, Luciano Foglio, Lorenzo Proietti, D Carminati, A J Vizcaíno, A. Lopez; Software, T Bongiorno, A J Vizcaíno, A Lopez, Supervision, Katia Parati, F J Alarcon López, M Vasconi, Vittorio Maria Moretti, G Acien Fernández; Validation, K Parati, , F J Alarcon López, D Carminati, V M Moretti; Visualization, Tiziana Bongiorno; Writing – original draft, Tiziana Bongiorno; Writing – review & editing, Tiziana Bongiorno, K Parati, D Carminati, M Vasconi, F J Alarcon López

References

1. FAO 2018. The State of World Fisheries and Aquaculture (2018). - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.
2. Tacon A.G.J., Metian M. (2015). Feed matters: satisfying the feed demand of aquaculture. Rev Fish Science Aquaculture, https://doi.org/10.1080/23308249.2014.987209
3. Oliva-Teles A, Enes P., Peres H. 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Davis DA (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
4. De Cruz C.R., Lubrano A., Gatlin D.M. III (2018). Evaluation of microalgae concentrates as partial fishmeal replacements for hybrid striped bass *Morone* sp. Aquaculture, https://doi.org/10.1016/j.aquaculture.2018.04.060

5. Perez-Velazquez M., Gatlin D.M. III, González-Félix M.L., García-Ortega A. (2018). Partial replacement of fishmeal and fish oil by algal meals in diets of red drum *Sciaenops ocellatus*. Aquaculture, https://doi.org/10.1016/j.aquaculture.2018.01.0016.
6. Shah M.R., Lutzu G.A., Alam A., Sarker P., Chowdhury M.K., Parsaeimehr A., Liang Y., Daroch M. (2018). Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of Applied Phycology, https://doi.org/10.1007/s10811-017-1234-z
7. Tulli F., Chini Zittelli G., Giorgi G., Poli B. M., Tibaldi E., Tredici M. R. (2012) Effect of the Inclusion of dried *Tetraselmis suecica* on growth, feed utilization, and fillet composition of European sea bass juveniles fed organic diets, Journal of Aquatic Food Product Technology, https://doi.org/10.1080/10498850.2012.664803
8. Sáez M.I., Vizcaíno A., Galafat A., Anguís V., Fernández-Díaz C., Balebona M.C., Alarcón F.J., Martínez T.F. (2020). Assessment of long-term effects of the macroalgae *Ulva ohnoi* included in diets on Senegalese sole (*Solea senegalensis*) fillet quality, Algal Research, https://doi.org/10.1016/j.algal.2020.101885.
9. Becker W. Microalgae for Aquaculture: The Nutritional Value of Microalgae for Aquaculture. In: Richmond, A. (eds.): Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Oxford, 2004, 380-391
10. Becker E. (2007). Micro-algae as a source of protein. Biotechnoly Advances, https://doi.org/10.1016/j.biotechadv.2006.11.002
11. Sales R., Galafat A., Vizcaíno A.J., Sáez M.I., Martínez T.F., Cerón-García M.C., Navarro-López E., Tsuzuki M.Y., Acién-Fernández F.G., Molina-Grima E., Alarcón F.J. (2021). Effects of dietary use of two lipid extracts from the microalga *Nannochloropsis gaditana* (Lubián, 1982) alone and in combination on growth and muscle composition in juvenile gilthead seabream, *Sparus aurata*. Algal Research, https://doi.org/10.1016/j.algal.2020.102162.
12. Teimouri M., Yeganeh S., Mianji G.R., Najafi M., Mahjoub S. (2019). The effect of *Spirulina platensis* meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation

of rainbow trout (*Oncorhynchus mykiss*). Fish Physiology Biochemistry, https://doi.org/10.1007/s10695-019-0608-3

13. Robin J.H., Vincent B. (2003). Microparticulate diets as first food for gilthead sea bream larva (*Sparus aurata*): study of fatty acid incorporation. Aquaculture, https://doi.org/10.1016/S0044-8486(03)00310-7
14. Valente L.M.P., Custódio M., Batista S., Fernandez H., Kiron V. (2019). Defatted microalgae (*Nannochloropsis* sp.) from biorefinery as a potential feed protein source to replace fishmeal in European sea bass diets. Fish Physiology Biochemistry, https://doi.org/10.1007/s10695-019-00621-w
15. Vizcaíno A.J., López G., Sáez M.I., Jiménez J.A., Barros A., Hidalgo L., Camacho-Rodríguez J., Martínez T.F., Cerón-García M.C., & Alarcón F.J. (2014). Effects of the microalga *Scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream, *Sparus aurata*, juveniles. Aquaculture, https://doi.org/10.1016/j.aquaculture.2014.05.010
16. Vizcaíno A.J., Rodiles A., López G., Sáez M.I., Herrera M., Hachero I., Martínez T.F., Cerón-García M.C., & Alarcón F.J. (2018). Growth performance, body composition, and digestive functionality of Senegalese sole (*Solea senegalensis* Kaup, 1858) juveniles fed diets including microalgae freeze-dried biomass. Fish Physiology Biochemistry, https://doi.org/10.1007/s10695-018-0462-8
17. Messina M., Bulfon C., Beraldo P., Tibaldi E., Cardinaletti G. (2019). Intestinal morpho-physiology and innate immune status of European sea bass (*Dicentrarchus labrax*) in response to diets including a blend of two marine microalgae, *Tisochrysis lutea* and *Tetraselmis suecica*, Aquaculture, https://doi.org/10.1016/j.aquaculture.2018.09.054.
18. Wu C., Xiao Y., Lin W., Li J., Zhang S., Zhu J., Rong J. (2017). Aqueous enzymatic process for cellwall degradation and lipid extraction from *Nannochloropsis* sp. Bioresource Technology https://doi.org/10.1016/j.biortech.2016.10.063

