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Cell wall disruption: An effective strategy to improve the nutritive quality of microalgae in African catfish (*Clarias gariepinus*)

Jeleel O. Agboola[1,2] | Emma Teuling[1,3] | Peter A. Wierenga[3] | Harry Gruppen[3] | Johan W. Schrama[1]

[1]Aquaculture and Fisheries (AFI) group, Wageningen University and Research, Wageningen, The Netherlands

[2]WorldFish Center, Abbassa, Egypt

[3]Laboratory of Food Chemistry, Wageningen University and Research, Wageningen, The Netherlands

Correspondence
Johan W. Schrama, Aquaculture and Fisheries (AFI) group, Wageningen University and Research, Wageningen, The Netherlands.
Email: Johan.schrama@wur.nl

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Abstract

The rigid cell walls of microalgae may hinder their utilization in fish feeds. The current experiment assessed the correlation between the accessibility of microalgae nutrients and their in vivo digestibility in African catfish. *Nannochloropsis gaditana* biomass was subjected to physical or mechanical treatments to weaken its cell wall; untreated—no disruption treatment (UNT), pasteurization (PAS), freezing (FRO), freeze-drying (FRD), cold pasteurization (L40) and bead milling (BEM). Six experimental diets formulated from differently treated and untreated microalgae (at 30% diet inclusion level) were tested on growth performance and apparent nutrient digestibility (ADCs) in juvenile African catfish. A basal diet (REF) containing no microalgae was used as reference diet. Results showed that biomass gain and feed conversion ratio of fish fed L40 and BEM diets increased by 13% and 11%, respectively, relative to the UNT diet. Additionally, FRD, FRO, L40 and BEM cell wall disruption treatments improved protein digestibility by 0.5%, 5.9%, 8.4% and 16.3%, respectively, compared to the UNT treatment. There was a positive correlation between accessibility of microalgal nutrients and their digestibility in African catfish. Nutrient digestibility of microalgae was dependent on extent of cell disruption. Also, the impact of cell disruption on nutrient digestibility of microalgae differs between African catfish and Nile tilapia.

KEYWORDS
accessibility, algae, digestibility, disruption treatments, nutrient utilization, rigid cell wall

1 | INTRODUCTION

There is a limited supply of fishmeal/fish oil for feed in aquaculture. Stagnation in total supply from fish bycatch (FAO, 2016), as well as increasing demand of fishmeal/oil for aquaculture feed production (Belton, Bush, & Little, 2017; FAO, 2016), are two major reasons responsible for the limited supply. As a result, there is a renewed interest in the use of novel feed resources for aquafeed. Microalgae, one of the novel feed ingredients, have been studied during the larvae stages (Eryalçın, Ganuza, Atalah, & Cruz, 2015; Rocha, Ribeiro, Costa, & Dinis, 2008) and during the grow-out phases of fish (Gong, Guterres, Huntley, Sørensen, & Kiron, 2018; Sarker, Gamble, Kelson, & Kapuscinski, 2016; Teuling, Schrama, Gruppen, & Wierenga, 2017). In digestion trials for fish, microalgae are often fed in intact forms (Nandeesha, Gangadhara, Manissery, & Venkataraman, 2001; Sørensen, Berge, Reitan, & Ruyter, 2016; Teuling et al., 2017;

Tibaldi et al., 2015). However, for intact algae, the cell walls may be an important factor limiting the digestibility. Previously, it has been shown that cell wall structure is an important factor determining lipid extraction from microalgae for biofuel production (Lee, Lewis, & Ashman, 2012; Lee, Yoo, Jun, Ahn, & Oh, 2010). Knowledge on effect of microalgal processing on cell wall integrity and subsequent effects on nutrient accessibility and digestibility will enhance better utilization of microalgae in fish feeds. Therefore, the aim of this study was to assess the effects of cell wall disruption on accessibility and digestibility of nutrients from microalga in African catfish. In addition, the results from African catfish were compared to those from Nile tilapia (Teuling, Wierenga, Agboola, Gruppen, & Schrama, 2019), to determine the effects of fish species on utilization of the disrupted microalga.

There is variability in digestibility of microalgae in fish. The digestibility of nutrients from microalgae has been suggested to be influenced by their intrinsic cell wall structure (Scholz et al., 2014; Teuling et al., 2017), their nutritional composition (Becker, 2007) and the digestive physiology of fish species (Burr, Barrows, Gaylord, & Wolters, 2011; Teuling et al., 2017). Protein apparent digestibility coefficients (ADCs) of *Arthrospira sp*., *Chlorella sp*., *Nannochloropsis sp*. and *Scenedesmus sp*. were reported to be 81%, 80%, 72% and 67%, respectively, in both Nile tilapia and African catfish (Teuling et al., 2017). The lower ADCs reported for *Nannochloropsis sp*. and *Scenedesmus sp*. were attributed to the rigidity of their cell walls (Teuling et al., 2017). The cell wall properties determine to which extent the nutrients are made accessible to the digestive enzymes. Based on this, microalgae with less rigid cell walls or membranes (such as peptidoglycan in *Arthrospira*) are hypothesized to have better nutrient digestibility coefficients than those with very rigid (or: poorly digestible) cellulosic cell wall (e.g. the cell wall of *Scenedesmus spp*.). Similar nutrient apparent digestibility coefficients observed in tilapia and African catfish could be attributed to the dominance of rigid microalgae cell wall over other factors, such as nutritional composition or the digestive physiology of fish. *Nannochloropsis gaditana* contains cellulosic cell walls with additional mass of lipids known as "algaenan" covering the cell wall (Scholz et al., 2014). These cell walls can be degraded using cellulases (Scholz et al., 2014), but these are not secreted by the digestive system of fish (Lindsay & Harris, 1980; Shi et al., 2017; Yigit & Olmez, 2011). Studies have suggested that cellulase can be formed by microflora present in the intestines (Hlophe, Moyo, & Ncube, 2014; Lindsay & Harris, 1980).

To increase better utilization of microalgae, that is increase nutrient accessibility and digestibility from microalgae in fish, cell wall disruption could be an effective strategy. The impact of cell wall disruption on nutrient accessibility and digestibility from microalgae has received little attention in digestion trials. The few available studies have shown that cell disruption increases accessibility of astaxanthin (Sommer, Potts, & Morrissy, 1991) and protein (Komaki et al., 1998; Teuling et al., 2019) from different microalgae by 2%–60%. In addition, the extractability of lipids from microalgal cells can be improved by up to 3–4 times by disrupting the cells (Lee et al., 2012 Lee et al., 2010). The variability observed in these studies is due to the differences in microalgae species, types of disruption treatment and the impact generated on the microalgae cells. To determine the impact of cell wall disruption treatments, methods were developed by Teuling et al. (2019) to quantify nutrient accessibility from disrupted microalgae. That study also shows a strong positive correlation between in vitro accessibility and in vivo digestibility of nutrients from disrupted microalgae in Nile tilapia (Teuling et al., 2019).

Cell wall disruption is the process of disrupting the native, intact cell wall structures, leaving the inner cell intact, but the structure of the cell wall damaged. Cell disintegration, on the other hand, entails rupturing the entire cells, where the cells are no longer recognized as intact cells under the microscope. Both methods can be used to release nutrients or bioactive constituents embedded within the cells. Cell wall disruption and cell disintegration can be done through mechanical and non-mechanical treatments (Günerken et al., 2015; Lee et al., 2012). Maintaining the nutritive quality of bioactive components within the algae cells should be of utmost concern during the selection of disruption treatments. Enzymatic and chemical treatments are believed to modify the nutritive quality of intracellular constituents (Lee et al., 2010). Thus, these are less preferable than mechanical treatments such as bead milling (Engler, 1985; Günerken et al., 2015; Kula & Schütte, 1987; Lee et al., 2012), ultrasonication (Borthwick et al., 2005; Chandler et al., 2001; Günerken et al., 2015) and homogenization (Kula & Schütte, 1987; Shirgaonkar, Lothe, & Pandit, 1998). Mechanical treatments also have varying effects on microalgal cells. Mechanical treatments such as bead milling (Engler, 1985; Günerken et al., 2015; Kula & Schütte, 1987; Lee et al., 2012) can be used to completely disrupt the cell wall leading to full disintegration of the cell. Other physical treatments such as freezing (Lee et al., 2012; Mazur, 1969), freeze-drying (Lee et al., 2012) and thermal (Mendes-Pinto, Raposo, Bowen, Young, & Morais, 2001; Ometto et al., 2014) keep the cells intact, but can create partial disruption or little perforation of the microalgal cell wall structure.

