



©Asian Fisheries Society
ISSN: 0116-6514
E-ISSN: 2073-3720
<https://doi.org/10.33997/j.afs.2020.33.2.009>

Digestibility of Diets Micro-supplemented with Whole *Chlorella vulgaris* Beijerinck, 1890 in Hybrid Red Tilapia (*Oreochromis mossambicus* × *O. niloticus*)

MUHAMMAD AHMAD TALBA^{1,2,*}, MOHAMED SHARIFF^{1,3}, YONG MENG GOH¹, FATIMAH MD. YUSOFF^{3,4,5}

¹Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

²Present affiliation: Veterinary Teaching Hospital, Ahmadu Bello University, Samaru, Zaria, Kaduna, Nigeria

³Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

⁴Department of Aquaculture, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

⁵International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

*E-mail: mutalgsh80@gmail.com | Received: 25/02/2020; Accepted: 20/06/2020

Abstract

Microalgae are rich in carotenoids, immunomodulating metabolites and other nutrients suitable as fish feed. *Chlorella* sp. has all the essential amino acids, and its micro-supplementation enhances fish performance. While there are several studies on the role of *Chlorella* sp., little information is known about its digestibility at micro-supplementation levels. The present study was aimed at investigating digestibility of diets micro-supplemented with whole *Chlorella vulgaris* Beijerinck, 1890 at different inclusion levels in hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). The experiment was carried out using whole *C. vulgaris* to determine the nutrient apparent digestibility coefficient (ADC) of the diet. A total of 84 hybrid red tilapia (105 ± 7 g) were assigned randomly into four groups with 21 fish each for a 21-day feeding trial. The treatment groups were; control (basal diet only), test diet I, II and III (basal diet + 1 %, 3 %, and 5 % *C. vulgaris* w/w, respectively). The result showed that micro-supplementation of *C. vulgaris* at all the inclusion levels significantly improved the ADC values for dry matter and protein, which was also associated with an increase in the micro-supplementation level. Thus, the result of the present study showed the ability of hybrid red tilapia to digest diets micro-supplemented with whole *C. vulgaris* at different inclusion levels.

Keywords: apparent digestibility coefficient, fish feeds, low-level supplementation, microalgae

Introduction

The global aquaculture production has been increasing over the past decades. The high production of farmed fish is linked to the increasing worldwide demand for protein of fish origin alongside limited wild catch, which is one of the primary sources of protein for the aquaculture industry and has resulted in exorbitant fishmeal prices (Kim, 2015). Therefore, the demand for alternative fish feed ingredients like microalgae has been increasing (Kim, 2015). Microalgae are known as a good source of protein and lipids (De Silva et al., 2018). Vitamin and amino acid profile of most microalgae are better than other ingredients used as alternative protein in fish feeds (Becker, 2007). Microalgae enhance the nutritional content of conventional animal feeds and act as probiotic agents, in addition to improvement of fish performance. However, macro-supplementation (high-level supplementation) of microalgae results in suboptimal growth and other

performances in crustaceans and fish (Shields and Lupatsch, 2012; Maliwat et al., 2017; Ahmad et al., 2018). Nevertheless, microalgae at low-level supplementation improve fish performances and resistance to diseases (Becker, 2007).

Digestibility and palatability are the key factors for the selection of an ingredient as an alternative component in aquaculture feed (Glencross et al., 2007; Shields and Lupatsch, 2012). The selection of microalgae for use as aquaculture feed depends on its digestibility, ease of culture, size and rapid production of biomass (Spolaore et al., 2006; Guedes et al., 2015). Extracellular polysaccharides in the cell wall of microalgae interfere with nutrient digestion and its subsequent absorption (Spolaore et al., 2006; Guedes et al., 2015). Among the several microalgae species, *Chlorella* spp. is reported to have a rigid and indigestible cell wall that hampers its use (Takashi and Kenji, 1982; Doucha and Lívanský, 2008). Besides other

factors, effects of microalgae on growth performance and fish health is associated with nutrient digestibility (Teuling et al., 2017).