19. Osundeko O., Ansolia P., Gupta S. K., Bag P., & Bajhaiya A. K. (2019). Promises and challenges of growing microalgae in wastewater. Water Conserv Recyc Reuse, Issues and Challenges. https://doi.org/10.1007/978-981-13-3179-4_2

20. Pizzera A., Scaglione D., Bellucci M., Marazzi F., Mezzanotte V., Parati K., Ficara E. (2019). Digestate treatment with algae-bacteria consortia: a field pilot-scale experimentation in a sub-optimal climate area. Bioresource Technology, https://doi.org/10.1016/j.biortech.2018.11.067

21. Acién Fernández F.G., Gómez-Serrano C. and Fernández-Sevilla J.M. (2018). Recovery of nutrients from wastewaters using microalgae. Frontiers Sustainable Food System 2:59. https://doi.org/10.3389/fsufs.2018.00059

22. Bongiorno T., Foglio L., Proietti L., Vasconi M., Lopez A., Pizzera A., Carminati D., Tava A., Vizcaíno A.J., Alarcón F.J., Ficara E., Parati K.. (2020). Microalgae from Biorefinery as Potential Protein Source for Siberian Sturgeon (*A. baerii*) Aquafeed. Sustainability, https://doi.org/10.3390/su12218779.

23 Kiron V., Phromkunthong W., Huntley M., Archibald I., De Scheemaker G. (2012). Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. Aquaculture Nutrition, https://doi.org/10.1111/j.1365-2095.2011.00923.x

24 Zhang Y., Kong X., Wang Z., Sun Y., Zhu S., Li L., & Lv P. (2018). Optimization of enzymatic hydrolysis for effective lipid extraction from microalgae *Scenedesmus* sp. Renewable Energy, https://doi.org/10.1016/j.renene.2018.01.078

25 Galafat A., Vizcaíno A.J., Sáez M.I. *et al*. (2020). Evaluation of *Arthrospira* sp. enzyme hydrolysate as dietary additive in gilthead seabream (*Sparus aurata*) juveniles. Journal of Applied Phycoly, https://doi.org/10.1007/s10811-020-02141-0

26. Montone C.M., Capriotti A.L., Cavaliere C., La Barbera G., Piovesana S., Chiozzi R., Laganà A. (2018). Peptidomic strategy for purification and identification of potential ACE-inhibitory and antioxidant peptides in *Tetradesmus obliquus* microalgae. Analytical and Bioanalytical Chemistry https://doi.org/10.1007/s00216-018-0925-x

27. Wang X., Wang H., Pierre J.F., Wang S., Huang H., Zhang J., Liang S., Zeng Q., Zhang C., Huang M., Ruan C., Lin J., Li H. (2018). Marine microalgae bioengineered *Schizochytrium* sp. meal hydrolysates inhibits acute inflammation. Scientific Reports, https://doi.org/10.1038/s41598-018-28064-y

28 Miller G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry, https://doi.org/10.1021/ac60147a030

29. AOAC (1996). Official methods of analysis of the association of official analytical Chemists. Arlington (U.S.A.): Association of Official Analytical Chemists, https://doi.org/10.1016/0924-2244(96)10017-0

30. Folch J.; Lees M.; Sloane Stanley G.H. A. A. (1957). Simple method for the isolation and purification of total lipid from animal tissues. Journal of Biological Chemistry, https://doi.org/10.12691/jfnr-5-1-6

31. Christie W.W. (2003). Preparation of derivatives of fatty acids. In Lipid Analysis. Isolation, Separation, Identification and Structural Analysis of Lipids, Third Edition; Christie, W.W., Ed.; The Oily Press: Bridgwater, UK, 2003; pp. 205–215. ISBN 0-9531949-5-7

32. Graser T.A., Godel H.G., Albers S., Földi P. and Fürst P. (1985). An ultra rapid and sensitive high-performance liquid chromatographic method for determination of tissue and plasma free amino acids, Analytical Biochemistry, https://doi.org/10.1016/0003-2697(85)90064-8

33. ISO 4833-1, 2013. Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 °C by the pour plate technique. International Organization for Standardization, Geneva, Switzerland.

34. ISO 21528-2, 2004. Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae* -Part 2: Colony-count method. International Organization for Standardization, Geneva, Switzerland.

35. ISO 16649-2, 2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at

44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Organization for Standardization, Geneva, Switzerland.

36. ISO 7937, 2004. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Clostridium perfringens* - Colony-count technique. International Organization for Standardization, Geneva, Switzerland.

37. Ryzinska-Paier G., Sommer R., Haider J.M., Knetsch S., Frick C., Kirschner A.K.T., Farnleitner A.H. (2011). Acid phosphatase test proves superior to standard phenotypic identification procedure for *Clostridium perfringens* strains isolated from water. Journal of Microbiology Methods, https://doi.org/10.1016/j.mimet.2011.08.006

38. ISO 6579-1, 2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Detection of Salmonella spp. International Organization for Standardization, Geneva, Switzerland.

