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Digestibility and pricing of *Chlorella sorokiniana* meal for use in tilapia feeds

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ABSTRACT: Several microalgae contain in excess of 50 % crude protein with amino acid profile comparable to that of fish meal. In addition, high polyunsaturated fatty acid contents encourage their use in animal feeding and nutrition, particularly in the formulation and processing of aquafeeds. This study aims at estimating the feasibility of *Chlorella* meal as feed ingredient for the feeding and nutrition of farmed tilapia based upon digestibility data. Juvenile tilapia were stocked in conical-bottomed tanks (200 L) with superficial, continuous water flow, and fed to apparent satiation in three daily meals with a reference diet and a test diet containing 30 % lyophilized *Chlorella sorokiniana* added of an inert marker. Feces were collected overnight by sedimentation in refrigerated, plastic containers coupled to the tanks and analyzed for determination of chemical composition and inert marker contents to estimate apparent digestibility coefficients (ADCs) of protein and energy of *Chlorella* meal; registered ADCs of *Chlorella* meal were 90.5 and 84.22, respectively. A pricing model considering the quantity of digestible nutrient was proposed based on ADCs of *Chlorella* and compared with the price of fishmeal (FM) and soybean meal (SBM). The indicative prices to elicit the use of *Chlorella* as a protein source rather than FM or SBM for the feed and nutrition of tilapia were 2.65 USD kg^{-1} and 0.66 USD kg^{-1}, respectively.
Keywords: fish nutrition, microalgae, fishmeal, pricing model

Introduction

While the production of oils by plants such as palm, coconut and physic nut (*Jatropha curcass*) average 5,960, 2,689 and 1,982 L ha^{-1} yr^{-1}, respectively, productivity estimates from laboratory data for some species of microalgae range on 10,000 and 60,000 L ha^{-1} yr^{-1} (Chisti, 2007; Chisti, 2008; Ranjitha and Vijayalakshmi, 2015; Singh and Singh, 2014). The farming of microalgae as potential raw material for the production of biodiesel has thus expanded rather quickly in recent decades. However, production of biodiesel from microalgae was never shown economically competitive because of high costs associated with agglutination, centrifugation, drying and lysis of cells for extraction of lipids (Demirbas and Demirbas, 2011; Nascimento et al., 2014). In this scenario, research on the use of microalgae by the pharmaceutical and cosmetics industries, human and animal nutrition has gained grounds in recent years.

Several microalgae contain in excess of 50 % crude protein (CP) with amino acid profiles comparable to that of fishmeal, the standard feedstuff for aquafeeds, and are a rather rich source of polyunsaturated fatty acids (PUFA), especially of the n-3 series. The use of microalgae in fish nutrition has thus been considered as a tool to enhance filet quality via deposition of n-3 PUFA, and replacement of fish meal and fish oil in aquafeeds (Christaki et al., 2011; Becker, 2007; Sarker et al., 2016a; Sarker et al., 2016b; Tulli et al., 2012; Tibaldi et al., 2015; Roy and Pal, 2015).

Reports on the use of *Chlorella* sp. in fish nutrition have shown both its potential as immunostimulant (Zah-ran and Risha, 2014; Grammes et al., 2013), pigment (Sergejevova and Masojidek, 2012; Gouveia et al., 2003), provitamin A source (Gouveia et al., 1998; Gouveia et al., 2002), performance enhancer (Xu et al., 2014), protein feedstuff (Mahboob et al., 2012) and surrogate protein source to fish meal (FM) in diets for *Macrobrachium rosenbergii* (Radhakrishnan et al., 2014). However, no information was found on the costs of *Chlorella* meal targeting economic viability of its use in fish feeding and nutrition.

Although price of soybean meal (SBM) is lower than that of FM, the use SBM in fish diets could induce alterations in the intestine epithelium, already referred to as SBM-induced enteropathy (SBMIE) (Grammes et al., 2013; Krogdahl et al., 2015; Krogdahl et al., 2010). The search for novel protein sources with competitive prices thus has become an important research line.

The pricing of new ingredients should consider not only their biological value, but also the price of ingredients ordinarily used in feed formulation for animal nutrition (Chauton et al., 2015; Maisashvili et al., 2015). The aim of this study was to define the price range to make *Chlorella* meal viable as a feed ingredient in diets for tilapia based on digestibility data.