It was hypothesized that different cell wall disruption treatments lead to different accessibility of nutrients from the microalga. Additionally, it is expected that accessibility of nutrients after the cell wall disruption of microalgae will correlate positively with nutrient digestibility in African catfish. Irrespective of the disruption treatments, it is hypothesized that herbivorous fish (e.g. Nile tilapia) are able to access and digest nutrients from microalga better than omnivorous fish (e.g. African catfish). To test these hypotheses, six different microalgal treatments (1 untreated and 5 treated *N. gaditana*) were fed to African catfish (*Clarias gariepinus*) at a 30% inclusion level in the diet and these data were compared with earlier published data on Nile tilapia (*Oreochromis niloticus)* fed the same batch of diets (Teuling et al., 2019).

2 | MATERIALS AND METHODS

2.1 | *Nannochloropsis gaditana* treatments

The *N. gaditana* was treated based on the methods described by Teuling et al. (2019). In summary, the *N. gaditana* biomass was

TABLE 1 Formulation of experimental diets fed to juvenile African catfish

	Diets	
	Reference diet	Test diets
Basal ingredients (g/kg)		
Maize	134	93.8
Wheat	200	140
Wheat bran	50	56
Wheat gluten	125	87.5
Rape Seed Meal	125	87.5
Fish meal (CP > 68%)	125	87.5
Soya bean meal (RC < 50)	125	87.5
Fish oil	25	17.5
Soya oil	25	17.5
$CaCO_3$	8.0	5.6
Mono-calcium phosphate	12.0	8.4
L-Lysine HCl	2.0	1.4
DL-methionine	3.0	2.1
L-threonine	1.0	0.7
Vitamin-mineral premix[a]	10.0	7.0
Yttrium oxide	0.2	0.2
Test ingredients (w/w%)		
Nannochloropsis gaditana[b]	–	300

[a]Mineral premix composition (mg/kg reference diet): 50 iron (as $FeSO_4 \cdot 7H_2O$); 30 zinc (as $ZnSO_4 \cdot 7H_2O$); 0.1 cobalt (as $CoSO_4 \cdot 7H_2O$); 10 copper (as $CuSO_4 \cdot 5H_2O$); 0.5 selenium (as Na_2SeO_3); 20 manganese (as $MnSO_4 \cdot 4H_2O$); 500 magnesium (as $MgSO_4 \cdot 7H_2O$); 1 chromium (as $CrCl_3 \cdot 6H_2O$); 2 iodine (as $CaIO_3 \cdot 6H_2O$). Vitamin premix composition (mg/kg reference diet): 10 thiamine; 10 riboflavin; 20 nicotinic acid; 40 pantothenic acid, 10 pyridoxine; 0.2 biotine; 2 folic acid; 0.015 cyanocobalamin; 100 ascorbic acid (as ascorbic acid 2-phosphate); 100 IU alpha-tocopheryl acetate; 3,000 IU retinyl palmitate, 2,400 IU cholecalciferol; 10 menadione sodium bisulphite (51%); 400 inositol; 1,500 choline (as choline chloride); 100 butylated hydroxytoluene; 1,000 calcium propionate. [b]Untreated, pasteurized, frozen-thawed, freeze-dried, commercially processed (NutriSpring® Liquid 40) or bead-milled biomass of *Nannochloropsis gaditana*. With exception of the freeze-dried sample, all algae biomass was drum dried.

obtained from Algaspring after being harvested, washed and centrifuged (Almere, The Netherlands). Subsequently, the viscous algae paste of 20% (w/w DM) resulting from centrifugation was subjected to different physical or mechanical treatments in order to rupture or weaken the microalgae cell walls.

One portion of the viscous algae known as untreated group (UNT) received no further treatment. The other viscous algae paste received 1 out 5 physical or mechanical treatments: PAS, FRO, FRD, L40 and BEM representing pasteurization, frozen-thawed, freeze-dried, commercially processed (NutriSpring® Liquid 40, Algaspring, NL) and bead-milled biomass, respectively. A total of six different algal treatments were used in this experiment. The UNT ingredient was considered as the negative control and the BEM sample as a positive control. The conditions applied for the treatments were as follows: (a) the PAS sample was pasteurized using a heat exchanger at 80°C for 20 s. (b) The FRO and FRD samples were frozen at −18°C. The FRO samples were thawed at 4°C (after ~2 weeks frozen storage). Freezing and thawing were performed in small batches to ensure microbial safety. (c) The L40 sample is a commercially available product ("NutriSpring® Liquid 40") provided by AlgaSpring. The L40 *N. gaditana* biomass (grown and harvested by AlgaSpring) received a physical treatment, similar to pasteurization. No additives were used in the production of L40. Commercially processed (L40) is otherwise called cold pasteurized samples throughout this document. (d) The BEM samples were diluted to 14% [w/w DM] and subsequently bead milled to disrupt the algal cells. The bead milling was performed on a DYNO-Mill type ECM-AP05 LAB (Willy A. Bachofen Maschinenfabrik, Muttenz, Switzerland), using 0.5 mm yttria-stabilized zirconia grinding beads, type ZY Premium (Sigmund Lindner, Warmensteinach, Germany). The pump speed was set at 20 L/hr and milling speed at 14 m/s. During milling, the milling chamber was cooled with running water to prevent protein deteriorating reactions and to ensure microbial safety. The algae suspension was passed through the mill three times to break the majority of the algal cells. Cell disruption was monitored by microscopy (Teuling et al., 2019).

All the treated and untreated samples, except FRD were drum dried, during which the algae products were dried within ~7 s on drums heated to 130°C. The FRD sample was freeze-dried. All algae samples were dried (both drum drying and freeze-drying) to a product of >900 g/kg DM.

2.2 | In vitro accessibility measurements of nutrients

Microscopic analysis, in vitro protein hydrolysis, nitrogen solubility, ion leaching, fat extractability and buffering capacity tests were used to quantify the extent of disruption on microalgae cells. Extensive description of methods and results of these accessibility tests can be found in Teuling et al. (2019). These results were used to assessed the relationship between the in vitro accessibility of nutrients and their corresponding digestibility in African catfish.

2.3 | Fish trial

In the current experiment, animals were not exposed to invasive techniques or discomfort related to the experimental treatments. Fish were not anesthetized or euthanized as part of the experimental procedures. This experiment was evaluated by the Animal Welfare Body of Wageningen University. The Animal Welfare Body approved this experiment and judged the procedures applied to the animals in this experiment to be below the threshold for being an animal experiment.

TABLE 2 Analysed proximate composition of *Nannochloropsis gaditana*[a] in g/kg DM, except for energy and dry matter which are in KJ/g DM and g DM/kg wet weight, respectively. Values are presented as means

	UNT	PAS	FRD	FRO	L40	BEM
Dry matter	964.2	972.1	931.0	949.7	976.0	919.1
Gross energy	24.5	24.4	24.2	24.6	23.6	24.7
Crude protein[a]	500.0	508.6	533.2	488.8	487.2	490.7
Crude fat	160.9	162.1	129.7	173.3	156.6	146.3
Total carbohydrates[b]	160.4	168.0	135.6	158.3	165.3	123.9
Rhamnose	7.77	7.36	7.96	7.75	8.20	8.50
Fucose	1.65	1.25	1.60	1.55	1.80	2.00
Arabinose	1.46	1.65	1.42	1.79	1.80	1.21
Xylose	1.84	1.62	1.87	1.80	1.82	1.80
Mannose	26.0	28.7	16.2	35.5	28.6	37.2
Galactose	22.1	23.6	21.5	18.0	23.7	19.4
Glucose	83.6	85.2	68.2	76.1	82.9	36.6
Ribose	5.7	5.7	6.4	6.0	5.6	4.7
Uronic acids	10.4	10.4	10.5	9.8	10.9	12.5
Starch	1.0	2.6	2.3	4.5	5.7	5.9
NSP[c]	159.4	165.4	133.2	153.8	159.7	118.0
Ash	72.2	70.3	81.6	77.7	95.8	90.9
Phosphorus	12.8	13.1	8.3	11.0	11.8	11.3
Calcium	4.65	4.69	4.60	3.28	5.40	5.95
Copper	<0.01	<0.01	<0.01	0.01	0.00	<0.01
Iron	1.14	1.14	1.00	0.75	0.99	0.60
Magnesium	3.39	3.27	4.02	3.65	4.35	4.29
Manganese	0.21	0.21	0.24	0.18	0.20	0.22
Zinc	0.03	0.03	0.03	0.04	0.03	0.03

Notes. DM and NSP represent dry matter and non-starch polysaccharides, respectively.
[a]UNT, PAS, FRD, FRO, L40 and BEM: untreated, pasteurized, freeze-dried, frozen-thawed, commercially processed (NutriSpring® Liquid 40, Algaspring, NL) and bead-milled biomass of *Nannochloropsis gaditana*, respectively. All *N. gaditana* biomass was drum dried, except FRD.
[a]Crude protein was calculated as nitrogen×6.25. [b]Total carbohydrates include starch and NSP. [c]NSP = total carbohydrates – starch.