39. Martin C., De la Noüe J., & Picard G. (1985). Intensive cultivation of freshwater microalgae on aerated pig manure. Biomass, https://doi.org/10.1016/0144-4565(85)90064-2

40. Snell-Castro R., Godon J.J., Delgenès J.P., & Dabert P. (2005). Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. *FEMS* Microbiology Ecology, https://doi.org/10.1016/j.femsec.2004.11.016

41. He H., Chen Y., Li X., Cheng Y., Yang C., & Zeng G. (2017). Influence of salinity on microorganisms in activated sludge processes: a review. International Biodeterioration & Biodegradation, https://doi.org/10.1016/j.ibiod.2016.10.007

42. Palmegiano G. B., Gai F., Daprà F., Gasco L., Pazzaglia M., Peiretti P.G. (2008). Effects of Spirulina and plant oil on the growth and lipid traits of white sturgeon (*Acipenser transmontanus*) fingerlings. Aquaculture Research, https://doi.org/10.1111/j.1365-2109.2008.01914.x

43. Palmegiano G. B., Agradi E., Forneris G., Gai F., Daprà F., Gasco L., Rigamonti E., Sicuro B., Zoccarato I. (2005). Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). Aquaculture Research, https://doi.org/10.1111/j.1365-2109.2005.01209.x

44. Caimi C., Renna M., Lussiana C., Bonaldo A., Gariglio M., Meneguz M., Dabbou S., Schiavone A., Gai F., Elia A.C., Prearo M., Gasco L. (2020) First insights on Black Soldier Fly *Hermetia illucens* L.) larvae meal dietary administration in Siberian sturgeon (*Acipenser baerii* Brandt) juveniles. Aquaculture, https://doi.org/10.1016/j.aquaculture.2019.734539

45. Rawski M.; Mazurkiewicz J.; Kierończyk B.; Józefiak D. (2020). "Black soldier fly full-fat larvae meal as an alternative to fish meal and fish oil in *Siberian Sturgeon* nutrition: The effects on physical properties of the feed, animal growth performance, and feed acceptance and utilization". Animals, https://doi.org/10.3390/ani10112119

46. .Sáez M.I., Vizcaíno A.J., Galafat A., Cerri R., Ruíz-Jarabo I., Tapia S.T., Suárez M.D., Martínez T.F., Alarcón F.J. (2019). Assessment of the effects of *Nannochloropsis gaditana* enzyme hydrolysates added into aquafeeds on growth, muscle composition, pigmentation and oxidative condition of sea bream (*Sparus aurata*) juveniles. Abstract in European Aquaculture 2019, pp 1333.

47. Vizcaíno A.J., Galafat A., Cerri R., Ruíz-Jarabo I., Tapia S.T., Suárez M.D., Martínez T.F., Alarcón F.J., Sáez M.I. (2019). Evaluation of enzymatically-hydrolysed *Nannochloropsis gaditana* as feed additive for feeding juvenile gilthead seabream: effect on intestinal functionality. Abstract in European Aquaculture 2019, pp 1339.

48. Karadag H., Fırat O., Fırat O. (2014). Use of oxidative stress biomarkers in *Cyprinus carpio* L. for the evaluation of water pollution in Ataturk Dam Lake (Adiyaman, Turkey). Bulletin Environmental Contam. Toxicology https://doi.org/10.1007/s00128-013-1187-0

49. Shrivastava A. (2015). Catalase Activity in Different Tissues of fresh water teleost *Heteropneustes fossilis* on exposure to Cadimum. IOSR Journal of Environmental. Science Toxicology and Food Technology, 1, 6–9 ISSN: 2319-2399

50. Filho D.W.; Boveris A. (1993). Antioxidant defenses in marine fish-II. Elasmobranchs. Comp. Biochem. Physiol., 106C, 415–418

51. Otto D.M.E.; Moon T.W. (1996). Endogenous antioxidant systems of two teleost fish, the rainbow trout and the black bullhead, and the effect of age. Fish Physiology Biochemistry, https://doi.org/10.1007/BF02112362

52. Nayak S.B.; Jena B.S.; Patnaik B.K. (1999). Effects of age and manganese (II) chloride on peroxidase activity of brain and liver of the teleost, *Channa punctatus*. Experimental Gerontology, https://doi.org/10.1016/S0531-5565(99)00021-2

53. Rueda-Jasso R., Conceicao L.E.C., Dias J., De Coen W., Gomes E., Rees J.F., Soares F., Dinis M.T., Sorgeloos, P. (2004). Effect of dietary non-protein energy levels on condition and oxidative status of Senegalese sole (*Solea senegalensis*) juveniles. Aquaculture, https://doi.org/10.1016/S0044-8486(03)00537-4

Figure
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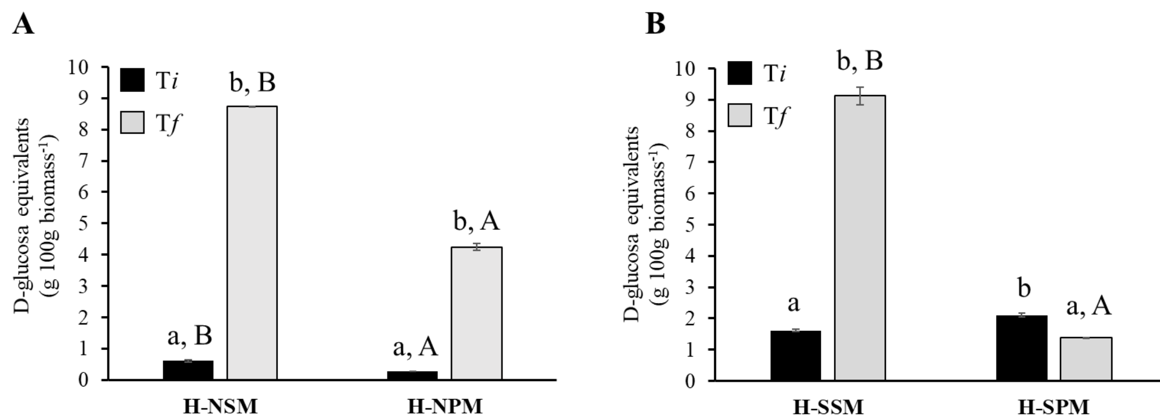

Fig. 1. Total D-glucose equivalents released from *N. gaditana* (A) and *S. almeriensis* (B) biomasses after enzymatic hydrolysis. Codes: Hydrolysed *N. gaditana* grown on Synthetic Medium (H-NSM), or on Pig manure (H-NPM); Hydrolysed *S. almeriensis* grown on Synthetic Medium (H-SSM) or on Pig Manure (H-SPM). Data represent mean ± SD. Different lowercase letters indicate significant differences ($P<0.05$) between initial and final time of enzymatic reaction (T*i* and T*f*, respectively). Different uppercase letters indicate significant differences ($P<0.05$) among microalgae grown on Synthetic Medium or Pig Manure.