Materials and Methods

Tilapia as a biological model

Fish of the genus *Oreochromis* sp. are universally known as tilapia and belong to the Cichlid family. They are originally from the African continent and have been introduced in several, tropical and subtropical countries

during the second half of the twentieth century (Eknath and Hulata, 2009). As a group, tilapia are the second most farmed fish in the world, recording 3×10^6 t in 2009 (Ng and Romano, 2013).

Experimental procedures

Digestibility trial was carried out in an open, continuous water flow system, under controlled temperature (27 $\pm$ 2 °C), oxygen (5 $\pm$ 0.5 mg L^{-1}), pH (7 $\pm$ 0.5) and photoperiod (12 h light + 12 h dark). Tilapia juveniles (78.01 $\pm$ 1.87 g) were stocked in a 500-L tank (n = 150) and acclimatized for 15 d feeding to apparent satiation on a commercial feed (32 % crude protein - CP) in three daily meals. Fish were then split into six groups, stocked in six, 200-L conical tanks (20 fish per tank, n = 3), and feed conditioned for 10 d to the experimental diets added of an inert marker, fed to apparent satiation three times a day (08h00, 12h00 and 18h00). After the last meal, tanks were cleaned, approximately 90 % of the water exchanged and 200-mL plastic flasks were coupled to the tank bottom, kept cooled within iceboxes, and feces where then collected by sedimentation (NRC, 2011) from 07:00 to 19h00 the next day. At the end of collection period, flasks where drained, centrifuged (3,100 g; 5 °C; 10 min) and fecal material immediately frozen-stored (-80 °C) until analysis. Frozen feces pellets were lyophilized and analyzed for proximate composition (protein, energy and dry matter), chromium oxide contents and amino acid profile (AOAC, 1999).

Experimental diets

A nutritionally complete, reference diet meeting all nutritional requirements for tilapia (Ng and Romano, 2013; Shiau, 2002) was processed by extrusion and experimental diets were obtained by adding *Chlorella sorokiniana* meal (CSM) to the reference diet at a 3:7 ratio. Both diets were added of 0.2 % chromic oxide (Cr_2O_3) as inert marker (Table 1). *Chorella* sp. meal was obtained from whole, lyophilized, non-commercial, animal nutrition grade cells harvested from autotrophic system (Algae Company; Piracicaba, SP, Brazil).

The reference diet was processed from finely ground (1 mm) ingredients and processed in experimental extruder, 3 mm die head. Pellets were dried in forced-air oven (45 °C) for 24 h, stored in opaque plastic bags and kept under refrigeration (4 °C) until use.

Estimation of apparent digestibility coefficients

Apparent digestibility coefficients (ADC) for protein, energy and amino acids of CSM were estimated as recommended in NRC (2011), as follows:

i) *ADCs of nutrients*

$$ADC = 1 - \frac{Cr_2O_3\ in\ feed}{Cr_2O_3\ in\ feces} \times \frac{Nutrient\ contents\ in\ feces}{Nutrient\ contents\ in\ feed}$$

Table 1 – Ingredient and proximate composition of the reference and test diets and the *Chlorella sorokiniana* meal.

Ingredients (%)	Reference diet	70 % Ref + 30 % *Chlorella* meal	*Chlorella* meal
Soybean meal	47.35	-	-
Corn	28.31	-	-
Corn gluten meal	11.20	-	-
Wheat bran	10.00	-	-
Soybean oil	1.71	-	-
Cellulose	0.24	-	-
Methionine	0.27	-	-
Chromium oxide III	0.20	-	-
Vitamin and mineral mix[1]	0.80	-	-
BHT[2]	0.02	-	-
NaCl	0.10	-	-
Proximate composition (%)[3]			
Crude protein	36.50	41.60	69.10
Lipids	3.10	3.60	0.60
Crude fiber	4.10	4.01	0.23
Ash	4.84	5.38	8.70
Non-protein nitrogen	0.39	0.41	0.77
Chromium oxide III	0.160	0.160	-
Energy (kJ g^{-1})	19.70	20.60	22.00
Essential amino acids (%)[4]	36.40	39.90	60.92
Arginine	2.40	2.65	3.71
Threonine	1.35	1.54	2.91
Valine	1.56	1.89	3.60
Methionine	0.76	0.81	1.08
Cystine	0.65	0.72	1.07
Isoleucine	1.47	1.67	2.73
Leucine	3.38	3.88	5.69
Phenylalanine	1.90	2.11	3.40
Lysine	1.67	2.14	5.17

