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Cell wall disruption increases bioavailability of *Nannochloropsis gaditana* nutrients for juvenile Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

In this study the correlation between the accessibility of nutrients and *in vivo* nutrient digestibility was tested on the marine microalga *Nannochloropsis gaditana* in juvenile Nile tilapia (*Oreochromis niloticus*). It was hypothesized that disrupting the cell walls of microalgae increases the nutrient accessibility and digestibility. *N. gaditana* biomass was subjected to physical treatments (pasteurization, freezing, freeze drying) or mechanical treatments (bead milling) to influence its cell wall integrity. These treatments resulted in an up to 4 x increase in *in vitro* accessibility of *N. gaditana* nutrients, assessed from measurements of leaching and susceptibility to protein hydrolysis. Apparent digestibility coefficients of macronutrients, dry matter, energy, phosphorus and calcium of untreated and treated microalgae biomass were determined in triplicate, at a 30% diet inclusion level. Bead milling the algae led to the highest increase in *in vivo* digestibility of dry matter, energy, protein, fat, ash and calcium on ingredient level, compared to untreated algae biomass. This includes an increase in apparent digestibility coefficient (ADC) of protein and fat from 62 to 78% and from 50 to 82%, respectively. ADCs of total carbohydrates and of phosphorus were not affected by algal cell disruption. *In vivo* digestibilities of *N. gaditana* dry matter, energy, protein, and fat were positively correlated ($p < .001$; $r \geq 0.91$) with the nutrient accessibility of *N. gaditana*, as estimated with *in vitro* nutrient leaching analyses. This shows that the *in vitro* methods used are effective ways to assess the effect of mechanical and physical treatments on *in vivo* nutrient quality of a single ingredient. The results of this study confirm that nutrient accessibility plays a significant role in the nutrient digestibility of the microalga *Nannochloropsis gaditana* in Nile tilapia.

1. Introduction

In aquaculture, microalgae are currently predominantly used as live food for larvae. From the limited data available on the use of algae in compound feeds for the grow-out phase of fish, nutrient digestion from microalgae was found to vary greatly among various microalgal species (Sarker et al., 2016; Teuling et al., 2017b). The assumption is that in some algae the nutrient digestion can be limited by the presence of the algae cell walls, limiting the *in vivo* accessibility of the intracellular nutrients. Consequently, it is expected that for those algae the *in vivo* digestibility can be improved by improving the nutrient accessibility after disruption of the cell wall structure. The aim of this study was to correlate the accessibility of microalgae nutrients measured *in vitro* to the *in vivo* nutrient digestibility in fish.

Reported apparent digestibility coefficients (ADCs) of microalgal protein in fish have a high variability between studies (67–86%) (Burr et al., 2011; Gong et al., 2017; Safari et al., 2016; Sarker et al., 2016; Teuling et al., 2017b; Tibbetts et al., 2017). In some of these studies, the protein digestibility was low. This low protein digestibility has been suggested to be related to differences in cell wall structure and associated nutrient accessibility of the algae and cyanobacteria. This cell wall hardness of microalgae and cyanobacteria has been quantified previously (Teuling et al., 2017b), but has not yet been quantitatively related to protein digestibility in fish or other animal studies. Studies have shown, however, that treating microalgae with (high pressure) homogenization (Janczyk et al., 2007; Komaki et al., 1998; Sommer et al., 1991; Tibbetts et al., 2017) can increase the *in vivo* accessibility and the digestibly of nutrients and natural colorants in rats and fish. Effect of cell wall disruption on nutrient digestibility in fish is limited to a single study by Tibbetts et al. (2017). They described how homogenization of the freshwater alga *Chlorella* sp., increased the ingredient protein ADC from 79.5% to 85.4% (at dietary inclusion levels of 30%)

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Table 1
Analyzed chemical composition of treated or untreated *Nannochloropsis gaditana* biomass included in diets (30% inclusion level) that were fed to juvenile Nile tilapia. Values are presented are means, in g/kg DM, unless stated otherwise.