Algae with minimal digestibility may require further processing to allow digestive enzymes to permeate the cell wall and to facilitate the utilisation of the vital nutrients. Extrusion process has been used to improve microalgal digestibility (Gong et al., 2018). Even though processing is crucial for digestion of *Chlorella* spp. (Shields and Lupatsch, 2012), subjecting the microalgae to high temperature during extrusion could interfere with the nutrient apparent digestibility coefficient (ADC) of microalgal diet. Thus, Maliwat et al. (2017) suggested that digestibility trials of diets supplemented with unprocessed microalgae be conducted. However, there are limited studies on the digestibility of microalgae at micro-supplementation levels (Teuling et al., 2017; Ahmad et al., 2018). Thus, the assessment of microalgal digestibility will give a better understanding of its potentials as a feed ingredient/supplement in aquaculture feeds (Becker, 2007). The current study was designed to investigate the digestibility of feeds supplemented with unprocessed *C. vulgaris* at micro-supplementation levels.

Materials and Methods

Fish and experimental protocol

Eighty-four, hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*, 105 ± 7.0 g) of mixed sex were obtained from the University Agriculture Park, Puchong, Malaysia. The experiment was carried out at the wet-lab of Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia. The fish were acclimatised to experimental condition 2 weeks prior to commencement of the trial in a fibreglass tank of

2000 L capacity, during which the control diet was fed to the fish. After 2 weeks of acclimatisation, the fish were randomly distributed into 12 V-bottom shaped rectangular glass tanks of 200 L capacity (7 fish per tank) at 4 g.L⁻¹ stocking density (three tanks per treatment group). The fish were maintained at a photoperiod of 12 h light and 12 h dark cycle. The water temperature was at a range of 27.0–28.0 °C throughout the experiment. Both the pH and dissolved oxygen were within the range of 6.5–7.9 and 5.8–6.7 mg.L⁻¹, respectively. Daily 25–30 % of the tank water was exchanged. The experimental protocol was reviewed and approved by the Universiti Putra Malaysia, Institutional Animal Care and Use Committee (UPM/IACUC/AUP-R052/2016).

Experimental diets, design and feeding

Four isonitrogenous and isolipidic diets were formulated (Table 1) using a commercial feed (Star Feed mill, Malaysia). The diet was ground and divided into four parts. Inert digestive marker (titanium dioxide) was added into each portion at 1 g.kg⁻¹ (0.1 %) as described by (Guzman-Cedillo et al., 2017), with slight modification. A portion was used as the control diet (basal diet and 0.1 % digestive marker only). In the *Chlorella vulgaris* Beijerinck, 1890 diets, the microalgal powder (Daesang Corporation, South Korea), was added into the remaining three portions at the following supplementation levels; 10 (1 %), 30 (3 %) and 50 (5 %) g.kg⁻¹ (test diets I, II and III, respectively). The diets were made by mechanically stirring the ingredients into a homogenous mixture using a stand mixer (Faber, Malaysia). Cool distilled water was added to achieve a consistency suitable for cold pelletising, using a manual pelletiser (Ajanta, India). Diets were dried in an oven at 37 °C for 24 h as described by Gong et al. (2018) with slight modification. At the

Table 1. Composition and proximate contents (as dry matter basis) of the control and test diets micro-supplemented with *Chlorella vulgaris* fed to hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) for 14 days.

Ingredients	Control diet ¹	Test diet I ²	Test diet II ³	Test diet III ⁴
Basal diet (g)	1000	1000	1000	1000
<i>Chlorella vulgaris</i>	-	10 g	30 g	50 g
Titanium dioxide (g)	0.1	0.1	0.1	0.1
Proximate analysis				
Dry matter (%)	90.82	92.11	92.73	92.08
Crude protein (%)	39.85	39.93	40.82	40.56
Ether extract (%)	11.48	11.72	11.69	12.09
Crude fibre (%)	7.01	6.91	6.75	7.02
Nitrogen free extract (%)	22.13	23.31	23.43	22.35
Total Ash (%)	10.35	10.24	10.04	10.06
Gross energy (KJ.g ⁻¹)	18.19	18.28	18.22	18.29

¹Control diet = Basal diet + 0 % *Chlorella vulgaris*, ²Test diet I = Basal diet + 1 % *Chlorella vulgaris*, ³Test diet II = Basal diet + 3 % *Chlorella vulgaris*, ⁴Test diet III = Basal diet + 5 % *Chlorella vulgaris*.

commencement of feeding the fish with the experimental diets, fish in each tank were hand fed appropriate diet at 3 % (percentage feed given per day) of their body weight twice daily at 0930 and 1700 h. An equal amount of feed was provided to the fish in the morning and afternoon. Uneaten feed was collected 30 min after feeding so as not to mix with the faecal samples.