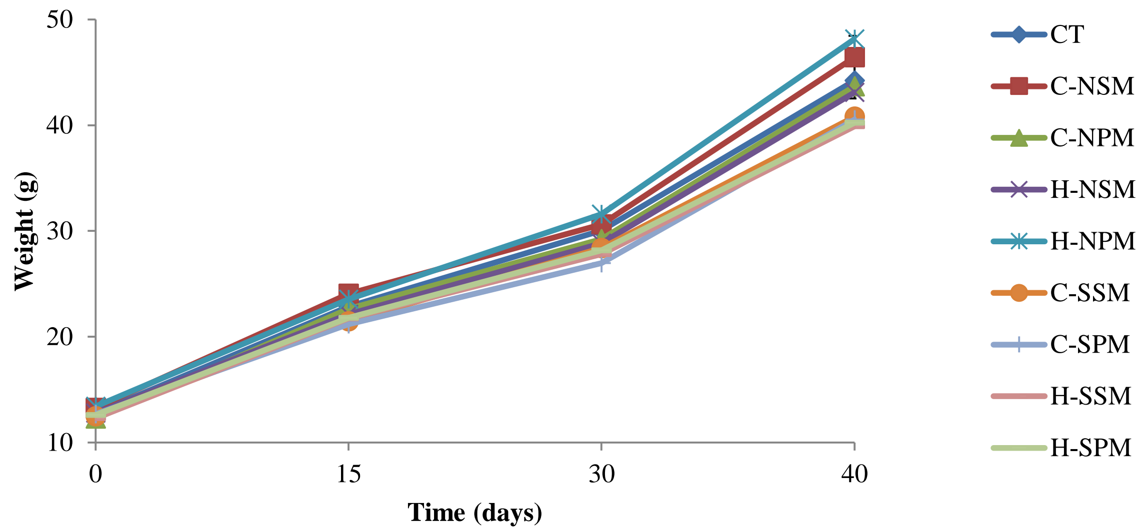

Fig. 2. Time course variation of body weight of *A. baerii* fingerlings fed the different experimental diets for 40 days (n =3, 16 fish per tank).

Table
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Table 1. Ingredient composition of experimental diets used in the feeding trial.

Ingredients (g kg^{-1} dry matter, DM)	CT	C-NSM	C-NPM	H-NSM	H-NPM	C-SSM	C-SPM	H-SSM	H-SPM
Fish meal[1]	274.0	225.0	225.0	225.0	225.0	211.0	211.0	211.0	211.0
Soybean protein concentrate[2]	250.0	250.0	250.0	250.0	250.0	250.0	250.0	250.0	250.0
Gluten meal[3]	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Dried microalgae	**0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Attractant premix[4]	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Fish solubles CPSP 90[5]	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Fish oil	50.0	41.8	41.8	41.8	41.8	51.0	51.0	51.0	51.0
Wheat meal[6]	40.5	3.1	3.1	3.1	3.1	3.5	3.5	3.5	3.5
Soybean oil	27.0	21.6	21.6	21.6	21.6	26.0	26.0	26.0	26.0
Soy lecithin[7]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin and mineral premix[8]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Guar gum	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Alginate	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride[7]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Betaine	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Lisine	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Methionine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Stay C Roche 0.2%	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

1 (protein: 69.4%; lipids: 12.3%), Norsildemel (Bergen, Noruega); 2 (protein: 51.5%; lipids: 8.0%); 3 (protein: 76.0%; lipids: 1.9%); 4 (50% squid meal, 50% krill meal); 5 (protein: 84.1%; lipids: 8.8%), Sopropeche (France);6 (protein: 12.0%; lipids: 2.0%); 7 SigmaAldrich (Madrid, Spain); 8 Vitamin and mineral premix.

Table 2. Proximate composition, amino acid (AA), fatty acid profile and Antioxidant Capacity of *N. gaditana* and *S. almeriensis* freeze dried biomass growth on both Synthetic Medium (NSM, SSM) and Pig Manure (NPM, SPM) (data expressed on dry matter basis).

