Nutritional Evaluation of Crambe Meal as a Partial Replacement of Soybean Meal in Nile Tilapia Diets

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Abstract

This study evaluated the apparent digestibility coefficient (ADC) of crambe meal (CM) and its potential to partially replace soybean meal (SM) protein in Nile tilapia diets. The ADC for dry matter, crude protein, ether extract, energy, amino acids, calcium and phosphorus of CM were assessed in fish (n=80; 65.30 ± 5.32 g). Subsequently, an 80-day feeding trial was conducted with Nile tilapia (n=140; 6.04 ± 0.25 g) randomly distributed in 20 experimental cages (70 L; seven fish cage^{-1}) allocated in five circular tanks (1000 L) in recirculation water system, to evaluate the effects of replacement of SM by CM (0, 6, 12, 18 and 24% in isonitrogenous and isoenergetic diets) on growth, blood parameters, fillet yield and proximal composition. The CM shows good digestibility of protein (0.824) and amino acids (0.844) by Nile tilapia and its inclusion in the diet does not affect carcass and fillet yield or proximal composition. Fish fed diets with 24.0% of the SM replaced by CM showed the worst weight gain and feed conversion rate. The protein efficiency ratio decreased in fish fed diets with 12.0, 18.0 and 24.0% of the SM replaced by CM. Hemoglobin, mean corpuscular hemoglobin concentration, total plasma protein, glucose and alanine aminotransferase enzyme activity trend to increase at highest levels of CM in the diet. In conclusion, CM has potential to replace SM in Nile tilapia diets, due to high digestibility of protein and amino acids. However, anti-nutritional factors present in untreated CM interfere on the growth and nutrient utilization of Nile tilapia.

Introduction

Soybean meal (SM) is one of the most suitable plant protein sources used for the replacement of fish meal in aquafeeds formulations, especially for carnivorous species, due to its high protein content, reasonable amino acid profile, cost-effectiveness, consistent nutritional composition, and steady supply (Chou et al. 2004; Storebakken et al. 2000).

Although, world production of soybean has increased over the past two decades, the consumption has also expanded in the livestock sector (Hardy 2010). Furthermore, SM is an internationally traded commodity, and therefore subject to global market oscillations (Rana et al. 2009), which have increased its price in recent years, impacting the production cost of several fish species.

In consequence, several alternative protein sources have been evaluated in aquafeeds; however, such protein sources must demonstrate wide availability, competitive price, ease of logistics and storage, and good nutritional characteristics, such as low levels of fiber, starch and anti-nutrients, and high protein contents in order to be commercially viable (Gatlin et al. 2007). By-products of the ethanol and biodiesel production chain meet these requirements, and can potentially replace traditional aquafeed sources such as fishmeal (FM) and SM in fish diets (Naylor et al. 2009).

Crambe (*Crambe abyssinica* H.) is a native cruciferous plant of the Mediterranean region. Since 1990, interest in Crambe as a commercial crop has increased in several countries, including the USA, Canada and European countries, because of its high oil content (350-570 g kg^{-1}) and industrial lubricant

properties (Berzuini et al. 2021; Costa et al. 2019; Knights 2002; Yong-Gang et al. 1993). Crambe culture has been evaluated as potential oilseed for biodiesel production in Brazil (Cremonez et al. 2015). Its by-products (crambe meal/cake) are arousing interest from companies, research institutions and universities as an alternative feedstuff in animal production (Pitol et al. 2010). Defatted crambe meal/cake originating from the dehulled seeds has potential as alternative feedstuff for ruminants (Araújo et al. 2018; Moura et al. 2017) and non-ruminant animals (Barbosa et al. 2017; Pretto et al. 2014; Vieira et al. 2020) due to its high protein content (280-460 g kg^{-1} CP) and well-balanced amino acid profile (Baker et al. 1977; Carlson and Tookey 1983).

However, crambe meal (CM) has high glucosinolate (80–100 µmol g^{-1}) and erucic acid levels (55-60% of the total lipid) (Yong-Gang et al. 1993; Yong-Gang et al. 1994). High glucosinolate content, especially its derivative compounds such as isothiocyanates, thiocyanate anions, oxazolidinethiones and nitriles are responsible for deleterious effects on thyroid function, depressing growth in some fish species (Burel et al. 2001; Davies et al. 1990; Hossain and Jauncey 1989). On the other hand, the inclusion of 0.1 and 0.7 µmol g^{-1} of natural glucosinolate isolated from *Brassica napus* did not affect the growth and health of turbot, *Psetta maxima* (von Danwitz and Schulz 2020). Therefore, glucosinolates toxicity is concentration-dependent and may vary among fish species. The high erucic acid levels caused growth depression, mortalities, and histopathological alterations in the skin, gills, kidney, and heart of Coho salmon, *Oncorhynchus kisutch* (Hendricks 2002).

Detoxification of CM can reduce the levels of some anti-nutritional factors such as glucosinolate and erucic acid, but the process may also reduce the lysine digestibility and an amount of the glucosinolate will still be converted into its toxic derivative compounds (Yong-Gang et al. 1993). Furthermore, detoxification processes increase the cost of cake/meal production, which can impair their subsequent application.