Faecal collection

The faecal samples were collected twice daily, 30 min before the morning feeding and 30 min before the afternoon feeding, for 21 days. Faeces were collected using a suction tube attached to the bottom of the tanks and emptied into a sieve attached to the outlet of the suction tubes. The faeces collected in the initial six days were discarded. Therefore, a 14 day period of faecal collection provided a sufficient quantity of faeces for the analysis (Guelph system with slight modification) as described by Hernández et al. (2010). Uneaten feed was siphoned out of the faecal collection column after each feeding. The collected faeces were transferred into 50 mL conical tubes (Eppendorf, Germany) and centrifuged at 5000 g for 10 min. The pellets were collected and the supernatant was discarded. The pellets collected for each tank were pooled and kept at -20 °C. The pooled pellets were freeze-dried at the end of the experiment and used for proximate analysis.

Chemical analysis

The analysis of feeds (diets) and faecal samples were conducted at the Nutrition Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia, according to the following methods: moisture, crude protein, ether extract, ash, crude fibre, energy (automated oxygen bomb calorimeter)(AOAC, 1990). In addition, analysis of titanium dioxide (TiO₂) concentration in feed and faeces was done according to the methods of Guzman-Cedillo et al. (2017). The ADCs for the nutrients and gross energy of the control and the test diets were analysed using the standard method as described by Hernández et al. (2010):

$$\text{ADC}(\%) \text{Nutrient/Energy} = [1 - (\text{ID} \times \text{NF}) / (\text{IF} \times \text{ND})] \times 100$$

ADC of dry matter was calculated according to the formula:

$$\text{ADC of dry matter}(\%) = 100 - (100 \times (\text{ID} / \text{IF}))$$

Where; ID = % indicator in diet; IF = % indicator in faeces; ND = % nutrient or gross energy in diet; NF = % nutrient or gross energy in faeces.

Statistical analysis

All data were subjected to one-way ANOVA and reported as the mean ± standard error (mean ± SE). Significantly different means were further clarified

using Tukey multiple comparison test. All statistical procedures were conducted at 95 % confidence level. The statistical analysis was performed using IBM SPSS Statistical package version 22.0 (USA).

Results

The apparent digestibility coefficient of *C. vulgaris* diets (test diets I–III) fed to fish were significantly higher ($P < 0.05$) than fish fed the control diet (Table 2). Nevertheless, the crude protein ADC value was significantly higher ($P < 0.05$) in the fish fed test diet III (80.51 ± 0.58) compared to those fed test diet I (78.52 ± 0.47)(Table 2).

Chlorella vulgaris as micro-supplement in fish diet presented different nutrient/energy ADC values (Table 2). Dry matter digestibility was significantly higher ($P < 0.05$) in the fish fed test diets than those fed control diet, however, there was no significant difference ($P > 0.05$) between the various treatment groups. Similarly, the crude protein digestibility of all the test diets was also significantly higher ($P < 0.05$) than the control diet and was seen to improve with increasing microalgal supplementation (Table 2). There was no significant difference ($P > 0.05$) in the gross energy ADC values between the treatment diets, but all the test diets were significantly higher than the control diet. There was no significant difference ($P > 0.05$) in the digestibility of lipid for all the diets tested.

Discussion

Despite the diets being isonitrogenous on a dry matter basis, changes in apparent digestibility coefficient (ADC) values were observed. The present study revealed higher crude protein ADC values in all the test diets. This is consistent with the finding of Teuling et al. (2017), who reported a higher crude protein ADC value in Nile tilapia fed extruded diets supplemented with *Chlorella* at higher dietary inclusion level. *Oreochromis* spp. are reported to have a feeding habit consistent with herbivorous fish (El-Sayed, 2006), therefore the fish could digest intact cell walls of microalgae. El-Sayed (2006) reported that *Oreochromis* spp. mainly feeds on phytoplankton and do feed on microalgae found within their vicinity. Cichlids have a uniquely flexible gut morphology that allows them to accommodate dietary changes with ease, which suggest their ability to digest and utilise various dietary contents (Wagner et al., 2009).