Proximate composition (g/100 g microalgae), n=3	**NSM**	**NPM**	**SSM**	**SPM**
Water	4.5±0.0	4.7±0.01	8.0±0.0	3.4±0.02
protein	35.8±0.2	48.4±0.6	29.1±0.1	33.8±0.2
Lipid	14.6±0.2	18.6 0.8	2.12±0.0	8.39±0.2
Ash	32.8±0.1	23.8±0.1	25.2±0.0	13.8±0.0
Carbohydrate	8.2±0.03	4.5±0.1	18.5±0.0	29.2±0.0
Essential AA (g/100 g microalgae)	**NSM**	**NPM**	**SSM**	**SPM**
Arginine	4.06±0.01	5.52±0.04	3.29±0.02	4.27±0.06
Histidine	1.38±0.00	2.30±0.01	1.69±0.01	1.61±0.02
Isoleucine	1.60±0.04	2.10±0.03	1.26±0.01	1.59±0.01
Leucine	1.09±0.07	1.74±0.03	1.03±0.01	1.39±0.05
Lysine	1.61±0.08	2.30±0.05	1.43±0.02	1.80±0.10
Methionine	1.11±0.03	1.31±0.02	0.95±0.07	1.17±0.06
Phenylalanine	0.94±0.06	1.08±0.03	0.83±0.01	0.95±0.02
Tyrosine	0.99±0.05	1.24±0.01	0.72±0.07	1.02±0.02
Threonine	3.09±0.02	4.31±0.02	2.07±0.01	3.32±0.04
Valine	0.91±0.01	1.11±0.05	0.71±0.05	0.86±0.01
Non Essential AA (g/100 g microalgae)	**NSM**	**NPM**	**SSM**	**SPM**
Alanine	0.55±0.00	0.69±0.00	0.46±0.01	0.54±0.01
Aspartic acid	4.26±0.03	4.90±0.1	5.07±0.01	4.44±0.07
Glutamic acid	5.94±0.02	8.10±0.00	3.71±0.08	5.32±0.05
Glycine	0.59±0.01	0.73±0.00	0.49±0.00	0.61±0.02
Serine	1.99±0.04	2.53±0.05	1.47±0.03	2.17±0.01
Fatty acid composition (mg/100 g Fatty Acid)	**NSM**	**NPM**	**SSM**	**SPM**
14:0	0.7±0.0	0.8±0.0	5.6±0.3	0.7±0.1
16:0	22.4±0.5	20.2±0.4	15.8±0.3	29.4±0.3
16:1 n-7	0.6±0.0	1.0±0.0	5.3±0.2	1.8±0.1
17:0	12.2±0.5	11.9±0.9	15.2±0.5	2.1±0.1
16:2 n-4	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0
18:0	0.4±0.0	0.4±0.0	10.3±0.6	8.0±0.4
18:1 n-9	1.3±0.1	1.7±0.1	10.7±0.5	18.8±0.7
18:1 n-7	0.0±0.0	0.8±0.0	0.2±0.0	3.0±0.2
18:2 n-6 LOA	29.9±0.4	25.6±0.2	9.8±0.7	11.2±0.5
18:3 n-3 ALA	32.5±0.1	37.5±0.3	24.9±0.1	19.8±0.1
18:4 n-3	0.0±0.0	0.0±0.0	2.2±0.0	4.7±0.1
20:5 n-3 EPA	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.0
SFA	35.7±0.2	33.4±0.4	47.0±0.6	40.2±0.5
MUFA	1.9±0.0	3.5±0.1	16.2±0.4	23.6±0.7
PUFA	62.4±1.5	63.2±2.1	36.8±0.9	36.2±1.1
n3	32.5±0.7	37.5±1.0	27.0±0.9	25.0±0.5
n6	29.9±0.4	25.6±0.2	9.8±0.7	11.2±1.0
n3/n6	1.1±0.3	1.5±0.1	2.8±0.4	2.2±0.2
Total Antioxidant Capacity (μmol L^{-1} uric acid equivalent), n=2	**NSM**	**NPM**	**SSM**	**SPM**
	1040.5±51.7	1219.9±5.0	300.8±76.4	390.2±16.4

Data represent mean ± SD, n=3. Abbreviations: LOA, linoleic acid; ALA, α-linoleic acid; EPA, eicosapentaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 3 Microbial content of *N. gaditana* and *S. almeriensis* freeze dried biomass growth on both Synthetic Medium (NSM, SSM) and Pig Manure (NPM, SPM).

	NSM	**NPM**	**SSM**	**SPM**
Total viable aerobic count (log CFU g^{-1})	5.20 ±0.05	6.38±0.06	6.20±0.08	5.95±0.04
Enterobacteriaceae (log CFU g^{-1})	2.74 ±0.17	<2.00	2.04±0.21	<2.00
E. coli (log CFU g^{-1})	2.98 ±0.10	<2.00	<2.00	<2.00
Salmonella spp. (in 25 g)	absent	absent	absent	absent
Sulfite-reducing Clostridia spores (log CFU g^{-1})	<2.00	5.80±0.07*	2.60±0.16*	4.65±0.21*

Data represent mean ± SD, n=3. * Presence of *C. perfringens* colonies confirmed by positive reaction to acid phosphatase assay.

Table 4. Proximate composition and fatty acid profile of the experimental diets.