On the other hand, untreated CM can be used in broiler chicken diets at low levels (50-100 g kg^{-1}) without or with minimal adverse effects on weight gain and health (Ledoux et al. 1999). Additionally, no differences in growth or biochemical parameters were observed in jundiá, *Rhamdia quelen*, fed diets containing 208.4 g kg^{-1} of untreated CM, compared with a control diet (0% CM) (Pretto et al. 2014). Research into the use of CM in fish diets, and evaluation of possible deleterious effects caused by anti-nutritional factors is still scarce. This study aimed to determine the composition of nutrients and anti-nutritional factors in CM, the apparent digestibility coefficients (ADC) of nutrients, energy, amino acids and macro-minerals, and the effect of partial replace soybean meal (SM) by CM protein in Nile tilapia, *Oreochromis niloticus*, diets with regard to growth performance, blood parameters, body yield and proximal composition.

Materials And Methods

Experimental diets

In the digestibility assay, a control diet (0% CM) (Table 1) was used as the reference diet and the test diet was formulated to contain 70% of the reference diet and 30% CM, both marked with 0.1% of chromium oxide III (Cr_2O_3). Crambe meal (CM) was obtained from mechanically-extracted oilseed subjected to sequential solvent extraction, and was processed by Fundação MS, Campo Grande, Mato Grosso do Sul, Brazil.

Table 1
Ingredients and chemical composition of experimental diets

Ingredients (g kg^{-1})	0CM	6CM	12CM	18CM	24CM
Soybean meal[1]	530.0	498.2	466.4	434.6	403.0
Crambe meal[2]	-	44.4	88.9	133.3	177.5
Poultry by-product meal[3]	96.0	96.0	96.0	96.0	96.0
Corn[4]	87.1	67.4	44.0	23.5	48.0
Wheat middlings[5]	175.0	169.6	167.0	169.5	155.8
Broken rice[6]	22.5	51.7	80.5	95.5	84.4
Soybean oil[7]	37.6	30.8	24.5	20.0	13.0
Dicalcium phosphate[8]	22.0	19.0	16.9	13.5	11.9
Limestone[9]	-	-	-	5.6	2.00
Sodium chloride (NaCl)[10]	1.0	1.0	1.0	1.0	1.0
Cellulose[11]	21.0	14.3	7.2	-	-
L- threonine[12]	1.7	1.8	1.9	2.0	2.1
DL- methionine[13]	1.9	1.6	1.5	1.3	1.1
Vitamin-mineral premix[14]	4.0	4.0	4.0	4.0	4.0
Butyl hydroxy toluene[15]	0.2	0.2	0.2	0.2	0.2
	Composition (g kg^{-1})				
Digestible energy (MJ kg^{-1})[16]	13.39	13.39	13.39	13.39	13.39
Digestible protein[16]	300.0	300.0	300.0	300.0	300.0
Crude protein[17]	334.0	331.0	335.0	337.0	341.0
Digestible methionine[16]	6.3	6.2	6.2	6.2	6.2
Digestible threonine[16]	11.8	11.8	11.8	11.8	11.8
Digestible lysine[16]	15.0	15.0	15.0	15.0	15.0
Ether extract[17]	67.0	60.6	55.7	57.0	55.0

Ingredients (g kg^{-1})	0CM	6CM	12CM	18CM	24CM
Crude fiber[17]	64.6	62.6	62.3	65.8	69.8
Calcium[17]	11.3	10.8	10.5	12.1	10.6
Available phosphorus[16]	7.1	7.0	7.1	7.0	7.0
[1] Cargil, Uberlândia, MG, Brazil (g kg^{-1}): dry matter: 897.0; crude protein: 454.2; ether extract: 14.6; gross energy (MJ kg^{-1}):14.47; crude fiber: 62.1; Ca: 3.2; P: 5.4.					
[2] Fundação MS, Campo Grande, MS, Brazil.					
[3] BRF Ingredients, Chapecó, SC, Brazil.					
[4,5,7] Bunge, Santos, SP, Brazil.					
[6] Douramix, Dourados, MS, Brazil					
[8,9,10] BRNova- Trouw Nutrition, Campinas,SP.					
[11, 15] Êxodo Científica, Sumaré, SP, Brazil.					
[12, 13] Ajinomoto Biolatina, Valparaíso, SP, Brazil.					
[14] Composition of the vitamin-mineral premix (M Cassab, SP, Brazil) kg $diet^{-1}$: vitamin A: 500,000 UI, vitamin D3, 250,000 UI, vitamin E 5,000 mg, vitamin K3, 500 mg, vitamin B1 1.000 mg, vitamin B2: 1.000 mg, vitamin B6: 1,000mg, vitamin B12: 2,000 mg, niacin: 2.500, folic acid: 500 mg, biotin: 10 mg, vitamin C 10,000 mg, choline: 100,000mg, Inositol: 1,000 mg, selenium: 30 mg, iron: 5,000 mg, copper: 1,000 mg, manganese: 5,000 mg, zinc: 9,000 mg, cobalt: 50 mg, iodine: 200mg.					
[16] Calculated values based on ADC of nutrients of feed ingredients compiled by Furuya (2010), and CM was based on the results of this study.					
[17] Analyzed according to standard methods (AOAC 2000).					

The experimental diets used in the growth trial contained graded levels: 0; 6; 12; 18; and 24% of replacement of SM protein by CM protein. These levels corresponded to 0; 44.4; 88.9; 133.3 and 177.5 g kg^{-1} CM content and were designated as CM0, CM6, CM12, CM18, and CM24. The diets were formulated to be isonitrogenous and isocaloric with 300 g kg^{-1} of digestible protein (DP) and 13.39 MJ kg^{-1} of digestible energy (DE) based on dry matter (Table 1), according to Furuya (2010) and NRC (2011).