2.3.1 | Diets

The basal diet was formulated as in Table 1. From this basal diet, 6 other diets containing the differently treated or the untreated *N. gaditana* were formulated (based on test ingredient preparation explained in section 2.1). The control feed consisted of 100% basal diet, the treatment diets UNT, PAS, FRO, FRD, L40 and BEM contained 70% basal diet and 30% of the differently treated *N. gaditana*, respectively. Yttrium oxide (Y_2O_3) was included as an inert marker for digestibility studies. The diets were formulated to meet the nutrient requirement for African catfish (NRC, 2011). All diets were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands). The dried algae materials were mixed with other dietary ingredients, hammer milled, mixed with oil and subsequently extruded (through a 2 mm die) into sinking pellets. Prior to feeding, the diets were sieved (2 mm) to remove the fine particles. Proximate composition of the different treated *N. gaditana* biomass is represented in Table 2. Also, the composition and chemical constituents of the experimental diets are presented in Table 3.

2.3.2 | Fish, rearing and housing facilities

The experiment was performed at the Aquaculture Research Facility (ARF) of the Wageningen University and Research (WUR), The Netherlands. Mixed sex juveniles African catfish (*Clarias gariepinus*, Fleuren en Nooijen strain) were obtained from commercial breeder (Fleuren en Nooijen, The Netherlands) two weeks prior to the commencement of the trial. All tanks were connected to the same recirculation system, resulting in a common water supply and ensuring the same water quality for the inflow of each tank. The system consisted of a sump, settling tank and trickling filter with a water refreshment of 300 L/day. Each individual tank was connected to a swirl separator (AquaOptima AS, column height 44 cm; diameter 24.5 cm). The swirl separators, with detachable glass bottles, were used to collect faeces and count feed spills for each tank separately. The flow rate in each tank was set at 7 L/min using a hand-held liquid rotameter. All tanks were equipped with a cylindrical shaped air stone.

TABLE 3 Analysed proximate composition of experimental diets[a] fed to juvenile African catfish (*Clarias gariepinus*). Values are presented as means, in g/kg DM, unless stated otherwise

	REF	UNT	PAS	FRD	FRO	L40	BEM
Dry matter (g DM/kg wet weight)	966.7	963.3	951.7	953.8	979.24	965.8	941.3
Gross energy (kJ/g DM)	20.6	22.0	21.6	21.6	22.0	21.5	21.7
Crude protein[a]	371.0	410.4	418.0	415.5	404.1	403.2	406.10
Crude fat	93.0	108.8	111.3	103.7	113.8	111.6	122.7
Total carbohydrates[b]	408.8	328.0	333.1	326.9	330.5	326.8	324.3
Rhamnose	2.7	4.0	3.9	3.8	3.9	3.9	4.0
Fucose	0.79	0.77	0.77	0.92	0.80	0.85	0.96
Arabinose	22.2	15.4	16.3	18.3	17.2	17.2	17.2
Xylose	21.0	14.8	15.5	15.4	14.8	14.3	14.5
Mannose	3.9	10.4	10.3	7.4	13.1	10.2	12.0
Galactose	18.1	19.9	20.3	20.4	18.6	21.0	18.8
Glucose	322.6	246.8	250.4	244.3	245.9	243.5	240.5
Ribose	0.20	1.53	1.22	1.28	1.20	1.26	1.04
Uronic acids	17.3	14.4	14.5	15.0	15.1	14.4	15.3
Starch	240.6	181.4	176.8	192.5	181.4	177.1	178.8
NSP[c]	168.2	146.6	156.4	134.3	149.1	149.7	145.5
Ash	67.1	68.3	68.2	71.1	69.9	76.5	73.1
Yttrium	0.17	0.17	0.17	0.17	0.16	0.16	0.17
Phosphorus	9.75	10.8	10.9	9.36	10.2	10.4	10.3
Calcium	12.7	10.1	10.1	10.0	9.46	10.4	10.4
Copper	0.02	0.01	0.01	0.01	0.01	0.01	0.01
Iron	0.39	0.51	0.50	0.48	0.38	0.47	0.35
Magnesium	2.17	2.57	2.51	2.70	2.61	2.85	2.75
Manganese	0.05	0.11	0.11	0.11	0.09	0.11	0.11
Zinc	0.08	0.07	0.06	0.06	0.07	0.07	0.07

Notes. DM and NSP represent dry matter and non-starch polysaccharides, respectively.
[a]REF is reference diet. UNT, PAS, FRD, FRO, L40 and BEM: 70% REF, 30% untreated, pasteurized, freeze-dried, frozen-thawed, commercially processed (NutriSpring® Liquid 40, Algaspring, NL) and bead-milled biomass of *Nannochloropsis gaditana*, respectively. All *N. gaditana* biomass was drum dried, except FRD.
[a]Crude protein was calculated as nitrogen×6.25. [b]Total carbohydrates include starch and NSP. [c]NSP = total carbohydrates − starch.

Water quality parameters were checked regularly to ensure that the water quality remained within preset ranges. The means of measured water parameters were as follows: pH of 7.2, temperature of 27.2°C, conductivity of 2,176 µs, ammonium ion of 0.58 mg/L, nitrite of 0.21 mg/L and nitrate of 266 mg/L. On some days in which the pH fluctuated below the desirable limits, sodium carbonate was added to fish water to maintain the pH back to desirable range. The photoperiod was set to 12 hr light: 12 hr dark (lights on 7:00, lights off 19:00). All water parameters were kept within the optimal range for African catfish throughout the duration of the experiment.

2.3.3 | Experimental procedure

After two weeks on pre-experimental diets, the African catfish with an average initial body weight of 40 ± 2.96 g were sorted, batch weighed and randomly distributed to 21 tanks of 60 L water each. The 21 tanks were divided over three racks of seven tanks each. Each tank was randomly stocked with 30 fish. The seven experimental treatments were randomly distributed over all the tanks. Each treatment was replicated thrice. The fish were fed restrictively to keep the amount of feed on dry matter (DM) basis per tank per day the same. Fish were fed at 18 g/kg$^{0.8}$ BW/day. The daily amount of feed was increased throughout the experiment by predicting fish growth and weight, using the average start weight of the fish (all treatments) and an expected feed conversion ratio (FCR) of 1. These were used to create the feeding lists. The feeding list for a particular tank was subsequently adapted according to the mortality recorded in that tank. Fish were hand-fed twice a day at 9:00 and 15:30 hr. Uneaten pellets recovered from the settling units were recorded per tank after each feeding moment. Prior to each feeding, the collection bottles (21 in number) were attached to each settling tank to collect the uneaten pellets. The weight of the uneaten pellets was

correlated to equal weight of fresh pellets by multiplying the number of uneaten pellets with the average weight of a pellet. Daily feed intake was calculated from feed fed to fish and weight of recovered uneaten pellets. The diets were kept under refrigerated (4°C) conditions throughout the experiment. 100 g sample of each diet was taken every week. The feed samples were pooled per diet and stored (4°C) until further analysis.