Proximate composition (g/100 g feed on dry basis)	**CT**	**C-NSM**	**C-NPM**	**H-NSM**	**H-NPM**	**C-SSM**	**C-SPM**	**H-SSM**	**H-SPM**
Moisture	11.1±0.0	13.7±0.03	12.2±0.0	11.4±0.0	10.6±0.02	11.4±0.0	10.6±0.0	10.5±0.0	9.5±0.01
Crude protein	51.5±0.20	50.31±0.01	52.3±0.2	51.3±0.02	52.4±0.03	50.2±0.2	50.1±0.1	51.1±0.0	51.6±0.02
Crude lipid	14.0±0.3	13.2±0.0	14.0±0.6	13.8±0.1	14.6±0.47	13.7±0.0	13.9±0.3	14.4±0.3	13.7±2.68
Ash	7.6±0.0	9.1±0.0	8.2±0.01	9.6±0.01	8.5±0.00	8.8±0.01	8.2±0.0	8.7±0.0	7.8±0.04
Carbohydrate	15.8±0.5	13.7±0.3	13.2±0.6	13.9±0.0	13.0±0.4	16.0±0.3	17.2±0.2	15.3±0.3	17.3±0.00
Fatty acid composition (g/100 fatty acid)	**CT**	**C-NSM**	**C-NPM**	**H-NSM**	**H-NPM**	**C-SSM**	**C-SPM**	**H-SSM**	**H-SPM**
14:0	4.42±0.10	4.18±0.17	4.70±0.11	4.30±0.21	4.18±0.17	4.33±0.24	4.43±0.12	4.48±0.31	4.30±0.16
15:0	0.38±0.02	0.41±0.01	0.49±0.03	0.44±0.01	0.41±0.01	0.40±0.00	0.39±0.06	0.40±0.05	0.38±0.00
16:0	17.08±0.31	17.94±0.58	18.38±0.61	18.32±0.24	17.65±0.02	17.64±0.18	17.95±0.41	17.70±0.37	17.48±0.55
16:1 n-7	3.83±0.12	3.57±0.18	3.83±0.50	3.60±0.33	3.60±0.27	3.65±0.56	3.79±0.10	3.84±0.20	3.75±0.49
17:0	0.28±0.01	0.83±0.03	0.98±0.07	0.81±0.05	0.89±0.05	0.39±0.01	0.40±0.02	0.38±0.00	0.37±0.01
16:2 n-4	0.40±0.05	0.37±0.02	0.41±0.07	0.37±0.03	0.40±0.03	0.44±0.07	0.47±0.01	0.48±0.07	0.47±0.03
16:3 n-4	0.39±0.05	0.35±0.00	0.40±0.02	0.37±0.01	0.38±0.00	0.36±0.05	0.38±0.06	0.39±0.01	0.38±0.04
18:0	3.19±0.16	3.11±0.22	3.11±0.32	3.15±0.12	3.12±0.23	3.31±0.11	3.38±0.10	3.20±0.19	3.26±0.09
18:1 n-9	15.97±0.43	14.89±0.71	14.98±0.52	15.16±0.67	15.86±0.34	16.30±0.71	16.05±0.50	16.53±0.53	16.69±0.78
18:1 n-7	2.41±0.15	2.28±0.09	2.32±0.06	2.28±0.1	2.35±0.06	2.31±0.09	2.37±0.21	2.37±0.17	2.42±0.34
18:2 n-6 LOA	20.42±1.20	21.70±2.01	21.06±0.93	21.91±0.07	20.69±2.22	21.62±1.71	20.71±0.76	20.34±0.52	20.59±1.23
18:3 n-3 ALA	2.39±0.20	3.95±0.08	4.29±0.19	3.82±0.21	4.49±0.14	3.43±0.11	3.04±0.07	3.24±0.05	3.05±0.03
20:0	0.24±0.03	0.22±0.00	0.18±0.01	0.24±0.03	0.24±0.00	0.29±0.01	0.27±0.01	0.27±0.03	0.28±0.01
18:4 n-3	1.73±0.17	1.59±0.08	1.75±0.12	1.53±0.16	1.65±0.07	1.65±0.13	1.79±0.16	1.82±0.10	1.71±0.04
20:1n-11	0.33±0.01	0.30±0.07	0.30±0.02	0.31±0.00	0.30±0.01	0.29±0.05	0.29±0.01	0.29±0.03	0.29±0.00
20:1 n-9	3.52±0.32	3.22±0.42	3.08±0.20	3.16±0.17	3.09±0.09	3.13±0.41	3.21±0.14	3.16±0.23	3.29±0.11
20:4 n-6 ARA	0.54±0.06	0.49±0.02	0.49±0.10	0.47±0.05	0.50±0.02	0.50±0.02	0.52±0.11	0.53±0.09	0.53±0.05
22:1 n-11	4.71±0.51	4.28±0.21	4.12±0.20	4.18±0.31	4.26±0.12	4.15±0.19	4.23±0.11	4.29±0.25	4.34±0.27
22:1 n-9	0.40±0.04	0.37±0.03	0.28±0.00	0.36±0.00	0.34±0.01	0.35±0.02	0.36±0.04	0.37±0.01	0.41±0.02
20:5 n-3 EPA	7.42±0.31	6.77±0.40	6.44±0.19	6.50±0.32	6.59±0.22	6.69±0.30	6.91±0.42	6.82±0.47	6.86±0.36
24:1	0.48±0.01	0.45±0.01	0.43±0.00	0.43±0.05	0.47±0.06	0.41±0.00	0.45±0.02	0.45±0.02	0.46±0.04
22:5 n-3	0.75±0.06	0.68±0.01	0.62±0.03	0.66±0.07	0.72±0.01	0.69±0.07	0.71±0.1	0.76± 0.12	0.79±0.06
22:6 n-3 DHA	8.74±0.11	8.04±0.70	7.36±0.26	7.62±0.63	7.83±0.58	7.66±0.42	7.88±0.90	7.85±0.21	7.91±0.76
SFA	25.59±1.27	26.69±2.5	27.84± 3.11	27.26± 3.04	26.49±1.05	26.36±1.21	26.82±0.98	26.43±1.11	26.06±0.56
MUFA	31.63±2.42	29.37±1.89	29.34±1.75	29.49±1.54	30.26±1.93	30.59±2.10	30.75±1.64	31.30±2.32	31.65±2.41
PUFA	42.78±3.16	43.94±4.26	42.82±3.77	43.25±4.04	43.24±3.90	43.05±3.57	42.42±1.56	42.27±1.25	42.29±1.09
n3	21.04±1.01	21.03±1.33	20.46±0.71	20.13±0.95	21.28±1.98	20.13±0.72	20.34±1.52	20.51±1.61	20.32±1.33
n6	20.96±0.9	22.19±2.04	21.55±1.65	22.38±2.27	21.18±1.30	22.12±1.54	21.23±0.99	20.90±0.64	21.12±1.08
n3/n6	1.00±0.05	0.95±0.07	0.95±0.05	0.90±0.02	1.00±0.07	0.91±0.04	0.96±0.06	0.98±0.06	0.96±0.05

Data represent mean ± SD, n=3, Abbreviations: LOA, linoleic acid; ALA, α-linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 5. Microbial content values in the experimental diets.

	CT	C-NSM	C-NPM	H-NSM	H-NPM	C-SSM	C-SPM	H-SSM	H-SPM
Total viable aerobic count (log CFU g^{-1})	3.69±0.30	4.24±0.12	5.34±0.8	3.95±0.07	4.40±0.06	4.15±0.06	5.41±0.06	4.45±0.21	3.78±0.11
Enterobacteriaceae (log CFU g^{-1})	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
E. coli (log CFU g^{-1})	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Salmonella spp. (in 25 g)	absent	absent	absent	absent	absent	absent	absent	absent	absent
Sulfite-reducing Clostridia spores (log CFU g^{-1})	<2.00	<2.00	4.57±0.03*	<2.00	<2.00	<2.00	3.00±0.09*	<2.00	<2.00

Data represent mean ± SD, n=3. * Presence of *C. perfringens* colonies confirmed by positive reaction to acid phosphatase assay

Table 6. Growth performance, nutrient utilization and somatic indices of *A. baerii* fingerlings fed the test diets over 40 days.