The dietary ingredients were ground in a laboratory grinder (Marconi MA340, Piracicaba, SP, Brazil) to achieve a particle size of 0.5 mm, weighed, mixed in a Y vertical mixer (Marconi MA201, Piracicaba, SP, Brazil), moistened (20% of water) and processed in a meat grinder (2.5 mm) (G Paniz MCR22, Caxias do Sul, RS, Brazil). The diets were dried in a forced-air oven (55°C for 24 hours) (Marconi MA035, Piracicaba, SP Brazil) and stored under refrigeration (5°C) until use.

Fish, experimental condition and feeding

Apparent digestibility coefficient (ADC)

Sex-reversed male tilapia were obtained from a local hatchery (Piscicultura Sgarbi, Palotina, Brazil). Prior to feces collection, fish were acclimated at experimental conditions and experimental diets (reference and test) during seven days. Fish (n=80; 65.30 ± 5.32 g) were distributed randomly in four cages with 70 L and placed in 1,000 L-tank with recirculation water system (1 L min^{-1}), controlled temperature with digital thermostat and electrical resistance (4,000W) and supplementary aeration (373 W). Dissolved oxygen (5.70 ± 0.54 mg L^{-1}) and temperature (26.10 ± 0.22°C) were measured daily in all tanks with multiparameter YSI-55 (Yellow Springs, Ohio, USA). Total amonia nitrogen (0.02 ± 0.01 mg L^{-1}) was measured by colorimetric kit (Alfakit, Florianópolis, SC, Brazil) and pH (7.2 ± 0.30) (Marconi-MA522, Piracicaba, SP, Brazil) every 2 days.

Feces collections were performed in two 200 L-conical bottom tanks by sedimentation. Fish were fed with reference and test diets, and allocated in four cages (two cages for each diet), where the feces of each group were collected on alternate days. Fecal volume was sufficient to obtain four replicates for the reference and test diets. Animals were kept during the day in cages 70 L placed in 1,000 L-tank and fed experimental diets until apparent satiation from 8 to 17:30 h. After last feeding, fish were transferred to the conical bottom tanks (200 L) with controlled temperature with digital thermostat and electrical resistance (1,000W) and supplementary aeration (248 W). Fish were fed in an independent system to avoid the contamination of possible reminiscent feed in the fecal samples, as recommended by Pezzato et al. (2002). Collected feces were centrifuged and dried in an air forced circulation oven at 55°C for 24 hours and stored at -20.0°C until chemical analysis.

The apparent digestibility coefficients of nutrients (ADC), energy, amino acids, calcium and phosphorus were calculated according to equation described by Cho et al. (1985):

$$\mathrm{ADC} = 100 - \left[100 \left(\frac{\%\mathrm{Cr_2O_{3_d}}}{\%\mathrm{Cr_2O_{3_f}}} \right) \times \left(\frac{\%\mathrm{N_f}}{\%\mathrm{N_d}} \right) \right]$$

where ADC = ADC of a nutrient in the test diets, Cr_2O_3d = chromic oxide in the diet, Cr_2O_3f = chromic oxide in the feces, Nf = nutrients in feces and Nd = nutrients in the test diets.

Apparent digestibility coefficients of nutrients, energy, amino acids, calcium and phosphorus from ingredient (ADCI) were calculated to the following equation (Forster 1999):

$$\mathrm{ADC_I} = \left[\frac{(\mathrm{a} + \mathrm{b}) \times \mathrm{ADC_T} - \mathrm{a} \times \mathrm{ADC_R}}{\mathrm{b}} \right]$$

where: a = nutrient contribution of reference diet to nutrient content of test diet, b = nutrient contribution of test ingredient to nutrient content of test diet, (a + b) = level of nutrient in combined diet (%), ADC_T = apparent digestibility coefficient of a nutrient in the test diet and ADC_R = apparent digestibility coefficient of a nutrient in the reference diet.

Growth performance

Sex-reversed tilapia were obtained from the same hatchery of ADC study and acclimated to the laboratory conditions for five days. Fish (n=140; 6.04 ± 0.25 g) were randomly distributed in 20 experimental cages (70 L; 7 fish $cage^{-1}$) allocated in five circular tanks (1000 L) in laboratory recirculation water system (4 L min^{-1} per tank) with individual control (water valve), physical and biological filter, controlled temperature with digital thermostat and electrical resistance (5,000W), supplementary aeration (746 W) and 12h light:12h dark photoperiod. Fish were fed daily with experimental diets until apparent satiation, at 08:00, 11:00, 13:00 and 16:00 h, for 80 days. Diets were weighed daily before the first and after the last feeding to calculate the amount of food consumed.

An initial polled sample of 15 fish from the original population was euthanized (300 mg L^{-1} benzocaine) for fillet composition analysis. At the end of the trial all fish were anesthetized with benzocaine (100 mg L^{-1}), after fasting for 24 h, and weighed individually. Growth, nutrient retention and hepatosomatic index were calculated as follows: weight gain (WG) = (final body weight(g) - initial body weight(g)); feed conversion ratio (FCR) = feed intake(g)/weight gain(g); protein efficiency ratio (PER) = weight gain(g)/protein intake(g); specific growth rate (SGR %) =100×(ln final weight(g) - ln initial weight(g)/days of the trial); nitrogen retention (NR) = [(final N of fillet - initial N of fillet)/total N intake) x100] and hepatosomatic index= 100% (liver weight(g) / fish weight(g)).