The higher ADC values of the test diets might be due to lower stomach pH reported in most *Oreochromis* spp. which could help in digesting the microalgal rigid cell wall and allow for nutrient absorption (Teuling et al., 2017). Furthermore, the test diets in the present study contained relatively high protein, which could trigger an extended digestive enzyme activity further in the length of the intestine (Sklan et al., 2004). Therefore, the significant nutrient digestion of the *Chlorella* diets (test diets) might be due to combined

Table 2. Nutrient apparent digestibility coefficient of diets supplemented with *Chlorella vulgaris* (test diets) alongside the basal diet (control diet) fed to hybrid red tilapia (*Oreochromis* sp.) as dry matter basis.

Nutrients/Energy	Dietary <i>Chlorella vulgaris</i> (%)			
	Control diet ¹	Test diet I ²	Test diet II ³	Test diet III ⁴
Dry matter (%)	31.80 ± 1.67 ^a	36.24 ± 1.89 ^b	38.83 ± 0.38 ^b	40.62 ± 1.38 ^b
Crude protein (%)	73.71 ± 1.02 ^a	78.52 ± 0.47 ^b	78.79 ± 1.04 ^{bc}	80.51 ± 0.58 ^c
Ether extract (%)	76.33 ± 1.76 ^a	74.55 ± 1.22 ^a	75.36 ± 0.78 ^a	73.37 ± 1.14 ^a
Gross energy (%)	35.24 ± 0.39 ^a	39.86 ± 1.82 ^b	42.15 ± 0.45 ^b	40.36 ± 0.68 ^b

Values with different superscripts (alphabets) within the same row are significantly different at $P < 0.05$ (mean ± S.E). ¹Control diet = Basal diet + 0 % *Chlorella vulgaris*, ²Test diet I = Basal diet + 1 % *Chlorella vulgaris*, ³Test diet II = Basal diet + 3 % *Chlorella vulgaris*, ⁴Test diet III = Basal diet + 5 % *Chlorella vulgaris*.

activities of the acidic stomach (Teuling et al., 2017), extended digestive enzymes activity (Sklan et al., 2004; Skrede et al., 2011) and the long transit time of the diets in the distinctively long intestine of tilapia which is associated with prolonged fermentation and enzymatic activity (El-Sayed, 2006; Wagner et al., 2009). Among the supplementation levels investigated, 5 % *C. vulgaris* (test diet III) appeared to be the preferred inclusion level for fish with feeding habits similar to that of hybrid red tilapia due to the higher crude protein ADC value.

The digestibility of diets containing different microalgae have been assayed in several cultured fish. In comparison to the present study, a similar crude protein ADC value for *C. vulgaris* (79.7 %) was reported by Sarker et al. (2016), in a study on Nile tilapia. Teuling et al. (2017) reported crude protein ADC values for *C. vulgaris* (86.1 % and 82.4 %), when supplemented at 30 % inclusion level in Nile tilapia and African catfish, respectively. The ADC values of crude protein in the previous reports were relatively higher than the values obtained in the current study. Digestibility trial on rainbow trout and mirror carp fed diet containing *Spirulina maxima* (*Limnospira maxima* (Setchell and N.L. Gardner, 1917) Nowicka-Krawczyk, Mühlsteinová and Hauer, 2019) at 68 % inclusion level revealed protein ADC values of 83.1 % and 87.1 %, respectively (Atack et al., 1979). In comparison, the protein ADC values of all the test diets I, II and III (78.52 %, 78.79 % and 80.51 %, respectively) in the present study were lower than most of the previous trials stated earlier, which could be due to lower inclusion levels of *C. vulgaris* and easily digestible cyanobacterial cell wall of *Arthrospira* sp. (Teuling et al., 2017).