Growth parameters	**CT**	**C-NSM**	**C-NPM**	**H-NSM**	**H-NPM**	**C-SSM**	**C-SPM**	**H-SSM**	**H-SPM**
IBW (g)	12.8±0.2	13.3±0.2	12.3±0.4	12.8±0.3	13.4±0.0	12.5±0.2	12.7±0.2	12.3±0.6	12.6±0.4
FBW (g)	44.2±1.7^{cd}	46.5±3.8^{de}	43.9±4.3^{bc}	43.1±1.8^{bc}	48.2±1.4^{e}	40.8±0.8^{ab}	40.5±1.7^{ab}	39.9±1.2^{a}	40.2±0.9^{ab}
FI[1] (g)	346.2±3.7	321.5±3.1	327.0±1.6	319.0±4.7	354.7±4.4	320.1±4.5	319.8±11.9	322.7±6.0	329.5±2.3
SGR[2]	3.1±0.05^{b}	3.1±0.2^{b}	3.2±0.2^{c}	3.0±0.2^{ab}	3.2±0.1^{c}	3.0±0.1^{ab}	2.9±0.1^{a}	3.0±0.04^{ab}	2.90±0.03^{a}
FCR[3]	0.69±0.02^{ab}	0.70±0.1^{bc}	0.69±0.02^{ab}	0.72±0.03^{bc}	0.65±0.04^{a}	0.71±0.03^{bc}	0.72±0.01^{bc}	0.74±0.01^{c}	0.75±0.04^{c}
SR[4] (%)	100±0.0	95.8±7.2	95.8±7.2	95.8±7.2	95.8±3.6	97.9±3.6	100±0.0	97.9±3.6	97.9±3.6
Somatic indices	**CT**	**C-NSM**	**C-NPM**	**H-NSM**	**H-NPM**	**C-SSM**	**C-SPM**	**H-SSM**	**H-SPM**
Total lenght (cm)	25.2±1.7^{ab}	25.6±1.6^{ab}	25.8±1.8^{b}	25.4±1.5^{ab}	25.7±0.9^{b}	24.9±1.5^{ab}	24.3±1.4^{ab}	24.9±1.5^{ab}	24.0±2.0^{a}
K-factor	0.30±0.02	0.29±0.04	0.29±0.08	0.28±0.03	0.29±0.02	0.28±0.02	0.28 ±0.03	0.29±0.02	0.29±0.04

Data represent mean ± SD, n=3. Different lowercase letters in the same row indicate significant differences (P<0.05).
Abbreviations:
[1]IBW (g): Initial fish biomass in the tan k (g)/number of fish in the tan k
[2]FBW (g): Final fish biomass in the tan k (g)/number of fish in the tan k
[3]FI (g): Daily feed ingested (g) x days
[4]SGR:100 x [(ln final body weight - ln initial body weight) / days]
[5]FCR: feed intake /weight gain
[6]SR: final number of live fish/initial number of live fish x 100

Table 7. Proximate composition and fatty acid profile of fillets of *A. baerii* fingerlings fed the test diets over 40 days.