Dissolved oxygen (5.8 ± 0.30 mg L^{-1}) and temperature (26.34 ± 0.25°C) were measured daily. Total ammonia (0.1 ± 0.02 mg L^{-1}), nitrite (0.01 ± 0.00 mg L^{-1}), nitrate (0.10 ± 0.02 mg L^{-1}), alkalinity (60 ± 0.20 mg L^{-1} of $CaCO_3$) and pH (7.2 ± 0.30) were measured weekly. All parameters analyzed were within acceptable limits for Nile tilapia (El-Sayed 2006). All analyses were performed by using the same equipment or kit described previously. Cleaning management was periodically realized by siphoning and renewing 20% of the total volume of the system. All water parameters remained within acceptable values for Nile tilapia.

Hematological, biochemical and enzymatic analyses

At the end of the growth trial, and after 24 hours of fasting, fish were anesthetized with benzocaine (100 mg L^{-1}) before blood collection. Blood samples were collected by caudal puncture using a syringe containing EDTA (3%) from 12 fish per treatment (n=60).

The percentage hematocrit was determined by the microhematocrit method and the samples were processed in a microhematocrit-centrifuge (NI 1807 Nova Instruments, Piracicaba, SP, Brazil) for 5 min at 10,000 rpm. Hemoglobin content was determined using the cyanometahemoglobin method (Gold Analisa

Diagnóstica, Minas Gerais, Brazil) (Collier 1944) and erythrocyte counting was performed after blood dilution (1:200) in formalin-citrate solution, using a Neubauer hemocytometer. The hematimetric indexes were also calculated (Wintrobe 1934), comprising the Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Total cholesterol and triglycerides were determined using colorimetric kits (Gold Analisa Diagnóstica, Belo Horizonte, MG, Brazil) and analyzed in a semi-automatic spectrophotometer (Bioplus Bio200, Barueri, SP, Brazil). Total plasma proteins (TPP) were determined by the refractometry method, and glucose concentration was measured using an Accu-Chek performa handset (Roche, São Paulo, SP, Brazil). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using a commercial kinetic kit (Gold Analisa Diagnóstica, Belo Horizonte, MG, Brazil) and analyzed in a semi-automatic spectrophotometer (Bioplus Bio200, Barueri, SP, Brazil).

Carcass and fillet yield

Fish (12 per treatment; n=60) remaining of blood collection were euthanized with benzocaine (300 mg L^{-1}). Animals were eviscerated and weighed separately to determine the percentage of carcass yield (CY). Carcasses were identified and placed under refrigeration at 5°C until rigor mortis. Further, the fillets were processed by the same operator and weighed to obtain the percentage of skinless fillet yield (FY), packaged, identified and frozen at -20°C, until determination of proximate composition.

Chemical analysis

Test ingredient, experimental diets and feces samples were analyzed in duplicate according to standard methods (AOAC 2000) for dry matter, crude protein, ether extract crude fiber, ash. Calcium, phosphorus and chromium (III) oxide levels in the ingredients, diets and feces were analyzed in an atomic absorption spectrophotometer (Varian Spectra AA 2020FS, Mulgrave, VIC, Australia). Amino acids in the ingredients, diets and feces were determined by high-performance liquid chromatography (HPLC-Shimadzu LC-20AT, Kyoto, Japan) after acid and base digestion for the ion exchange chromatographic analysis method, according to Guimarães et al. (2008). Gross energy content was determined in an adiabatic calorimetric bomb (Parr Instrument Company, Moline-IL, EUA). The following anti-nutritional compounds of CM were analyzed: phytate according to Latta and Eskin (1980), glucosinolate as described by Leoni et al. (2003) and erucic acid content was performed by modified methodology proposed by Ackman et al. (1983).

Statistical analysis

All data were subjected to tests for normality and homogeneity of variance, and investigated using one-way analysis of variance (ANOVA). Variables related to growth performance were analyzed by polynomial regression followed by Tukey's tests. Blood parameters, proximate composition, and meat quality were subjected to Tukey's tests. Differences were considered significant when $P < .05$. All statistical analyses were performed using the software SPSS 13.0.

Results

Nutritional and anti-nutritional composition of CM

The nutritional composition and the amino acid profile of CM used in this study is presented in Table 2. The following anti-nutritional compounds were determined: phytate 20.84 ± 1.10 g kg^{-1}, erucic acid 10.8 ± 2.20 g kg^{-1} and glucosinolate 41.00 ± 3.44 µmol g^{-1}.

Table 2
Chemical composition of crambe meal (CM) and apparent digestibility coefficient (ADC) for Nile tilapia (based on dry matter)

Nutritional composition (g kg^{-1})	**CM[1]**	**ADC[2]**
DM	921.5 ± 17.64	0.626 ± 0.002
CP	363.3 ± 8.89	0.824 ± 0.002
EE	37.1 ± 2.53	0.815 ± 0.008
GE (MJ kg^{-1})	18.61 ± 1.12	0.770 ± 0.004
CF	173.6 ± 1.40	-
Ash	66.4 ± 0.25	-
Ca	8.1 ± 0.81	0.663 ± 0.006
P	7.1 ± 0.64	0.733 ± 0.005
Essential amino acids (g kg^{-1})		
Arginine	20.5 ± 0.41	0.926 ± 0.002
Isoleucine	11.8 ± 0.12	0.799 ± 0.001
Leucine	21.2 ± 0.42	0.789 ± 0.001
Lysine	19.9 ± 0.40	0.875 ± 0.002
Methionine	6.5 ± 0.06	0.986 ± 0.001
Phenylalanine	14.8 ± 0.15	0.789 ± 0.001
Threonine	10.1 ± 0.20	0.773 ± 0.001
Tryptophan	3.1 ± 0.03	0.835 ± 0.001
Valine	17.7 ± 0.50	0.794 ± 0.002
Histidine	8.0 ± 0.07	0.867 ± 0.001
Non-essential amino acids (g kg^{-1})		
Alanine	13.4 ± 0.27	0.803 ± 0.004
Aspartate	27.2 ± 0.22	0.951 ± 0.001
Glicine	19.8 ± 0.39	0.803 ± 0.002
Glutamic	59.2 ± 1.18	0.937 ± 0.002

DM = dry matter; CP = crude protein; EE = ether extract; GE = gross energy; CF = crude fiber; Ca = calcium; P = phosphorus; [1]Analyzed according to standard methods (AOAC 2000); [2] Mean (n=3).