Faeces were collected for digestibility measurements on the 3rd, 4th and 5th week of the experiment. Another set of collection bottles (different from the one used for collecting uneaten pellets) were attached to each of the settling tanks a day preceding the faecal collection. The collection bottles were placed in a styrofoam box filled with ice water to avoid bacteria degradation of nutrients in the faeces. At the end of afternoon feeding (ca. 16:30 hr), the faeces collection bottles were attached to settling tanks and removed before morning feeding (ca.8:30 hr) the next day. Excess water on top of each bottle was carefully removed, and the remaining sedimented faecal matter was collected in separate aluminium trays for each tank. These faecal samples were immediately frozen after each collection. Faeces from the same tank were pooled into the same aluminium trays per week and subsequently freeze-dried before further processing. Additionally, faeces from the same tanks for all the three weeks (i.e. weeks 3, 4 and 5) period were pooled together and used for laboratory analyses. The experiment lasted for 5 weeks, after which the fish were counted and group weighed to estimate the growth performance.

2.4 | Analytical procedure and calculations

2.4.1 | Growth performance

The average fish growth (g) was calculated as the difference between the average initial (W_i) and average final (W_f) body weight of fish. The specific growth rate (SGR) was calculated as SGR = (LNW_f – LNW_i) × 100/day, where d is the duration of the experiment in days. The daily feed intake was recorded by weighing the given feed and the uneaten pellets. The feed conversion ratio (FCR) was calculated using the total feed intake (on dry matter basis) and fish weight gain per tank. Protein efficiency ratio was calculated from total protein ingested and fish weight gain per tank. Fish survival per tank was calculated as (N_f/N_i) × 100%, where N_i and N_f represent initial and final number of fish, respectively.

2.4.2 | Digestibility

The apparent digestibility coefficients (ADCs) of dry matter (DM), crude protein (CP), crude fat, total carbohydrates, starch, non-starch polysaccharides (NSP), ash and minerals of all diets and test ingredients were calculated, with the use of Yttrium as inert marker. The ADCs of diets were calculated using the formula of Cheng and Hardy (2002); ADC (%) = 100% × [1 – (Y_d × N_f)/(Y_f × N_d)], where Y_d and Y_f are the Yttrium content in the diets and faeces, respectively. N_d and N_f represent the nutrient content in the diet and faeces, respectively. The Yttrium and nutrient contents in both feeds and faeces were expressed as g/kg DM. The ADCs in the test ingredients (microalgae) were calculated using the following formula (Sugiura, Dong, Rathbone, & Hardy, 1998);

$$ADCti = ADCtd + (ADCtd - ADCrd) \times \left(\frac{0.7 \times D_{ref}}{0.3 \times D_{ing}}\right) \times 100\%$$

where ADC_{ti} is the ADC of the test ingredient (%), ADC_{td} is the ADC of the test diet (%), ADC_{rd} is the ADC of the reference diet (%), D_{ref} is the nutrient content (g/kg DM) or the kcal/g gross energy of the reference diet and D_{ing} is the nutrient content (g/kg DM) or the gross energy (kJ/g) of the ingredient. All calculations were on dry matter basis.

2.4.3 | Chemical analysis

The freeze-dried samples of algae, feeds and faeces were ground to pass through a screen (1 mm size) of a mill grinder (Retsch ZM 200, Germany) at 1,200 rpm, prior to chemical analyses The DM content was determined by drying samples for at least 4 hr at 103°C until constant weight (ISO 6496, 1983). Ash content was determined by incineration using a muffle furnace for 4 hr at 550°C (ISO 5984, 1978). The CP (N × 6.25) was analysed by the Kjeldahl method (ISO 5983, 1979). Crude fat was measured by petroleum-ether extraction (Soxhlet method, ISO 5986). Energy content was measured using bomb calorimetry by direct combustion (IKA® werke, C7000; IKA analysentechnik, Weitershem, Germany). Yttrium and minerals in feed and faeces were analysed by inductively coupled plasma mass spectrometry (ICPOES) using the standard NEN 15510 (2007).

Total starch was determined in duplicate using the total starch assay method C (AOAC Method 996.11 (AOAC, 2012)) from Megazyme (Megazyme International, Ltd, Wicklow, Ireland). In this method, the total starch includes resistant starch, digestible starch, free glucose and maltodextrins. D-glucose was used for calibration and standardized regular maize starch as a control. Neutral carbohydrate composition was determined in duplicate based on the alditol acetates procedure, as previously described by Teuling et al. (2017). The monosugar constituents of the total carbohydrates were expressed in anhydrous form. Total uronic acid content was determined according to an automated colorimetric m-hydroxydiphenyl assay, which was described by Teuling et al. (2017). All chemical analyses were performed in triplicate unless otherwise stated.

2.5 | Statistical analysis

Statistical analyses were performed using the SPSS statistical software package version 22.0 (IBM Institute, Armonk, NY, USA). Data on fish performance and ADCs of nutrients were tested for treatment effect using the one-way analysis of variance (ANOVA). Significant differences ($p < 0.05$) between means were detected

TABLE 4 Growth performance of African catfish (*Clarias gariepinus*) fed experiment diets* containing untreated and differently treated *Nannochloropsis gaditana* biomass. Values are presented as means. *SEM* is the standard error of the mean

Parameters	REF	UNT	PAS	FRD	FRO	L40	BEM	*SEM*	*p*-values
Initial body weight (g/fish)	43.78	39.83	43.60	44.57	43.69	45.84	46.62	2.18	0.135
Final body weight (g/fish)	122.38^{ab}	111.81^{b}	114.51^{b}	116.96^{b}	113.10^{b}	130.24^{a}	131.01^{a}	3.37	<0.001
Biomass gain (g/d/fish)	2.25^{ab}	2.06^{bc}	2.03^{bc}	2.07^{bc}	1.98^{c}	2.41^{a}	2.41^{a}	0.69	<0.001
Dry matter feed intake (g/d/fish)	2.09^{b}	2.13^{b}	2.19^{a}	2.19^{a}	2.06^{b}	2.20^{a}	2.24^{a}	0.03	0.001
Feed protein intake (g/d/fish)	0.75^{c}	0.83^{ab}	0.87^{a}	0.87^{a}	0.80^{cb}	0.86^{a}	0.86^{a}	0.01	<0.001
Specific growth rate (%/d)	2.99	3.04	2.84	2.85	2.80	3.07	3.04	0.10	0.074
Feed Conversion Ratio (g/g)	0.93^{a}	1.04^{b}	1.08^{b}	1.06^{b}	1.04^{b}	0.92^{a}	0.93^{a}	0.03	<0.001
Protein Efficiency Ratio (g/g)	2.98^{a}	2.46^{b}	2.33^{b}	2.39^{b}	2.49^{b}	2.82^{a}	2.80^{a}	0.07	<0.001
% Survival rate	95.56	94.44	97.78	95.56	97.81	88.89	96.67	3.08	0.139

Notes. Means lacking a common superscript letters (^{abc}) differ significantly ($p < 0.05$).
*REF is reference diet. UNT, PAS, FRD, FRO, L40 and BEM: 70% REF, 30% untreated, pasteurized, freeze-dried, frozen-thawed, commercially processed (NutriSpring® Liquid 40, Algaspring, NL) and bead-milled biomass of *Nannochloropsis gaditana*, respectively. All *N. gaditana* biomass was drum dried, except FRD.

using the Tukey test. Correlation coefficients between the in vitro accessibility parameters (degree of hydrolysis, nitrogen solubility and ion leaching) and ADCs of nutrients (dry matter, crude protein, gross energy, crude fat, ash and phosphorus) were examined using Pearson's correlation coefficients. Linear and quadratic relationships between the in vitro accessibility measurements (degree of hydrolysis, nitrogen solubility and ion leaching) and ADCs of protein and fat were evaluated through the regression analysis. Probability levels of less than 0.05 were considered to be statistically significant, and levels between 0.05 and 0.1 were considered a trend. In addition, fish species, treatment and their interaction effect on ADCs of microalgae were tested using an 2-way analysis of variance (ANOVA) by combining the reported data on Nile tilapia (Teuling et al., 2019) and the data of the current study on African catfish.