	Fish groups								
Proximate composition (g/100g muscle on wet basis)	**CT**	**C-NSM**	**C-NPM**	**H-NSM**	**H-NPM**	**C-SSM**	**C-SPM**	**H-SSM**	**H-SPM**
Moisture	79.0±1.73	77.80±1.18	78.59±0.83	79.22±0.11	78.83±1.22	78.21±0.43	77.84±0.43	78.64±2.38	77.59±1.31
Total protein	16.55±1.10	17.33±0.31	16.52±0.60	16.86±0.23	16.94±0.78	17.17±0.61	18.18±0.17	17.33±0.95	18.11±0.97
Total lipids	3.48±1.06	3.91±0.96	3.93±0.27	2.95±0.17	3.28±0.34	3.62±0.60	3.19±0.44	3.13±1.53	3.33±0.77
Ash	0.92±0.11	0.95±0.10	0.96±0.07	0.98±0.06	0.95±0.10	0.99±0.11	0.80±0.22	0.90±0.12	0.97±0.05
Fatty acid composition (g/100g fatty acids)	**CT**	**C-NSM**	**C-NPM**	**H-NSM**	**H-NPM**	**C-SSM**	**C-SPM**	**H-SSM**	**H-SPM**
14:0	3.37±0.19[ab]	3.49±0.11[ab]	3.38±0.07[ab]	3.30±0.07[b]	3.41±0.26[ab]	3.48±0.20[ab]	3.48±0.14[ab]	3.77±0.05[a]	3.56±0.09[ab]
16:0	18.96±0.28[ab]	19.62±0.49[a]	18.68±0.91[ab]	19.44±0.38[a]	19.81±0.94[a]	17.61±0.20[b]	18.50±0.80[ab]	19.86±0.15[a]	19.86±0.49[a]
16:1n-7	3.67±0.17	3.91±0.37	3.84±0.06	3.60±0.13	3.80±0.20	4.02±0.16	4.09±0.16	4.14±0.08	3.79±0.27
18:0	3.35±0.24	3.05±0.31	2.95±0.23	3.30±0.08	3.34±0.29	2.83±0.20	3.22±0.05	3.10±0.22	3.34±0.42
18:1n-9 cis	19.73±037[ab]	19.90±0.77[ab]	19.69±0.47[ab]	19.25±0.24[b]	20.10±0.70[ab]	20.28±0.20[ab]	19.95±0.32[ab]	20.61±0.29[a]	20.51±0.27[ab]
18:1n-7	3.24±0.10	3.16±0.08	3.19±0.12	3.21±0.06	3.19±0.10	3.19±0.05	3.33±0.03	3.11±0.06	3.18±0.15
18:2n-6 LOA	17.04±0.27[ab]	16.99±0.32[ab]	17.14±0.52[b]	16.99±0.03[ab]	15.83±0.79[ab]	18.13±0.06[a]	16.38±0.75[b]	16.43±0.78[b]	16.66±0.91[ab]
18:3n-6	0.39±0.10[cd]	0.35±0.08[d]	0.73±0.06[ab]	0.41±0.01[cd]	0.57±0.08[abcd]	0.60±0.011[abc]	0.79±0.03[a]	0.53±0.12[bcd]	0.36±0.06[d]
18:3n-3 ALA	1.64±0.09[d]	2.32±0.04[ab]	2.60±0.13[a]	2.17±0.00[bc]	2.32±0.21[ab]	2.36±0.05[ab]	1.91±0.09[cd]	2.25±0.11[bc]	2.05±0.20[bc]
18:4n-3	0.90±0.09[c]	0.90±0.01[c]	1.06±0.11[abc]	0.89±0.03[c]	0.92±0.07[bc]	1.10±0.05[ab]	1.12±0.03[a]	0.97±0.07[abc]	0.98±0.07[abc]
20:1n-11	1.18±0.02[a]	1.01±0.05[b]	1.04±0.06[b]	1.07±0.04[ab]	1.00±0.06[b]	1.01±0.05[b]	0.96±0.03[b]	0.94±0.06[b]	0.97±0.04[b]
20:1n-9	3.53±0.02[a]	3.23±0.013[b]	3.26±0.11[ab]	3.32±0.02[ab]	3.31±0.14[ab]	3.14±0.09[b]	3.08±0.06[b]	3.14±0.10[b]	3.21±0.10[b]
20:3n-6	0.33±0.02	0.34±0.04	0.42±0.06	0.40±0.02	0.38±0.07	0.34±0.01	0.41±0.03	0.32±0.03	0.33±0.02
20:4n-6 ARA	0.92±0.11	0.85±0.08	0.95±0.04	0.96±0.04	0.95±0.018	0.90±0.02	01.04±0.02	0.85±0.07	0.91±0.09

22:1n-11	2.72±0.14[a]	2.53±0.13[a]	2.48±0.05[ab]	2.57±0.03[a]	2.50±0.19[ab]	2.20±0.03[bc]	2.11±0.07[c]	2.48±0.13[ab]	2.49±0.14[ab]
20:5n-3 EPA	5.40±0.07	5.31±0.19	5.32±029	5.34±0.22	5.20±0.34	5.43±0.10	5.48±0.20	5.15±0.25	5.17±0.07
22:5n-3	1.66±0.14	1.64±0.08	1.69±0.07	1.66±0.01	1.65±0.16	1.75±0.03	1.78±0.09	1.61±0.07	1.54±0.07
22:6n-3 DHA	11.99±0.92	11.40±0.40	11.57±0.67	12.13±0.38	11.72±1.57	11.63±0.19	12.37±0.30	10.74±0.50	11.10±0.78
SFA	25.68±0.22[ab]	26.16±0.42[a]	25.01±1.16[ab]	26.04±0.36[ab]	26.56±1.27[a]	23.92±0.50[b]	25.20±0.99[ab]	26.73±0.35[a]	26.76±0.80[a]
MUFA	34.06±0.54	33.74±0.77	33.51±0.52	33.02±0.34	33.90±1.22	33.84±0.19	33.52±0.35	34.43±0.46	34.15±0.58
PUFA	40.26±0.74	40.10±0.95	41.48±1.67	40.95±0.65	39.55±2.40	42.24±0.46	41.28±1.32	38.84±0.23	39.09±0.54
n-3	21.59±0.95	21.57±0.58	22.25±1.16	22.19±0.59	21.82±1.96	22.27±0.28	22.66±0.61	20.72±.73	20.84±0.54
n-6	18.67±0.24[ab]	18.53±0.37[ab]	19.23±0.51[ab]	18.76±0.06[ab]	17.73±0.87[b]	19.97±0.18[a]	18.62±0.75[ab]	18.12±0.63[b]	18.26±0.83[b]
n-3/n-6	1.16±0.06	1.16±0.01	1.16±0.03	1.18±0.03	1.23±0.011	1.11±0.01	1.22±0.03	1.15±0.08	1.14±0.08

Data represent mean ± SD, n=3. Different lowercase letters in the same row indicate significant differences ($P<0.05$).
Abbreviations: LOA, linoleic acid; ALA, α-linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 8. Catalase (CAT) and Superoxide dismutase (SOD) activity in liver of *A. baerii* fingerlings fed the test diets over 40 days.

	CAT activity (nmol min^{-1} 100 mg^{-1})*	**SOD activity (U 100 mg^{-1})****
CT	3.63±0.32	0.188±0.0.18
C-NMS	3.65±0.41	0.199±0.021
C-NPM	3.85±0.28	0.208±0.016
H-NSM	3.93±0.57	0.169±0.020
H-NPM	3.93±0.17	0.147±0.036
C-SSM	3.23±0.63	0.123±0.062
C-SPM	4.58±1.34	0.152±0.039
H-SMS	3.94±0.23	0.151±0.031
H-SPM	3.86±1.08	0.184±0.017

Data represent mean ± SD, n=3. *= nmol of formaldehyde formed by CAT per minute in 100 mg of liver; **=1U is defined as the amount of SOD needed to exhibit 50% of dismutation of the superoxide radical in 100 mg of