Nutritional composition (g kg^{-1})	CM[1]	ADC[2]
Cystine	4.2 ± 0.01	0.738 ± 0.003
Tyrosine	11.0 ± 0.33	0.849 ± 0.001
Proline	21.2 ± 0.42	0.822 ± 0.002
Serine	12.5 ± 0.25	0.853 ± 0.001
Mean	-	0.844 ± 0.002

DM = dry matter; CP = crude protein; EE = ether extract; GE = gross energy; CF = crude fiber; Ca = calcium; P = phosphorus; [1]Analyzed according to standard methods (AOAC 2000); [2] Mean (n=3).

Apparent digestibility of CM

The ADCs for energy and crude protein were 77% and 82%, respectively (Table 2). Amino acids showed ADCs values above 80%, except for isoleucine, leucine, phenylalanine, threonine, valine and cystine (Table 2).

Growth performance and nutrient utilization

The weight gain (WG) decreased linearly as CM was increased in the tilapia diets ($\hat{Y}$ = -1.2552x + 116.84; R^2 = 0.79) (Table 3). Fish fed the control diet (0% CM) showed a higher WG than those fed a diet with 24% replacement of SM by CM, but their WG was not significantly different from the other treatments (Table 3).

Table 3
Weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), nitrogen retention (NR) and hepatosomatic index (HSI) of tilapia fed diets with increased levels of crambe meal (CM) during 80 days

Parameters	0CM	6CM	12CM	18CM	24CM	P-value
$WG_{(g)}$	123.99^{a} ± 16.29	100.65^{ab} ± 13,22	98.74^{ab} ±12,97	97.72^{ab} ± 12.84	87.80^{b} ± 11.53	0.0005
FCR	1.31^{a} ± 0.09	1.46^{ab} ± 0.10	1.53^{b} ± 0.11	1.49^{ab} ± 0.19	1.57^{b} ± 0.21	0.001
SGR (%)	3.85 ± 0.16	3.58 ± 0.15	3.51 ± 0.17	3.54 ± 0.14	3.45 ± 0.13	ns
PER	2.30^{a} ± 0.18	2.04^{ab} ± 0.16	1.95^{b} ± 0.15	1.99^{b} ± 0.13	1.89^{b} ± 0.12	0.03
NR (%)	23.32 ± 1.27	24.41 ± 1.32	25.74 ± 1.40	23.87 ± 1.29	25.54 ± 1.39	ns
HSI	2.22 ± 0.24	2.44 ± 0.27	2.06 ± 0.22	1.81 ± 0.19	2.05 ± 0.22	ns
WG: $\hat{Y} = -1.2552x + 116.84$ ($R^2 = 0.79$), FCR: $\hat{Y} = 0.0092x + 1.362$ ($R^2 = 0.76$), PER: $\hat{Y} = -0.0145x + 2.208$ ($R^2 = 0.75$).						
Different letters in the same line indicate significant difference ($P < .05$) among treatments by Tukey's test.						

The feed conversion ratio (FCR) increased linearly with the levels of CM ($\hat{Y} = 0.0092x + 1.362$; $R^2 = 0.76$) (Table 3). Fish that received the control diet showed a better FCR, but did not differ than those fed with 6% or 18% replacement of SM by CM (Table 3); however, there was a significant difference compared with fish fed diets with 12 and 24% of replacement of SM by CM (Table 3).

The protein efficiency ratio (PER) decreased linearly as the CM increased in the diets ($\hat{Y} = -0.0145x + 2.208$; $R^2 = 0.75$) (Table 3). Fish fed diets with 0 and 6% of replacement of SM by CM showed the best responses for PER and were not significantly different from each other (Table 3). However, the control treatment was higher compared with 12, 18 and 24% replacement of SM by CM (Table 3).

The specific growth rate (SGR), nitrogen retention (NR) and hepatosomatic index (HSI) did not show significant differences among treatments (Table 3).

Fillet yield and chemical composition

The fillet and carcass yield, and fillet chemical composition of tilapia was not influenced by the replacement of SM by CM (Table 4).

Table 4
Fillet yield (FY), carcass yield (CY), moisture (M), crude protein (CP), ether extract (EE) and ash of fillets of tilapia fed diets with increased levels of crambe meal (CM) during 80 days (Based on natural matter)

Parameters	0CM	6CM	12CM	18CM	24CM	P-value
FY (%)	33.35 ± 0.62	33.28 ± 0.55	33.07 ± 0.63	34.03 ± 0.74	32.31 ± 0.67	ns
CY (%)	89.04 ± 0.48	87.68 ± 0.47	88.38 ± 0.54	88.33 ± 0.47	88.40 ± 0.48	ns
M (g kg^{-1})	763.21 ± 2.67	766.64 ± 2.58	761.15 ± 3.66	761.38 ± 2.18	766.27 ± 7.54	ns
CP (g kg^{-1})	192.84 ± 3.37	195.67 ± 3.44	201.42 ± 2.17	200.11 ± 4.22	197.45 ± 2.53	ns
EE (g kg^{-1})	80.42 ± 12.62	61.67 ± 9.67	54.18 ± 8.50	45.53 ± 7.15	44.51 ± 7.00	ns
Ash (g kg^{-1})	13.74 ± 0.24	13.7 3 ± 0.37	13.88 ± 0.25	13.39 ± 0.54	13.30 ± 0.23	ns

Different letters in the same line indicate significant difference ($P < .05$) among treatments by Tukey's test.