The energy ADC values of the test diets I, II and III (39.86 %, 42.15 % and 40.36 %, respectively) in the present study were significantly higher ($P < 0.05$) than the control (35.25 %). Sarker et al. (2016) reported higher energy ADC values of *Chlorella* diets at 84 % in Nile tilapia, whereas Teuling et al. (2017) reported 71.8

% and 78.5% in African catfish and Nile tilapia, respectively.

A study on diets containing defatted biomass of 20 % *Desmodesmus* sp. was reported to have the ADC values for protein, lipid and energy of 84 %, 94 % and 80 %, respectively (Kiron et al., 2016). In the present study, the protein, ether extract (lipid) and gross energy digestibility values of diets supplemented with 5 % *C. vulgaris* (test diet III) were 80.51 %, 73.37 %, and 40.36 %, respectively, which were relatively lower than the values in the existing literature on digestibility of *Chlorella* diets. The discrepancy could be due to the higher microalgae supplementation levels and/or extrusion processing of the microalgae diets used in the previous studies.

Conclusion

Compared to the control diet, the present study demonstrated higher nutrient and energy ADC values in all the test diets. Therefore, the current trial showed the hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) ability to digest diets micro-supplemented with unprocessed *C. vulgaris* at various micro-supplementation levels. This information is important in order to optimise the use of *C. vulgaris* on a small scale in the aquaculture industry. Thus, further studies are necessary to determine the effect of different dietary micro-supplementation levels of *C. vulgaris*, and duration of feeding (short, medium and long term) on growth performance, immunity and haemato-biochemical indices of various fish. In addition, investigating the presence or absence of interaction between the supplementation levels of the *C. vulgaris* and durations of feeding would be of value.

Acknowledgements

This study was funded by a grant from Ministry of Education, Malaysia through Universiti Putra Malaysia, project number PRGS/2015/WAB01/UPM/01/1 and the

first author thanks Ahmadu Bello University, Needs Assessment Fund, Federal Ministry of Education Nigeria for sponsoring his study. The study was also partially supported by the SATREPS-COSMOS (JICA-JST) research grant.