3 | RESULTS

3.1 | Fish performance

The survival rate (mean of 95%) was unaffected by the disruption treatments ($p > 0.05$) (Table 4). The feed conversion ratio (FCR), protein efficiency ratio (PER) and daily growth (g/d) were different ($p < 0.005$) among all the treatments. The average feed intake (in DM) was different among all the treatments. The average initial body weight of fish was 44 and statistically similar ($p > 0.05$) for all the treatments. The biomass gain increased by 13% in L40 and BEM diets, compared to the UNT, PAS, FRD and FRO diets. The FCR in REF (0.93) was lower than for UNT, PAS, FRD and FRO diets (1.06 ± 0.019). The FCR in L40 (0.92) and BEM (0.93) were decreased by 11% relative to the UNT diet, and thereby reached similar FCR as the REF diets (0.93).

3.2 | Digestibility

Tables 5 and 6 show the apparent digestibility coefficients (ADCs) of nutrients in the experimental diets and microalgae. On diet level, ADCs of total carbohydrates, starch, NSP, ash and calcium were similar ($p > 0.05$) for all the disruption treatments. The mean ADC of starch for all the diets was 98%. ADCs of DM, CP, GE, crude fat, phosphorus, copper and magnesium were affected ($p < 0.05$) by the disruption treatments. The ADCs (in %) of CP for BEM (84%) diet was better than that of L40 and FRO (mean ADC = 80%). The ADCs of CP for UNT, PAS and FRD diets (mean = 77%) were similar, but lower than of BEM, L40 and FRO diets. The ADC of crude fat for BEM (82%) diet was similar to REF diet (85%), and better than UNT, PAS, FRD, FRO and L40 (mean ADC = 68%).

On microalgae (ingredient) level, ADCs of total carbohydrates and ash were unaffected ($p > 0.05$) by the disruption treatments. ADCs of CP, GE, fat and phosphorus were enhanced ($p < 0.05$) by the disruption treatments, with a tendency for significance for DM ($p = 0.052$) digestibility. The FRO, L40 and BEM disruption improved protein digestibility by 5.9%, 8.4% and 16.3%, respectively, relative to the UNT microalgae. The BEM cell disruption enhanced fat digestibility by 36.5%, respectively, relative to the UNT microalga, while the other treatments had similar ADC fat as UNT.

TABLE 5 Apparent digestibility coefficients (%ADC) of experimental diets[a] containing untreated and treated *Nannochloropsis gaditana* in juvenile African catfish (*Clarias gariepinus*). Values are presented as means. *SEM* is the standard error of the mean

	REF	UNT	PAS	FRD	FRO	L40	BEM	*SEM*	*p*-values
Dry matter	72.7^{a}	65.1^{b}	64.0^{b}	65.1^{b}	65.8^{b}	68.8^{b}	70.1^{a}	1.63	<0.001
Crude protein	88.6^{a}	77.4^{d}	75.8^{d}	77.4^{d}	79.9^{c}	80.7^{c}	83.8^{b}	0.52	<0.001
Gross energy	76.0^{a}	65.7^{c}	64.6^{c}	66.0^{c}	66.5^{c}	68.1^{bc}	71.7^{ab}	1.3	<0.001
Crude fat	85.3^{a}	65.4^{bc}	66.9^{bc}	71.8^{b}	65.1^{c}	68.6^{bc}	81.8^{a}	1.92	<0.001
Total carbohydrates[b]	66.3	61	63.6	59.1	60.3	63.1	60.5	3.66	0.395
Starch	98.1	98.5	98.4	98.5	98	98.6	98.6	0.27	0.199
NSP[c]	20.9	14.7	24.1	2.6	14.5	21.2	13.6	8.62	0.196
Ash	51.3	53.0	45.4	54.8	54.5	66.3	58.1	6.5	0.137
Phosphorus	66.1^{c}	70.1^{b}	67.8^{c}	68.6^{c}	73.3^{ab}	72.5^{b}	73.0^{ab}	1.13	<0.001
Calcium	42.4	49.8	34.1	55.7	38.5	60.6	53.6	16.5	0.657
Copper	42.8^{ab}	24.5^{bc}	14.5^{c}	28.4^{bc}	25.1^{bc}	21.6^{bc}	22.6^{bc}	7.11	0.043
Magnesium	58.3^{a}	53.1^{b}	50.3^{b}	53.7^{b}	60.1^{a}	58.2^{a}	60.5^{a}	1.2	<0.001

Notes. Means lacking a common superscript letters (^{abcd}) differ significantly ($p < 0.05$).
[a]REF is reference diet. UNT, PAS, FRD, FRO, L40 and BEM: 70% REF, 30% untreated, pasteurized, freeze-dried, frozen-thawed, commercially processed (NutriSpring® Liquid 40, Algaspring, NL) and bead-milled biomass of *Nannochloropsis gaditana*, respectively. All *N. gaditana* biomass was drum dried, except FRD. [b]Total carbohydrates include starch and NSP. [c]NSP represents non-starch polysaccharides, NSP = total carbohydrates - starch.

TABLE 6 Apparent digestibility coefficients (%) of untreated and treated *Nannochloropsis gaditana*[a] in African catfish (*Clarias gariepinus*) fed the experimental diets. Values are presented as means. *SEM* is the standard error of the mean

	UNT	PAS	FRD	FRO	L40	BEM	*SEM*	*p*-values
Dry matter	48.3	45.2	47.0	50.2	60.3	63.7	5.54	0.052
Crude protein	59.3^{c}	55.5^{c}	59.8^{c}	65.2^{b}	67.7^{b}	75.6^{a}	1.37	<0.001
Gross energy	46.6^{b}	43.6^{b}	46.7^{b}	48.8^{b}	53.0^{ab}	63.5^{a}	3.91	0.003
Crude fat	40.3^{b}	44^{b}	49.9^{b}	41.2^{b}	47.2^{b}	76.8^{a}	4.79	<0.001
Total carbohydrates[b]	31.7	48.7	43.3	28.1	45.4	34.9	18.23	0.790
Ash	56.4	33.1	61.2	60.7	88.9	69.5	18.22	0.153
Phosphorus	76.5^{bc}	70.5^{c}	74.9^{bc}	87.4^{a}	83.5^{ab}	86.3^{a}	2.86	<0.001

Notes. Means lacking a common superscript letters (^{abc}) differ significantly ($p < 0.05$).
[a]UNT, PAS, FRD, FRO, L40 and BEM: 70% REF, 30% untreated, pasteurized, freeze-dried, frozen-thawed, commercially processed (NutriSpring® Liquid 40, Algaspring, NL) and bead-milled biomass of *Nannochloropsis gaditana*, respectively. All *N. gaditana* biomass was drum dried, except FRD. [b]Total carbohydrates include starch and non-starch polysaccharides.

3.3 | Correlation between the in vitro accessibility measurements and in vivo nutrient digestibility

Accessibility of nutrients varied among the disruption treatments (Teuling et al., 2019). Nitrogen solubility, degree of protein hydrolysis and ion leaching of microalgae increased by 1%–12%, 1%–10% and 6%–35%, respectively, with different cell wall disruption treatments (Teuling et al., 2019). Table 7 shows the correlation between in vitro accessibility measurements (nitrogen solubility, degree of hydrolysis and ion leaching) and in vivo nutrient digestibility of microalgae (ADCs of DM, CP, GE, fat, ash and P). Protein digestibility strongly correlated ($r > 0.90$) with degree of hydrolysis, nitrogen solubility and ion leaching. Also, fat digestibility has a strong positive correlation ($r > 0.85$) with degree of hydrolysis and nitrogen solubility. Furthermore, it was tested whether the relationships were best described by a linear or quadratic equation (Table 8, Figures 1 and 2). ADCs of protein and crude fat had significant linear and quadratic relationships with the degree of hydrolysis and ion leaching of the microalgae. Protein ADC had significant linear relationships with nitrogen solubility of the microalgae, but they were not quadratically related. Fat ADC had both linear and quadratic relationships with nitrogen solubility of the microalgae.