Hematological, biochemical and enzymatic variables

The replacement of SM by CM in the tilapia diet did not influence hematocrit (Htc), red blood cell count (RBC) and mean corpuscular volume (MCV) values (Table 5). However, differences were observed in fish hemoglobin (Hb) and Mean Corpuscular Hemoglobin Concentration (MCHC) among the different treatments (Table 5). Fish fed with 6, 12 and 18% replacement of SM by CM showed higher values for Hb and MCHC, when compared to fish in the control treatment (Table 5).

Table 5
Hematological, biochemical and enzymatic parameters of tilapia fed diets with increased levels of crambe meal (CM) during 80 days

Parameters	0CM	6CM	12CM	18CM	24CM	P-value
Hematological						
Htc (%)	29.00 ± 1.74	30.67 ± 1.84	31.36 ± 1.88	33.91 ± 2.03	32.83 ± 1.97	ns
Hb (g dL^{-1})	6.29^{a} ± 1.06	9.79^{b} ± 1.65	9.43^{b} ± 1.59	9.86^{b} ± 1.66	8.61^{ab} ± 1.45	0,001
RBC (10^6 μL^{-1})	1.57 ± 0.18	1.67 ± 0.19	2.02 ± 0.23	1.83 ± 0.21	2.04 ± 0.23	ns
MCV (fL)	204.10 ± 19.33	186.23 ± 15.67	162.39 ± 17.44	189.08 ± 14.65	162.64 ± 18.22	ns
MCHC (%)	21.13^{a} ± 3.21	32.09^{b} ± 4.89	29.77^{b} ± 4.53	29.12^{b} ± 3.78	26.22^{ab} ± 4.57	0.004
Biochemical and enzymatic						
TPP (g dL^{-1})	5.2^{a} ± 0.34	5.3^{a} ± 0.27	5.3^{ab} ± 0.44	6.0^{b} ± 0.38	5.6^{ab} ± 0.21	ns
Gluc (mg dL^{-1})	29.40^{a} ± 5.82	30.80^{a} ± 6.10	41.80^{b} ± 8.28	44.20^{b} ± 8.75	45.20^{b} ± 7.14	0.002
Chol (mg dL^{-1})	104.09 ± 8.65	121.67 ± 10.11	113.62 ± 9.22	128.05 ± 8.25	109.30 ± 8.95	ns
TG (mg dL^{-1})	147.38 ± 21.45	123.69 ± 18.00	166.28 ± 24.21	180.07 ± 26.21	174.90 ± 25.24	ns
ALT (U L^{-1})	27.80^{ab} ± 8.21	20.07^{a} ± 5.90	41.87^{b} ± 7.37	42.00^{b} ± 12.39	42.12^{b} ± 9.21	0.02
AST (U L^{-1})	110.70 ± 32.35	95.01 ± 17.74	131.44 ± 36.24	123.94 ± 37.22	195.30 ± 51.12	ns

Hematocrit (Htc), hemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total plasma proteins (TPP), glucose (Gluc), cholesterol (Chol), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT). Different letters in the same line indicate significant difference ($P < .05$) among treatments by Tukey's test.

There were no significant differences in biochemical variables such as cholesterol, triglycerides or aspartate aminotransferase (AST) activity (Table 5). Fish fed diets with 12, 18 and 24% of replacement of SM by CM showed the highest glucose levels and differed from fish fed with the control or with the diet containing 6% replacement of SM by CM (Table 5). Fish fed diets with 18% replacement of SM by CM

showed a difference in the total plasma protein (TPP) compared with the control and with the diet with 6% replacement of SM by CM (Table 5). The alanine aminotransferase (ALT) activity in fish fed the control diet did not differ from those fed the 12, 18 and 24% replacements of SM by CM (Table 5). However, a trend of increasing ALT and AST was observed towards the highest level of CM (24%).

Discussion

The present study demonstrated the potential of CM as an alternative feedstuff to replace SM in tilapia diets, which in recent years has increase the consumption for animal feeding and its price in the international market. The DM, CP and Ca in CM in the present study (921.5, 363.3 and 8.1 g kg^{-1}) were similar to CM evaluated by Ledoux et al. (1999) (DM 910.0, CP 366.0 and Ca 10.0 g kg^{-1}), and lower than those analyzed by Liu et al. (1995) (CP 442.0 and Ca 73.0 g kg^{-1}). However, crude fiber (CF) content of CM of the present study was about three times higher than those showed by Liu et al. (1995), and lower than that reported by Carlson and Tookey (1983), whose values ranged between 220 and 260 g kg^{-1}. According to these same authors, whole seed with shell presents 221 g kg^{-1} of CF, while the dehulled seed shows 36 g kg^{-1} of CF. All essential and non-essential amino acid concentrations of CM analyzed in the present study were lower compared to those reported by Liu et al. (1995). Therefore, the nutritional composition of CM differs according to the type of cultivar, and amounts of shell present with the seeds during processing, which influence the fiber and protein content.