References

- Ahmad, M.T., Shariff, M., Yusoff, F.M., Goh, Y. M., Banerjee, S. 2018. Applications of microalgae *Chlorella vulgaris* in aquaculture. Reviews in Aquaculture 12:328–346. <https://doi.org/10.1111/raq.12320>
- AOAC. 1990. Official methods of analysis of the Association of the Official Analytical Chemists AOAC. 15th Edition. Arlington, VA, USA. 1230 pp.
- Atack, T.H., Jauncey, K., Matty, A.J. 1979. The utilization of some single cell proteins in fingerlings of mirror carp. Aquaculture 18:337–348. [https://doi.org/10.1016/0044-8486\(79\)90037-1](https://doi.org/10.1016/0044-8486(79)90037-1)
- Becker, E.W. 2007. Micro-algae as a source of protein. Biotechnology Advances 25:207–210. <https://doi.org/10.1016/j.biotechadv.2006.11.002>
- De Silva, A.Z., Shariff, M., Banerjee, S., Yusoff, F.M. 2018. Growth and quality enhancement of *Chlorella vulgaris* Beyerinck (Beijerinck) 1890 using simple cost-effective medium. Asian Fisheries Science 31:61–72.
- Doucha, J., Livanský, K. 2008. Influence of processing parameters on disintegration of *Chlorella* cells in various types of homogenizers. Applied Microbiology and Biotechnology 81:431–440. <https://doi.org/10.1007/s00253-008-1660-6>
- El-Sayed, A.-F. M. 2006. Basic biology and ecology. In: Tilapia culture. CABI Publishing, Cambridge, Wallingford, Oxfordshire, pp. 29–34.
- Glencross, B.D., Booth, M., Allan, G.L. 2007. A feed is only as good as its ingredients – A review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition 13:17–34. <https://doi.org/10.1111/j.1365-2095.2007.00450.x>
- Gong, Y., Guterres, H.A.D.S., Huntley, M., Sørensen, M., Kiron, V. 2018. Digestibility of the defatted microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon, *Salmo salar*. Aquaculture Nutrition 24:56–64. <https://doi.org/10.1111/anu.12533>
- Guedes, A. C., Sousa-pinto, I., Malcata, F. X. 2015. Application of microalgae protein to aquafeed. In: Handbook of marine microalgae; biotechnology advances, Kim, S. (Ed.), Elsevier Academic Press, Oxford, UK, pp. 93–125. <https://doi.org/10.1016/B978-0-12-800776-1.00008-X>
- Guzman-Cedillo, A.E., Corona L., Castrejon-Pineda, F., Rosiles-Martínez, R., Gonzalez-Ronquillo, M. 2017. Evaluation of chromium oxide and titanium dioxide as inert markers for calculating apparent digestibility in sheep. Journal of Applied Animal Research 45:275–279. <http://dx.doi.org/10.1080/09712119.2016.1174124>
- Hernández, A., Bórquez, A., Alcaíno, L., Morales, J., Dantagnan, P., Saez, P. 2010. Effects of autoclaving on the apparent digestibility coefficient of dehulled pea seed meal (*Pisum sativum* L.) in rainbow trout (*Oncorhynchus mykiss* W.). Ciencia e Investigacion Agraria 37:39–46. <https://doi.org/10.4067/S0718-16202010000300003>
- Kim, S. 2015. Handbook of marine microalgae biotechnology advances. 1st Edition. Elsevier Academic Press, Oxford, UK. 604 pp. <https://doi.org/10.1016/B978-0-12-800776-1.00001-7>
- Kiron, V., Sørensen, M., Huntley, M., Vasanth, G.K., Gong, Y., Dahle, D., Palihawadana, A.M. 2016. Defatted biomass of the microalgae, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. Frontiers in Marine Science 3:1–12. <https://doi.org/10.3389/fmars.2016.00067>
- Maliwat, G.C., Velasquez, S., Robil, J.L., Chan, M., Traifalgar, R.F., Tayamen, M., Ragaza, J.A. 2017. Growth and immune response of giant fresh-water prawn *Macrobrachium rosenbergii* (De Man) postlarvae fed diets containing *Chlorella vulgaris* (Beijerinck). Aquaculture Research 48:1666–1676. <https://doi.org/10.1111/are.13004>
- Sarker, P.K., Gamble, M.M., Kelson, S., Kapuscinski, A.R. 2016. Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from fresh-water *Spirulina* sp. feed ingredients. Aquaculture Nutrition 22:109–119. <https://doi.org/10.1111/anu.12230>
- Shields, R.J., Lupatsch, I. 2012. Algae for aquaculture and animal feeds. TATuP – Zeitschrift für Technikfolgenabschätzung in Theorie und Praxis 21:23–37. <https://doi.org/10.14512/tatup.21.1.23>
- Sklan, D., Prag, T., Lupatsch, I. 2004. Structure and function of the small intestine of the tilapia *Oreochromis niloticus* x *Oreochromis aureus* (Teleostei, Cichlidae). Aquaculture Research 35:350–357. <https://doi.org/10.1111/j.1365-2109.2004.01020.x>
- Skrede, A., Mydland, L.T., Ahlstrøm, Ø., Reitan, K., Gislerød, H.R., Øverland, M. 2011. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. Journal of Animal and Feed Sciences 20:131–142. <https://doi.org/10.22358/jafs/66164/2011>
- Spolaore, P., Joannis-cassan, C., Duran, E., Isambert, A. 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering 101:87–96. <https://doi.org/10.1263/jbb.101.87>
- Takashi, Y., Kenji, S. 1982. Comparative studies on *Chlorella* cell walls: Induction of protoplast formation. Archives of Microbiology 132:10–13. <https://doi.org/10.1007/BF00690809>
- Teuling, E., Schrama, J.W., Gruppen, H., Wierenga, P.A. 2017. Effect of cell wall characteristics on algae nutrient digestibility in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarus gariepinus*). Aquaculture 479:490–500. <https://dx.doi.org/10.1016/j.aquaculture.2017.06.025>
- Wagner, C.E., McIntyre, P.B., Buels, K.S., Gilbert, D.M., Michel, E. 2009. Diet predicts intestine length in Lake Tanganyika's cichlid fishes. Functional Ecology 23:1122–1131. <https://doi.org/10.1111/j.1365-2435.2009.01589.x>