[1]Premix (Nutrifish Guabi®; Campinas, SP, Brazil), warranty levels: Fe 1,500 mg; Cu 1,500 mg; Zn 12,500 mg; I 375 mg; Mn 12,500 mg; Se 87.5 mg; Co 125 mg; vit. A 2,500,000 IU; vit. D 3,600,000; vit. E 37,500 IU; vit. K 3,750 mg; vit.C 50,000 mg; vit. B1 4,000 mg; vit. B2 4,000 mg; vit. B6 4,000 mg; vit. B12 4,000 mg; pantothenic acid 12,000 mg; biotin 15 mg; folic acid 1,250 mg; niacin 22,500 mg; BHT 15,000 mg; [2]butylhydroxytoluene; [3]as determined by analysis of raw material; [4]except tryptophan.

ii) *ADCs of ingredients*

$$ADC_{TI} = ADC_{TD} + \left[\left(ADC_{TD} - ADC_{RD} \right) \times \left(\frac{0.7 \times N_{RD}}{0.3 \times N_I} \right) \right]$$

where: *ADC* = apparent digestibility coefficient; *TI* = test ingredient; *TD* = test diet; *RD* = reference diet; *N* = quantity or percentage of ingredients or nutrients in the diet; *I* = ingredient.

Pricing model for the use of CSM as ingredient

The model of price estimation for the use of CSM as dietary protein source was benchmarked against the main protein feedstuffs of the aquafeed industry, soybean meal (SBM) and fish meal (FM). From information derived from the World Bank database on historic prices of commodities (http://www.worldbank.org/en/research/commodity-markets), the price of digestive

protein contents of these feedstuffs were estimated as follows:

$$D_i = (100 \times C) / (CP \times ADC_{(p)})$$ Equation 1

where: D_i = average price of digestive protein of feedstuffs; C = average price of feedstuff (USD kg⁻¹); CP = crude protein contents of feedstuff (%); $ADC_{(p)}$ = apparent digestibility coefficient of feedstuff protein.

Values of $ADC_{(p)}$ and CP of FM and SBM were obtained from Gonçalves et al. (2009); values of C were estimated from the World Bank database on historic prices of commodities considering the average price of feedstuffs for the last five years. Price of CSM (UDS kg⁻¹) was estimated from these data as follows:

$$D_{(chl)} = D_i \times (CP_{(chl)} \times ADC_{(chl)}) / (CP_i \times ADC_i)$$ Equation 2

where: D_i = price of digestive protein of feedstuffs; $D_{(chl)}$ = price of digestive protein of CSM; CP_i = crude protein contents of feedstuff (%); $CP_{(chl)}$ = crude protein contents of CSM; $ADC_{(chl)}$ = apparent digestibility coefficient of the protein of CSM; $ADC_{(i)}$ = apparent digestibility coefficient of the protein of feedstuff.

Results and Discussion

Amino acid and macronutrients digestibility of *Chlorella sorokiniana* meal

The composition and digestibility of protein, energy and amino acids found for CSM were similar to those of FM and SBM (Table 2). Except for histidine and methionine, essential amino acids contents of CSM were higher than contents of FM and SBM. Although the methionine content is comparatively lower than that of FM, the methionine digestibility of CSM was higher, which contributes with a greater amount of this amino acid in

feed formulations. The nutritional methionine requirement of tilapia is 0.7 % of the dietary protein. However, methionine is precursor of cystine, thus, it is recommended supplying 0.9 % methionine to tilapia diets, and CSM meal can then be a good methionine source of diets as it may supply up to 1.02 % digestible methionine (Diogenes et al., 2016; Michelato et al., 2013; Shiau, 2002).