Regarding the anti-nutritional factors, CM of this study showed 10.8 g kg^{-1} of erucic acid content. On the other hand, CM showed 41 μmol g^{-1} of glucosinolate, and this value was lower than that reported by Yong-Gang et al. (1993) (45-70 μmol g^{-1}). Due to lack of information about anti-nutrients and ADC of CM for tilapia, SM was used as reference value. Furthermore, other meals prepared from other cruciferous species, such as canola meal (CaM) *Brassica* sp., and cultivated radish meal *Raphanus sativus* L. were used to compare the results of the present study by their similarity in nutritional profile and anti-nutritional compounds (erucic acid and glucosinolates). CM of this study contained 20.84 g kg^{-1} of phytate, and this value was higher that SM (10-15 g kg^{-1}), and lower that rapeseed meal (RM) (50-75 g kg^{-1}) (Francis et al. 2001).

The ADC of CM of this study for DM, CP and GE were similar to the ADC of SM for Nile tilapia (DM 65.49%, CP 89.28% and GE 71.38%) reported by Boscolo et al. (2002) and to CaM for tilapia (DM 66.38% and CP 87.00) found by Pezzato et al. (2002). The phosphorus availability in CM was higher than the values reported by Furuya et al. (2001) for CaM (59.68%). Since plant protein sources contain up to 80% of phosphorus in the form of phytate, which is unavailable to fish (NRC, 1993), phosphorus from CM can be considered more available for Nile tilapia than other cruciferous species. The ADC of methionine of CM in the present study was higher (98.56%) than the values determined by Guimarães et al. (2008) for SM (93.4%), while the ADC of cystine of CM (73.82%) was lower in comparison with SM (89.3%) (Guimarães et al. 2008). In general, ADC of nutrients and amino acids of CM and SM showed similarity.

The presence of some anti-nutritional compounds, such as glucosinolate, phytate and erucic acid in CM negatively influenced the ADC of some nutrients and amino acids, and consequently, the growth performance and feed efficiency. Therefore, some technologies for removing anti-nutritional compounds have been studied and considered in plant-based feedstuffs. The use of heating with or without chemical additives and aqueous extraction can remove glucosinolates present in CM (Yong-Gang et al. 1994), resulting in a product with good properties to be used in animal diets. The CM extracted by the isoelectric pH method showed higher protein content, better amino acid profile and lower concentrations of phenolic compounds (Lovatto et al. 2017).

In the present study, a reduction in the WG of tilapia with increasing inclusion of CM in the diets was verified. Similarly, Ledoux et al. (1999) observed reduction in WG of chicken fed with 150.0 g kg^{-1} of CM in diets, and Yong-Gang et al. (1994) in pigs fed with 30.0 g kg^{-1} of CM. On the other hand, Burel et al. (2001) also reported a decrease in the growth of rainbow trout fed 30.0 g kg^{-1} of RM (cruciferous) in diets. However, Pretto et al. (2014) observed no differences on growth parameters of jundia fed diets containing 208.4 g kg^{-1} of CM, in comparison with a control diet (0 g kg^{-1} CM) and chemically treated CM.

The increased replacement of SM by CM reduced the nutrient utilization efficiency by Nile tilapia juveniles. Similar results were described by Santos et al. (2009), who evaluated diets for Nile tilapia that replaced SM protein by cultivated radish meal protein at 12.5, 25.0, 50.0 and 75%, obtaining FCRs of 1.27, 1.17, 1.53 and 1.59, respectively. Furthermore, Pretto et al. (2014) also observed the worst FCR results in jundia fed diets with high levels of CM (208.4 g kg^{-1}). The partial replacement of animal protein by CM protein concentrate (25 and 50%) in diets for *Rhamdia quelen*, worsened feed conversion and reduced the hepatic glycogen content of fish (Lovatto et al. 2018). Nagel et al. (2012), evaluated different levels of canola protein isolate in partial or total replacement (0, 33, 66 and 100%) of protein of fish meal (FM) for turbot, *Psetta maxima*, diets and determined values for PER of 2.31, 2.17, 1.55 and 1.45, respectively, similar to those in the present study.

Linear decreases in WG and PER, reinforcing those anti-nutritional factors of CM, reduced nutrient utilization, growth and protein efficiency. In addition, it is feasible that deleterious effects have been boosted by the complementation of the various anti-nutritional in CM. Furthermore, metabolites of glucosinolate hydrolysis can be consider the major toxic compound, which limit the use of non-detoxified CM in Nile tilapia diets.

Growth and nutrient utilization were negative affected by the anti-nutritional factors of CM. According to Mawson et al. (1994), the hydrolysis of glucosinolates by myronsinase generates toxic compounds such as isothiocyanates, thiocyanate anions, oxazolidinethiones and nitriles that may contribute to glucosinolate-induced hyperthyroidism. High levels of glucosinolates lead to depressed growth in fish, since thyroid hormones (T3 and T4) affect the metabolic utilization of energy, amino acids and possibly carbohydrates (Burel et al. 2000). Furthermore, isolated isothiocyanates promoted negative effects on the digestive utilization of nutrients in common carp, *Cyprinus carpio* (Hossain and Jauncey 1989). Thus, the

adverse effects of glucosinolate and their breakdown compounds on metabolism were the major reason to decrease growth and feed efficiency of tilapia fed with highest levels of CM.