3.4 | Effects of fish species on digestibility of the microalgae

The differently treated and untreated microalgae were previously tested in Nile tilapia (Teuling et al., 2019). Comparing the results obtained in tilapia with the current experiment showed that microalgae were differently digested in both fish species (Table 9). For CP and

fat, there was a significant interaction between cell wall disruption treatments and fish species. The ADCs of protein of microalgae (with different treatment) was 1%–5% higher in Nile tilapia than in African catfish. Also, the ADCs of crude fat were higher (6%–12% higher) in Nile tilapia than in African catfish. For energy, ash and P, the interaction was absent, but there was a significant fish species effect. Similar to ADCs of protein and fat, ADCs of DM, energy and phosphorus were higher in Nile tilapia than in African catfish.

TABLE 7 Pearson correlations between apparent digestibility coefficient (ADC) of treated or untreated *Nannochloropsis gaditana* in juvenile African catfish (*Clarias gariepinus)* and corresponding in vitro accessibility measurements

	Pearson correlation coefficients (*r*)[a]		
ADC of nutrients	Nsol.[b]	DH[b]	Ion[b]
Dry matter	0.73***	0.73***	0.68**
Crude protein	0.93***	0.94***	0.89***
Gross energy	0.85***	0.85***	0.76***
Crude fat	0.86***	0.85***	0.75***
Ash	0.40*	0.38*	0.45*
Phosphorus	0.61**	0.62**	0.65**

[a]Pearson correlation coefficients between apparent digestibility coefficient values (n = 18) and the mean values of each in vitro measurement (n = 6). [b]Nsol.—nitrogen solubility in pH 8 buffer; DH—degree of hydrolysis using pancreatin; Ion—ion leaching in water. ***p < 0.001 **p < 0.009 *p > 0.05.

4 | DISCUSSION

Microalgae are gaining increasing attention as new protein source in aquafeed. The rigidity of the microalga cell walls could have a strong influence on the accessibility of the nutrients to digestive enzymes. The current study examined the effect of cell wall disruption on nutrient accessibility and digestibility of *N. gaditana* in African catfish.

Inclusion of 30% intact (UNT diet) microalgae decreased growth performance in African catfish compared to the REF diet. FRO, FRD and PAS diets showed similar growth performance in African catfish as the UNT diet. However, cell disintegration by bead milling (BEM) and disruption of cell walls by cold pasteurization (L40) showed identical growth performance in African catfish as the reference diet (REF). This shows that 30% of the diet of African catfish could be substituted with any of the BEM or L40 algae biomass while maintaining the same performance. Compared to the UNT diet, L40 and BEM diets increased biomass gain (g/fish), FCR and PER of African catfish by 17%, 11% and 15%, respectively. The FCR (mean FCR = 1.01) observed in this trial generally performed better than diets containing *Spirulina* (FCR = 1.28) and *Cladophora* (FCR = 1.09) biomass previously used elsewhere for African catfish (Promya & Chitmanat, 2011).

The apparent digestibility coefficients (ADCs) of experiment diets and microalgae support the growth performance indices earlier reported in this experiment. As expected, increased digestibility of nutrients led to better feed efficiency and biomass gain of

TABLE 8 Regression equations (linear and quadratic) showing relationships between protein and fat apparent digestibility coefficients (ADC) (*Y*) in juvenile African catfish (*Clarias gariepinus)* and in vitro nutrient accessibility measurements (*X*)[a] from *Nannochloropsis gaditana* biomass

Y	*X*	Equation	R^2	*p* (linear component)*	*p* (quadratic component)*
Protein ADC (%)	Nsol. (%)	$Y = 48.35\ (1.44) + 1.44\ (0.14)\ X$	0.86	<0.001	-
		$Y = 41.20\ (6.17) + 2.72\ (1.07)\ X - 0.05\ (0.041)\ X^2$	0.88	–	0.247
	DH (%)	$Y = 53.40\ (1.14) + 1.90\ (0.18)\ X$	0.88	<0.001	-
		$Y = 46.95\ (2.66) + 4.27\ (0.92)\ X - 0.16\ (0.061)\ X^2$	0.92	–	0.020
	Ion (%)	$Y = 42.83\ (2.79) + 0.51\ (0.07)\ X$	0.79	<0.001	-
		$Y = 65.64\ (7.81) - 0.68\ (0.39)\ X + 0.01\ (0.001)\ X^2$	0.87	–	0.008
Crude fat ADC (%)	Nsol. (%)	$Y = 21.68\ (4.61) + 2.63\ (0.40)\ X$	0.73	<0.001	-
		$Y = 58.51\ (15.03) - 3.95\ (2.61)\ X + 0.25\ (0.09)\ X^2$	0.81	–	0.023
	DH (%)	$Y = 31.09\ (3.37) + 3.43\ (0.53)\ X$	0.73	<0.001	-
		$Y = 50.48\ (7.82) - 3.68\ (2.71)\ X + 0.48\ (0.18)\ X^2$	0.81	–	0.018
	Ion (%)	$Y = 15.08\ (8.10) + 0.85\ (0.19)\ X$	0.56	<0.001	-
		$Y = 87.80\ (21.16) - 2.94\ (1.06) + 0.05\ (0.013)\ X^2$	0.76	–	0.003

[a]Nsol.—nitrogen solubility in pH 8 buffer; DH—degree of hydrolysis using pancreatin; Ion—ion leaching in water. *p < 0.001 relationships were considered significant, *p < 0.05 relationships were considered a tendency for a significant relationship.

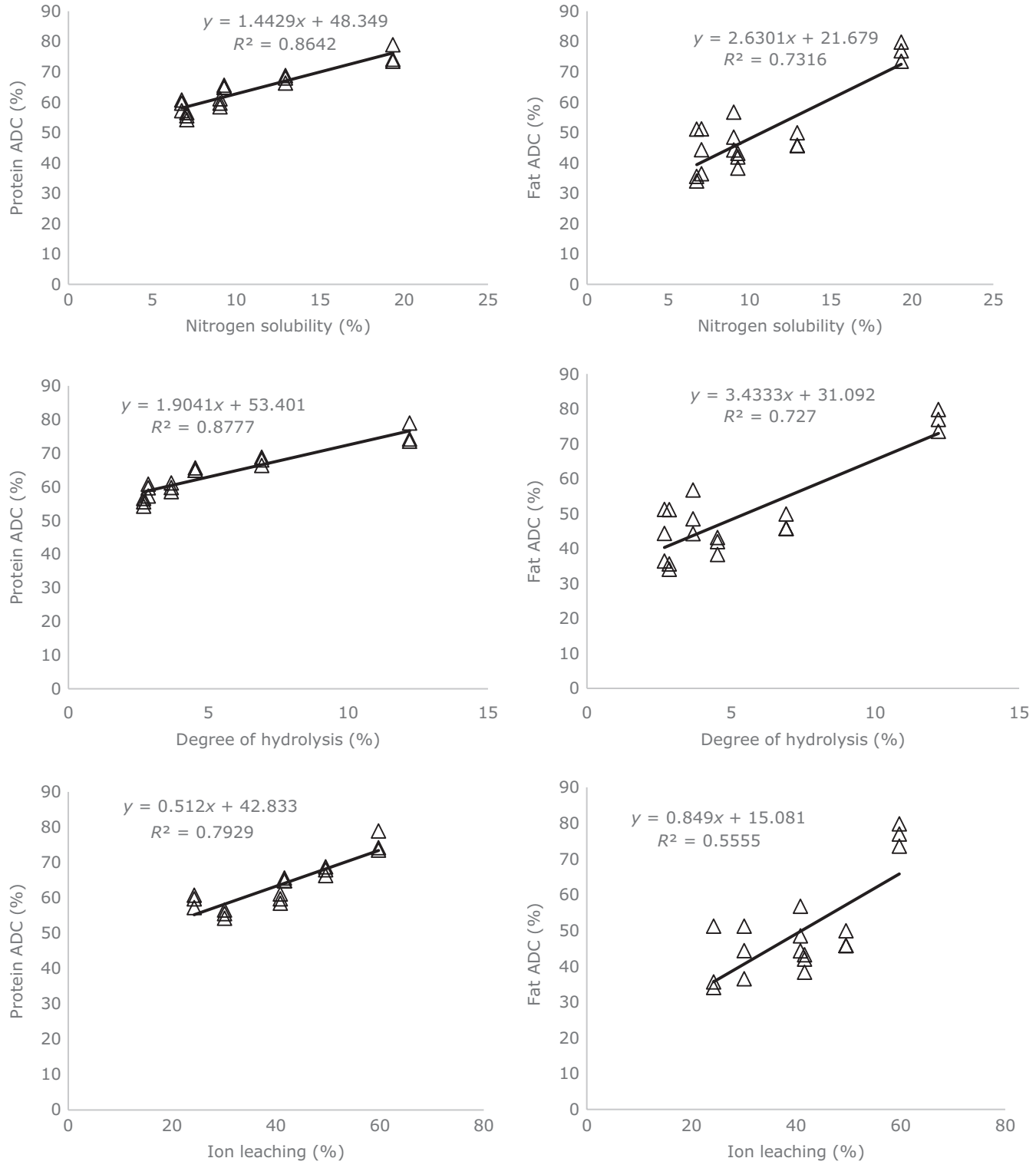

FIGURE 1 Relationships (linear) between the apparent digestibility coefficients (%ADC) of protein and fat from Nannochloropsis gaditana in juvenile African catfish and in vitro nutrient accessibility measurements of Nannochloropsis gaditana—nitrogen solubility, degree of protein hydrolysis and ion leaching. Significant ($p < 0.05$) relationships are indicated by solid lines