Protein contents of microalgae are ordinarily determined by Kjeldahl method, which uses the 6.25 factor to convert total nitrogen (N) into protein, an arguable procedure. When non-protein N present in chlorophyll, nucleic acids, free amino acids and amino saccharides molecules of microalgae are converted into crude protein with the use of the Kjeldahl method conversion factor, considerable biases can occur. However, the definition of a more realistic conversion factor for microalgae depends on the species, production system, growth stage of algae crop, harvesting and processing methods.

The suggested conversion factors for *Chlorella* sp. range between 5.02 for those harvested in the log phase of growth (highest protein concentration) and 4.84 for those harvested in the phase of logarithmic growth decline – death phase (higher concentration of lipids) (Templeton and Laurens, 2015; Angell et al., 2016). When using the conversion factor of 5.00, suggested by Angell et al. (2016), CP contents of the CSM drop from 69 % to 55 %, dry matter basis, yet a high protein content level. However, given that establishing a correlation between the CP levels of feed with protein levels of feces samples is rather challenging, values determined by the Kjeldahl method were herein conserved and used.

Lysine is the most limiting amino acid in fish diets, especially when plant protein sources are used as FM surrogates. Dietary lysine considerably affects growth performance, health and retention of N by fish (Li et al., 2009; Diogenes et al., 2016; NRC, 2011; Shiau, 2002). Lysine contents of CSM are considerably high – 5.17 % of

Table 2 – Comparison of CSM, fishmeal and soybean meal in terms of nutritional composition, apparent digestibility coefficient and digestible nutrients for tilapia.

Nutrient (%)	Chlorella meal[1]		Fishmeal[2]		Soybean meal[2]	
	Composition[3]	ADC	Composition[3]	ADC	Composition[3]	ADC
Protein	69.06	90.51 ± 0.89 (62.50)	60.15	82.59 (49.68)	51.70	94.13 (48.67)
Energy (kJ g⁻¹)	22.70	84.22 ± 4.78 (19.10)	17.90	95.29 (17.10)	20.30	84.12 (17.10)
Dry matter	-	82.71 ± 5.28 (78.83)	-	82.60 (74.79)	-	85.30 (74.82)
Essential amino acids[4]						
Arginine	3.71	96.10 ± 0.79 (3.57)	3.64	89.39 (3.26)	3.55	94.53 (3.35)
Phenylalanine	3.41	91.78 ± 1.08 (3.13)	2.68	88.89 (2.39)	2.83	97.58 (2.76)
Histidine	1.03	93.85 ± 1.69 (0.96)	1.28	92.24 (1.18)	1.43	94.40 (1.35)
Isoleucine	2.74	93.29 ± 0.96 (2.55)	2.57	89.70 (2.31)	2.63	92.64 (2.44)
Leucine	5.70	90.69 ± 1.28 (5.17)	4.47	92.59 (4.14)	4.44	93.32 (4.14)
Lysine	5.17	90.35 ± 0.81 (4.67)	4.51	93.63 (4.22)	3.06	97.01 (2.96)
Methionine	1.08	94.40 ± 0.60 (1.02)	1.73	87.26 (1.51)	0.49	90.70 (0.44)
Threonine	2.92	91.82 ± 1.13 (2.68)	2.47	83.93 (2.08)	2.02	90.40 (1.82)
Valine	3.61	91.65 ± 0.99 (3.31)	2.78	92.46 (2.57)	2.62	90.00 (2.36)

[1]Values determined by analysis ± standard error; [2]Reference: Gonçalves et al. (2009); [3]Dry matter basis; [4]Except tryptophan; ADC = apparent digestibility coefficient. Values within brackets represent % of digestible nutrient, CSM = *Chlorella sorokiniana* meal.

the protein content – and dietary requirements of tilapia are 1.6 % of the dietary protein (Shiau, 2002). Therefore, CSM can also be used as dietary lysine source, reducing, or even sparing the inclusion of synthetic lysine in tilapia diets. In addition to reducing costs, this characteristic can improve the efficiency of diets, since synthetic amino acids are more easily leached and their addition to diets can influence the balance of amino acids, not to mention that tilapia does not seem to use synthetic amino acids efficiently (Zarate et al., 1999; Liebert, 2009; Liebert and Benkendorff, 2007).