On the other hand, tannin has the ability to inhibit the action of proteases, and to complex with proteins, as well as phytate, impairing the absorption of amino acids (Richardson et al. 1985). Several *in vitro* studies have demonstrated that phytate-protein complexes are more resistant to proteolytic enzymes (Selle and Ravindran 2007). A decrease in protein digestibility was found by Sajjadi and Carter (2004), when 8 g kg^{-1} of phytate was included in a diet for Atlantic salmon, *Salmo salar*. In addition, phytate may negatively influence nutrient uptake, due to its ability to chelate divalent ions and to form complexes with proteins. This may limit or reduce its availability and damage the ceca-pyloric region by interfering with the absorption of nutrients (Francis et al. 2001). Thus, the relation of phytate to protein uptake may be the most reasonable explanation for nutrient ADC interference in CM, which may have also interfered on the growth. Moreover, high erucic acid levels impair the growth of Coho salmon and promoted histopathological alterations in important organs (Hendricks 2002).

The average fillet yield was 33.21% among the different treatments, within the range (25.4 to 42.0%) previously observed for Nile tilapia (Clements and Lovell 1994). The inclusion of the CM in the diet did not influence the fillets composition, but with a decreasing trend in fillet ether extract levels. Hossain and Jauncey (1989) reported similar results for common carp fed diets containing graded levels of isothiocyanate (isolated) and mustard oilcake, showing a decreasing trend in carcass crude lipid content with increasing allyl isothiocyanate (isolated) or mustard oilcake. This observation suggest that decrease in the ADC of lipids is provided by breakdown compounds of glucosinolates and tannin.

Variations in hemoglobin concentration can be related to the interaction of phytic acid with proteins that can modify the biological action of hemoglobin and thus the oxygen dissociation curve, reducing the affinity of hemoglobin for oxygen (Rivera-CH et al. 1995). In this study, the MCHC was raised as a function of CM increase in the diet. This change possibly occurred due to an increase in hemoglobin production by erythrocytes to compensate for the low levels of oxygen available to tissues. Feldman et al. (2006) describe reference values for healthy Nile tilapia in the range of 1.91 to 2.83 for RBC, 7.0 to 9.8 g dL^{-1} for Hb and 27.0 to 37.0% for Htc. Despite significant variations in hemoglobin and MCHC, the values obtained in the present study for all hematological parameters were within the range considered normal for the species. Thus, the different levels of CM evaluated in this study did not interfere on the health status of Nile tilapia.

The replacement of 6% of SM by CM showed lower ALT activity compared to the other treatments, however, it did not differ from the control. A trend of increasing ALT and AST was observed towards the highest level of CM (24%). Similarly, Pretto et al. (2014) also observed an increase in the numerical values of ALT and AST in fish fed diets containing untreated and treated CM, but without significant differences. High ALT and AST activity is indicative of injury to some specific organs. Because of the high concentrations of these enzymes in hepatocytes, increased membrane permeability of these cells by necrosis or inflammation can be identified by the release of these enzymes into the plasma (Grizzle and

Lovshin 1996). Thus, increased AST and ALT activity in plasma may indicate liver damage (Asztalos et al. 1988).

In the present study, no significant difference was observed in the hepatosomatic index for tilapia fed diets with graded levels of replacement of SM by CM. According to Quinsac et al. (1994), deleterious effects of glucosinolates in broilers fed with treated or untreated RM caused hypertrophy in the liver. Although AST and ALT showed an increasing trend with glucosinolate levels, the percentages of CM used in this study were not high enough to provoke severe damage such as liver hypertrophy.

The increase in glucose levels observed with increased replacement of SM by CM could be indicative of the metabolic reflex of the animal due to the physical effort needed for the degradation of toxic substances in the liver, which demanded a greater energy supply, and not necessarily due to stress (Landman et al. 2006).

The interest in crambe for biodiesel production has prompted researchers to evaluate its by-products (meal and cake) for use in animal feed. According to Barros et al. (2006), the use of cakes and meals derived from oilseed processing as feedstuffs is essential to the biodiesel production chain. However, studies on the use of CM in fish diets are still scarce. Even considering the presence of anti-nutritional factors in CM, the present study selected the use of untreated meal because it can be directly used and is less costly. On the other hand, it is also important to evaluate different processing techniques that detoxify or reduce the anti-nutrient content of CM, in order to increase its potential as an alternative protein source.

Currently, there is no CM price reference in the Brazilian and international market. However, some projections indicate that the estimated value of CM is about one-third of SM price (Salsgiver 1997), and its replacement by SM would reduce the formulation cost. Based on the results of the present study, replacement of up to 18.0% of the SM protein by CM protein (133.3 g kg^{-1} in the diet) could be used in Nile tilapia diets, but a rigorous cost-benefit evaluation is necessary, because the reduction on growth and feed efficiency caused by increasing CM in diets needs to be offset by the lower dietary cost expected from the CM inclusion.

Conclusion

In conclusion, CM has potential to replace SM in Nile tilapia diets, due to high digestibility of protein and amino acids. However, anti-nutritional factors present in untreated CM interfere on the growth and nutrient utilization of Nile tilapia.

Declarations

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Competing Interests The authors declare no conflict of interest.

Data Availability All data generated or analyzed during this study are included in this published article.

Ethics approval The experimental procedures were in accordance with the ethical principles in animal research and was approved by the Committee for Ethics in Animal Experimentation at the Universidade Federal da Grande Dourados - UFGD, Mato Grosso do Sul, Brazil (Protocol number: 003/2011), and complies with the ethical principles issued by the Brazilian National Council for Animal Experimentation Control - CONCEA, Brasília, Brazil.

Consent to participate

Not applicable.

Consent to publish

All authors agree to the content of paper for publication.

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