fish. On diet level, fish on diets containing BEM and L40 microalgae had better protein digestibility and better FCR than the rest of the algal treatments. The nutrients of BEM and L40 were digested to the highest extent (68%–84%) and the nutrients of PAS to the lowest extent (63%–75%) among the microalgae diets. As expected, starch was almost fully digested in all the experimental diets. The NSP contents of the diets are poorly digested by the African catfish. Study has shown that African catfish possess the minimal capacity

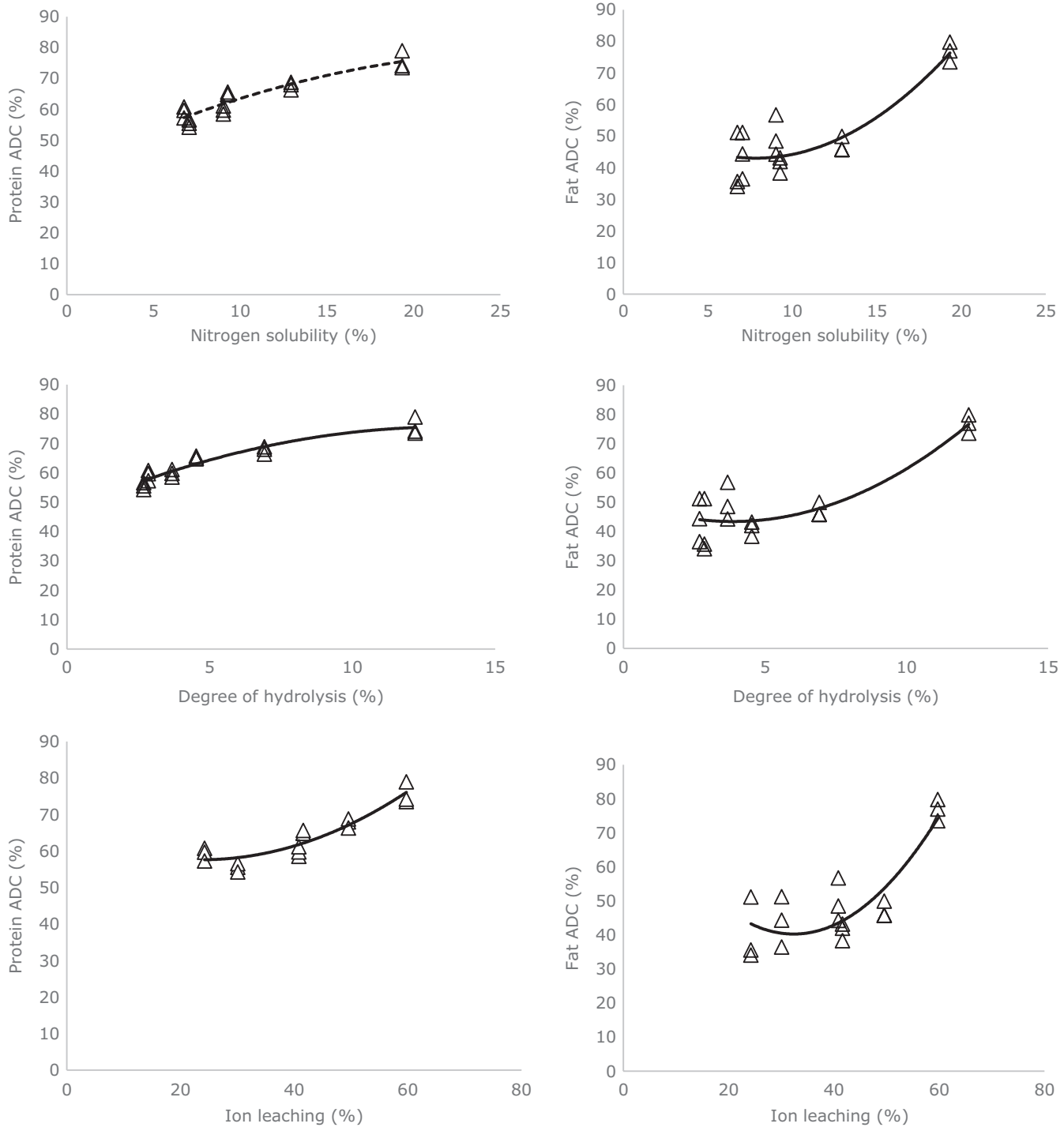

FIGURE 2 Relationships (quadratic) between the apparent digestibility coefficients (%ADC) of protein and fat from Nannochloropsis gaditana in juvenile African catfish and in vitro nutrient accessibility measurements of Nannochloropsis gaditana—nitrogen solubility, degree of protein hydrolysis and ion leaching. Significant ($p < 0.05$) and non-significant ($p > 0.05$) relationships are indicated by solid and dotted lines, respectively

to hydrolyze NSP from vegetal sources (Leenhouwers, Veld, Verreth, & Schrama, 2007). The ADCs values for NSP (3%–24%) in all the experimental diets are similar to or lower than values recorded in previous experiments (Leenhouwers et al., 2007; Teuling et al., 2017). Cell disintegration by bead milling (BEM) and disruption of cell walls by freezing (FRO) and cold pasteurization (L40) increased protein digestibility of microalgae relative to the intact microalgae (UNT). Cell wall disruption by pasteurization (PAS) and freeze-drying (FRD) had no effect on protein digestibility compared to the untreated microalgae (UNT). The ADC of protein for intact *N. gaditana* was lower than those measured for fishmeal and for soybean meal in African catfish by Fagbenro and Davies (2001). Fat digestibility of

TABLE 9 Apparent digestibility coefficient (%ADC) of nutrients from untreated and treated *Nannochloropsis gaditana*[a] in juvenile Nile tilapia (*Oreochromis niloticus)* and African catfish (*Clarias gariepinus)*. Values are means and the pooled standard error of the mean (*SEM*)

		Algal treatments							*p*-values		
ADC	Fish specie	UNT	PAS	FRD	FRO	L40	BEM	*SEM*	Algae	Fish specie	Algae*fish specie
Dry matter	Tilapia	48.35	50.22	50.64	55.23	61.19	66.28	2.60	<0.001	0.056	0.904
	African catfish	48.27	45.22	47.03	50.20	60.25	63.68				
Crude protein	Tilapia	61.51	60.66	60.61	66.25	72.87	78.01	0.80	<0.001	<0.001	0.013
	African catfish	59.27	55.46	59.82	65.15	67.71	75.57				
Gross energy	Tilapia	50.96	50.11	53.04	57.04	60.61	69.20	1.87	<0.001	<0.001	0.930
	African catfish	46.60	43.64	46.69	48.78	53.03	63.47				
Fat	Tilapia	50.44	56.13	57.77	53.05	66.39	82.02	2.17	<0.001	<0.001	0.034
	African catfish	40.32	44.04	49.87	41.19	47.19	76.80				
Total carbohydrates[b]	Tilapia	34.89	37.99	38.50	40.46	46.55	56.73	10.34	0.555	0.458	0.512
	African catfish	31.66	48.70	43.28	28.11	45.41	34.93				
Ash	Tilapia	13.19	29.09	29.58	26.13	55.01	37.91	8.72	<0.001	<0.001	0.319
	African catfish	56.42	33.08	61.22	60.70	88.94	69.52				
Phosphorus	Tilapia	91.99	110.89	94.75	109.12	110.70	120.87	4.85	<0.001	<0.001	0.091
	African catfish	76.52	70.48	74.89	87.41	83.54	86.34				