When comparing original data on CSM of this study with FM and SBM data from the reference literature (e.g. Gonçalves et al., 2009), it is important to consider the array of variables that possibly influence the results of both digestibility trials especially type and processing method of the reference diets (Table 3). Gonçalves et al. (2009) used semi-purified diets formulated from gelatin, casein and dextrin plus a lipid source to prevent possible interactions between ingredients and avoid the presence of antinutritional factors. However, the use of semi-purified diets, which are not usually as palatable as practical diets, may decrease feed intake, a source of disturbance in digestibility trials results, since animals may not ingest the proper amount of feed eliciting normal functions of the digestive system (NRC, 2011).

The extrusion process of diets causes denaturation of some proteins and gelatinization of the starch thus yielding better use of nutrients and, consequently, higher values of digestibility comparatively to pelleted diets (Ma et al., 2016). Comparing digestibility values recorded by Gonçalves et al. (2009), who used pelleted diets, and results herein reported from the use of extruded diets, recorded ADCs in both studies do not vary, that is, the processing method had less influence than dietary ingredients did on diet digestibility, or no influence at all. This observation corroborates Sklan et al. (2004) who studied the digestibility of different feedstuffs (fishmeal included) in extruded and pelleted feeds for tilapia and reported no significant differences in the ADCs of protein and lipids.

As a matter of fact, Koprucu and Ozdemir (2005) reported ADCs of protein of 90 % and 87 % for anchovy meal (71 % CP) and soybean meal (53 % CP), respectively, for juvenile Nile tilapia (15 $\pm$ 0.1 g). Sklan et al. (2004) has also reported ADCs of dietary protein of 90 % and 96 % for FM (63 % CP) and SBM (44 % CP), respectively, when working with hybrid tilapia (*O. niloticus* × *O. aureus*) with 150 g of initial body weight.

The protein contents of fish meal may vary from 59 - 72 % (NRC, 2011) depending on fish species, processing methods and storage conditions. Considering the composition of the fish meal (60 % CP) used by Gonçalves et al. (2009), it is safe to say that the authors have used a low-quality protein source in their assays, as some fish meal can contain 72 % CP. However, the pricing model proposed in the present study can be used for different comparisons and data reported by Gonçalves et al. (2009) were chosen for comparison purposes because of the similarity of the experimental conditions, especially tilapia strain and initial body weight.

Estimation of price range for the use CSM as dietary protein source

Prices of FM have detached from that of SBM in recent years. Therefore, average price estimations were considered for the last five years. A steady plateau of prices at 1.68 USD kg^{-1} and 0.40 USD kg^{-1} for FM and SBM, respectively, was identified (Figure 1). From the calculation below, the price of digestible protein of FM and SBM were estimated at 3.38 USD kg^{-1} and 0.82 USD kg^{-1}, respectively.

$$D_{FM} = (100 \times C) / (CP \times ADC_{(FM)}) \quad D_{SBM} = (100 \times C) / (CP \times ADC_{(SBM)})$$

$$D_{FM} = (100 \times 1.68) / (54.46 \times 82.59) \quad D_{SBM} = (100 \times 0.4) / (45.35 \times 94.13)$$

$$D_{FM} = 3.38 \text{ USD kg}^{-1} \quad D_{SBM} = 0.82 \text{ USD kg}^{-1}$$

where: D_{SBM} = price of SBM digestible protein; D_{FM} = price of FM digestible protein; C = average ingrediente price; CP = ingrediente crude protein; ADC = apparent digestibility coefficient.

Table 3 – Comparison of the experimental procedures used in the current work with the reference literature.