	Nannochloropsis gaditana[a]						
	UNT	PAS	FRD	FRO	L40	BEM	%CV[d]
Dry matter (g DM/kg wet weight)	964.2	972.1	931.0	949.7	976.0	919.1	0.05
Gross energy (kJ/g)	24.5	24.4	24.2	24.6	23.6	24.7	0.35
Crude protein (N * 6.25)	500.1	508.6	533.2	488.8	487.2	490.7	0.42
Crude protein (N * 4.84)	387.3	393.9	412.9	378.5	377.3	380.0	0.42
Crude fat	160.9	162.1	129.7	173.3	156.6	146.3	2.15
Total carbohydrates[b]	160.4	168.0	135.6	158.3	165.3	123.9	1.45
Rhamnose	7.77	7.36	7.96	7.75	8.2	8.50	3.99
Fucose	1.65	1.25	1.60	1.55	1.8	2.00	13.26
Arabinose	1.46	1.65	1.42	1.79	1.8	1.21	9.94
Xylose	1.84	1.62	1.87	1.80	1.8	1.80	7.34
Mannose	26.0	28.7	16.2	35.5	28.6	37.2	1.97
Galactose	22.1	23.6	21.5	18.0	23.7	19.4	3.69
Glucose	83.6	85.1	68.2	76.1	82.9	36.6	1.13
Ribose	5.7	5.7	6.4	6.0	5.6	4.7	3.11
Uronic acids	10.4	10.4	10.5	9.8	10.9	12.5	8.40
Starch	1.0	2.7	2.3	4.5	5.7	5.9	19.93
NSP[c]	159.4	165.4	133.2	153.8	159.7	118.0	1.91
Ash	72.2	70.3	81.6	77.7	95.8	90.9	0.20
Phosphorus	12.8	13.1	8.3	11.0	11.8	11.3	0.47
Calcium	4.65	4.69	4.60	3.28	5.40	5.95	0.54
Copper	< 0.01	< 0.01	< 0.01	0.01	0.00	< 0.01	35.24
Iron	1.14	1.14	1.00	0.75	0.99	0.60	0.64
Magnesium	3.39	3.27	4.02	3.65	4.35	4.29	0.44
Manganese	0.21	0.21	0.24	0.18	0.20	0.22	0.40
Zinc	0.03	0.03	0.03	0.04	0.03	0.03	15.32

[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. With exception of FRD, all *N. gaditana* biomass was drum dried.
[b] Total carbohydrates comprise starch and NSP.
[c] NSP = total carbohydrates – starch.
[d] Coefficient of variation.

in juvenile Atlantic salmon (*Salmo salar* L.). Methods that can be used to disrupt algal cell walls can in general be divided into four categories: enzymatic, chemical, physical and mechanical methods (Lee et al., 2012). Examples of each are the use of cellulases (enzymatic), alkaline, acid and organic solvents (chemical), bead milling, high-pressure homogenization and microfluidics and ultrasonication (mechanical) and thermal treatments and freeze drying (physical) (Lee et al., 2012; Middelberg, 1995; Tibbetts et al., 2017). To allow comparison of the effect of nutrient accessibility, the chemical composition and molecular structure of the nutrients should be the same between the treated and untreated algae. Enzymatic and chemical methods are believed to affect the integrity of inner-cell nutrients. For this reason, the use of physical and mechanical methods is preferred over the use of chemicals and enzymes. Mechanical methods like bead milling can be used to completely disrupt cell walls (Doucha and Lívanský, 2008; Middelberg, 1995), while keeping the composition intact. Physical methods like freezing and freeze drying (Mazur, 1969) and thermal treatments (Mendes-Pinto et al., 2001; Ometto et al., 2014) are milder since they can be employed to damage cell wall structures without completely disrupting the walls. A disadvantage of thermal processing is the chance of Maillard reaction product formation. The Maillard reaction is a chemical reaction between amino acids and reducing sugars. The reaction is accelerated by heat and is known to reduce the nutritional quality of proteins and amino acids present (van Rooijen et al., 2013).

To clearly discern the effect of the disruption methods on nutrient digestibility it is important to quantify the extent of cell disruption. Cell disruption can be measured directly by microscopy or particle size analysis, or indirectly by measuring the release of intracellular products (Middelberg, 1995). Disruption refers to treatments where the cell wall structure is compromised while the cells may still appear 'intact' in shape and size. Disruption can be quantified by the release of intracellular products (Ometto et al., 2014; Tibbetts et al., 2017). Other treatments like bead milling can result in complete breakdown of algae cells. The 'broken cells' can be analyzed or quantified using light microscopy and particle size analysis.

To test the effect of cell wall damage on microalgae nutrient digestibility, one type of microalgae (*Nannochloropsis gaditana*) was treated in 5 different ways. These cell disruptive treatments aimed to increase the protein accessibility and subsequent protein digestibility. Nutrient accessibility of the treated and of untreated *N. gaditana* was measured *in vitro*, and nutrient digestibility was measured *in vivo* in triplicate in juvenile Nile tilapia (*Oreochromis niloticus*).

2. Materials and methods

2.1. Materials

All chemicals used for the *in vitro* study and for analyses of ingredients, feeds and feces were of analytical grade and purchased from either Merck (Darmstadt, Germany) or Sigma Aldrich (St. Louis, MO, USA), unless stated otherwise. Commercially available biomass of marine *Nannochloropsis gaditana* (strain number AS1405) was kindly provided by AlgaSpring B.V. (Almere, The Netherlands). The biomass was provided in 2 batches that were harvested in June and July of the same year. No stabilizers or other chemicals were added to the biomass. After harvesting, the *N. gaditana* biomass was centrifuged to a paste of 20% [*w*/w DM]. The biomass was washed by diluting the paste 1:1 with tap water, following by another centrifugation to a final DM of 20% [*w*/w]. Pancreatin used originated from porcine pancreas (Sigma product number P3292).

2.2. Nannochloropsis gaditana treatments

N. gaditana biomass was treated by 5 different methods, aimed at decreasing the cell wall integrity and increasing the accessibility of