[a]UNT, PAS, FRD, FRO, L40 and BEM: 70% REF, 30% untreated, pasteurized, freeze-dried, frozen-thawed, commercially processed (NutriSpring® Liquid 40, Algaspring, NL) and bead-milled biomass of *Nannochloropsis gaditana*, respectively. All *N. gaditana* biomass was drum dried, except FRD. [b]Total carbohydrates include starch and non-starch polysaccharides.

microalgae was only increased by bead milling (36% increase from UNT). The increase in growth performance and nutrients digestibility of microalgae in African with L40 and BEM treatments shows that disruption treatments could be an effective strategy to reduce the negative impact of rigid cell walls on utilization of microalgae in fish. In the case of *N. gaditana*, the cellulosic algaenan bilayer cell walls are very resistant to digestive enzyme hydrolysis (Scholz et al., 2014; Staehelin & Pickett-Heaps, 1975). There are variations between this study and an earlier study on intact *N. gaditana* in African catfish (Teuling et al., 2017). In the current experiment, the FCR and ADC of protein (1.04 and 59%) for intact *N. gaditana* were relatively lower than the measured values in the previously cited experiment (0.89 and 72%). It should be mentioned that the *N. gaditana* used in the present study was a different strain (AS1405) than the algae used in the previous study (AS1301), and that the average initial fish size (44 g) in the current experiment was lower than in the previous experiment (70 g).

It has been previously shown that the different disruption treatments used in this study result in different levels of nutrient release from the microalgae (Teuling et al., 2019). The different disruption treatments were found to increase the release of different nutrients from microalgae by a factor of 0.2–4.3 compared to the intact microalgae (Teuling et al., 2019). Milder physical treatments (PAS, FRD, FRO and L40) causing partial porosity of the cells are sufficient to cause the release of protein, nitrogen and ions, while fat accessibility requires complete breakdown of the cells (Teuling et al., 2019). Despite the increase in nutrients accessibility after the disruption treatments (Teuling et al., 2019), cell wall disruption treatments such as PAS, FRD and FRO did not improve growth performance (as seen in their FCR and biomass gain) and digestibility of microalgae in African catfish relative to intact microalgae. The maillard reaction products (MRP) quantified by Teuling et al. (2019) showed that this observation is unconnected with formation of MRP after the microalgae processing. MRP contents of microalgae were higher in PAS and FRO treatments, but lower in FRD treatment compared to the UNT (Teuling et al., 2019). Also, there was no correlation between MRP contents in microalgae (data from Teuling et al. (2019)) and ADCs of microalgae (protein and fat; Table 10). Thermal treatments of feeds and feed ingredients are generally known to cause formation of MRP, with reduced bioavailability of amino acids (especially lysine), and overall nutritional quality of protein (Hulshof, Bikker, Poel, & Hendriks, 2016; van Rooijen, 2015; Rutherfurd, 2010; Villanea, 2017). Since it has been ascertained that utilization of microalgae after the cell wall disruption seems to be unconnected to MRP formation, it is therefore difficult to conclude on those factor(s) or elements responsible for the lower utilization of microalgae after the PAS, FRO and FRD cell wall disruption treatments.

The variability in nutrient release with different disruption treatments was in line with the performance and nutrient digestibility data observed in this experiment. Correlation tests showed that microalgal nutrient digestibility was influenced by the accessibility of nutrient from microalgae. Nitrogen solubility, degree of hydrolysis and ion leaching measurements positively correlated with the ADCs of protein and fats (see Table 8, Figure 1). The positive, but rather weak correlation between the ion leaching and fats digestibility was an indication that relatively large porous cells are needed to increase the accessibility and digestibility of fats. Fat extractability improved by 400% with BEM treatment, but no effect was observed with other treatments (Teuling et al., 2019). It can be argued that the difference in accessibility of fats and other nutrients was due to the variation in their structural conformation. Lipids (predominately triglycerides) in plant materials, including microalgae are embedded within the intracellular spaces (Maurer et al., 2013). Therefore, one could speculate that cell wall disruption causing partial perforation of algal cells only allows release of protein, but not phospholipids, and triglycerides embedded within the intracellular spaces. This could be the ultimate reason why bead milling is a widely used disruption technique to improve lipid extractability during biofuel production from microalgae.

There was variation in the use of treated and untreated microalgae by African catfish and tilapia. Protein and fat digestibility of microalgae were both affected by the interaction effect (algal treatments vs. fish species). This implies that the difference in ADCs between the two fish species was dependent on the different cell wall disruption treatments. Irrespective of algal treatments, tilapia had better capacity to digest the microalgae than African catfish. ADCs of protein for microalgae was higher by 1%–5% in tilapia compared to catfish. In addition, ADCs of fats was also higher by 6%–12% in tilapia than catfish. As expected, herbivorous fish (e.g. Nile tilapia) are able to digest plant ingredients better than omnivorous fish, like catfish. Hlophe et al. (2014) show that tilapia through their intestinal microflora has more capacity to produce endogenous cellulase than catfish before feeding. In addition, the low pH conditions in Nile tilapia (which can reach pH 2 at 7 hr after feeding) (Saravanan et al., 2013) are more suited to digest

TABLE 10 Pearson correlations (*r*) between apparent digestibility of treated or untreated *Nannochloropsis gaditana* in juvenile African catfish (*Clarias gariepinus)* and corresponding contents of maillard reaction products (MRP) formed after the cell disruption treatments of microalgae

	Pearson correlation coefficients (*r*)[a]		
	FUR[b]	CEL[b]	CML[b]
Dry matter	−0.02 (0.94)*	0.13 (0.61)*	−0.14 (0.59)*
Crude protein	0.24 (0.35)*	0.13 (0.61)*	0.08 (0.75)*
Gross energy	0.08 (0.75)*	0.11 (0.68)*	−0.05 (0.84)*
Crude fat	−0.05 (0.84)*	0.18 (0.48)*	−0.11 (0.66)*
Ash	−0.08 (0.75)*	−0.13 (0.62)*	−0.17 (0.51)*
Phosphorus	0.54 (0.02)**	0.06 (0.82)*	0.38 (0.12)*

Notes. Values in parenthesis are *p*-values for the correlation tests.
[a]Pearson correlation coefficients (*r*) between apparent digestibility coefficient values ($n = 18$) and the mean values of MRP measurement ($n = 6$).
[b]Level of MRP in the microalgae; FUR—furosine; CEL—carboxyethyllysine; CML—carboxymethyllysine. $^{**}p < 0.01$ $^{*}p > 0.1$.

plant materials than African catfish (pH 3.5 at 8 hr after feeding) (Hlophe et al., 2014). Therefore, the marginal increase in nutrient ADCs for all the microalgae in Nile tilapia compared to African catfish could be attributed to the combined effects of endogenous cellulase production and low pH conditions in the digestive tract. In both fish species, BEM and L40 microalgae are better digested as shown in the protein ADCs (67% – 78%) compared to the UNT (51%–59%). It can be inferred from the above discussion that cell wall characteristic is not the only overriding factor affecting the utilization of microalgae. The digestibility of microalgae is also influenced by the digestive systems of fish. The impact of rigid cell wall on nutrient digestibility of microalgae seems to have a domineering effect over the differences in digestive systems between herbivorous and omnivorous fish.

5 | CONCLUSIONS

Partial and complete disruption of cell walls of microalgae resulted in increased nutrient digestion in African catfish. Bead milling and cold pasteurization cell wall disruption improved performance and digestibility of microalgae in African catfish more than the other treatments and increased growth performance to similar values as the reference diet. The impact of cell wall disruption on nutrient digestibility from microalgae differs between African catfish and Nile tilapia. Also for other microalgae, especially those with rigid cell walls, it is therefore expected that cell wall disruption can increase nutrient accessibility from microalgae.

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ORCID

Jeleel O. Agboola https://orcid.org/0000-0003-4763-592X

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