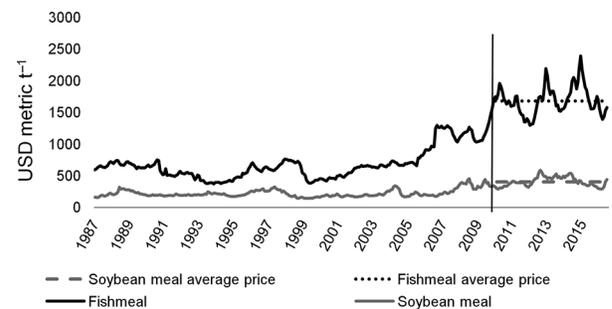|	Original research data	Gonçalves et al. (2009)
Tilapia juvenile initial weight (g)	78.01 ± 1.87	82.00 ± 5.00
Feces collection method	Indirect/passive	Indirect/passive
Marker	Cr_2O_3	Cr_2O_3
Marker quantity (%)	0.20	0.10
Feed processing	Extruded	Pelleted
Diet	Practical	Semi-purified
Proximate composition of control diets in dry matter (%)		
Crude protein	36.50	36.05
Energy (kJ g^{-1})	20.40	17.60
Fiber	4.10	5.24
Lipids	3.10	4.72

Figure 1 – Price history of fish meal (FM) and soybean meal (SBM) from 1987 to June 2016 and average price of the last 5 years of these ingredients. Source: World Bank database on historic of prices of commodities (http://www.worldbank.org/en/research/commodity-markets).

It is thus estimated that CSM can replace FM and SBM as dietary protein source when digestible protein contents are priced at a maximum of USD 4.25 kg⁻¹ or USD 1.06 kg⁻¹, respectively (Equation 2). When the value of the whole product is considered, CSM should be priced at a maximum of 2.65 USD kg⁻¹ and 0.66 USD kg⁻¹ to elicit replacement of FM and SBM, respectively. These estimates are based upon the unconfirmed hypothesis that CSM can replace both ingredients without hampering growth performance of fish.

Although no reports on the replacement of FM by *Chlorella* sp. were found, other microalgae species such as *Isochrysis* sp. (Tibaldi et al., 2015); *Nanofrustulum* sp. and *Tetraselmis* sp. (Kiron et al., 2012); *Navicula* sp. and *Nannochloropsis salina* (Patterson and Gatlin, 2013), and *Spirulina* sp. (Palmegiano et al., 2005) have already been evaluated. The highest level of substitution tested was 60 % (Palmegiano et al., 2005), and growth performance of fish were not hampered at any dietary level.

Although ADC of CSM by tilapia herein registered was 91 %, values recorded for the digestibility of *Chlorella* meal by other animals ranged from 68 to 80 % (Becker, 2007; Kotrbacek et al., 2015). However, tilapia is an herbivorous fish that may behave as an omnivore when adult. The ADC of CSM for many other farmed, neotropical species, such as the omnivore pacu, *Piaractus mesopotamicus*, or the carnivore dourado, *Salminus brasiliensis*, are probably lower than those recorded for tilapia. In this case, using CSM in the feed and nutrition of these species is feasible only when the price of digestible protein of CSM does not exceed 4.25 USD kg⁻¹.

Future evaluations of CSM as alternative protein source to FM in tilapia nutrition should take into account that adding FM to tilapia feed formulations is rather low – ordinarily circa 2 - 5 % (Chiu et al., 2013). As a matter of fact, the use of FM in tilapia diets is possibly unnecessary (Koch et al., 2016). Consequently, it is logical to use CSM as replacement to SBM as protein source, or as it is a good source of digestible lysine, CSM could replace costly synthetic lysine sources.

Pricing of microalgae as source of eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids in replacement to fish oil in aquafeeds was studied by Chauton et al. (2015), who reported that it is feasible to use microalgae meal in aquafeeds when priced at 2.4 USD kg⁻¹ or 8.0 USD kg⁻¹ of the equivalent amount in EPA and DHA, respectively. Considering the contribution in protein, energy and amino acids in formulations, and using the hedonic model of price prediction, Maisashvili et al. (2015) reported that the use of *Chlorella pyrenoidosa* meal in aquafeeds is feasible when priced at a maximum of 0.64 USD kg⁻¹ for the period studied (2005-2012). The results of these studies to a certain extent corroborate those reported here, since the estimated prices or values ranged between the main, traditional protein sources of aquafeeds - FM and SBM.

The price range between USD 4.25 kg⁻¹ and USD 1.06 kg⁻¹ estimated in this study still requires validation in growth performance studies and assessment of complete FM replacement. However, these values can already be used as suggestive and benchmarking in the market price formation for the use of CSM in tilapia nutrition.

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