Nutritional evaluation of crambe meal as a partial replacement of soybean meal in Nile tilapia diet

Hamilton Hisano1 · Pamela Souza de Pietro2 · Márcia Mayumi Ishikawa1 · Alex Júnio da Silva Cardoso1 · Arielle Cristina Arena3

Received: 23 November 2021 / Accepted: 11 July 2022 / Published online: 23 August 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
A variety of plant protein sources have been evaluated in aquafeeds. Crambe meal (CM) has potential for inclusion in fish diets because of its nutritional composition. This study evaluated the apparent digestibility coefficient (ADC) of crambe meal and its potential to partially replace soybean meal (SM) protein in Nile tilapia Oreochromis niloticus 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 a 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 high digestibility of protein and amino acids for Nile tilapia. However, anti-nutritional factors present in untreated CM interfere on the growth and nutrient utilization of Nile tilapia.

Keywords Alternative feedstuff · Anti-nutrients · Biodiesel · Crambe abyssinica · Digestibility · Growth performance · Oreochromis niloticus

Introduction
Soybean meal (SM) is one of the most suitable plant protein sources used for the replacement of fish meal (FM) 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, 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). Some by-products of the ethanol and biodiesel production chain meet these requirements, and can
potentially replace traditional aquafeed sources such as 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 North America and Europe, 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 a potential oilseed for biodiesel production in Brazil (Cremonex 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, 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 by different fish species. The high erucic acid levels caused growth depression, mortalities, and histopathological alterations in the skin, gills, kidney, and heart of Coho salmon, Oncorhyncus 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.

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 replacement of 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\(_2\)O\(_3\)). 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.

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 pelleted diets were dried in a forced-air oven (55 °C for 24 h) (Marconi MA035, Piracicaba, SP Brazil) and stored under refrigeration (5°C), during the experimental period.

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) for seven days. Fish (n=80; 65.30±5.32 g) were distributed randomly in four cages (70 L) and placed in a 1,000 L-tank with
recirculating water system (1 L min⁻¹), with digital thermostat and electrical resistance (4,000 W) 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 a multiparameter YSI-55 (Yellow Springs, Ohio, USA). Every two days, 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).

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 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 (1,000 W), supplementary aeration (248 W) and 12 h light:12 h dark photoperiod. 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 h 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):

\[
ADC = 100 - \left[ 100 \left( \frac{\% Cr_2O_3_d}{\% Cr_2O_3_t} \right) \times \left( \frac{\% N_f}{\% N_d} \right) \right]
\]

where \( ADC \) = ADC of a nutrient in the test diets, \( Cr_2O_3_d \) = chromic oxide in the diet, \( Cr_2O_3_t \) = chromic oxide in the feces, \( N_f \) = nutrients in feces and \( N_d \) = nutrients in the test diets.

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

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

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.

Table 1  Ingredients and chemical composition of experimental diets

| Ingredients (g kg⁻¹) | CM (%) |
|----------------------|--------|
|                      | 0      | 6      | 12     | 18     | 24     |
| Soybean meal¹        | 530.0  | 498.2  | 466.4  | 434.6  | 403.0  |
| Crambe meal²         | -      | 44.4   | 88.9   | 133.3  | 177.5  |
| Poultry by-product meal³ | 96.0   | 96.0   | 96.0   | 96.0   | 96.0   |
| Corn⁴                | 87.1   | 67.4   | 44.0   | 23.5   | 48.0   |
| Wheat middlings⁵     | 175.0  | 169.6  | 167.0  | 169.5  | 155.8  |
| Broken rice⁶         | 22.5   | 51.7   | 80.5   | 95.5   | 84.4   |
| Soybean oil⁷         | 37.6   | 30.8   | 24.5   | 20.0   | 13.0   |
| Dicalcium phosphate⁸ | 22.0   | 19.0   | 16.9   | 13.5   | 11.9   |
| Limestone⁹           | -      | -      | -      | 5.6    | 2.00   |
| Sodium chloride (NaCl)¹⁰ | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    |
| Cellulose¹¹          | 21.0   | 14.3   | 7.2    | -      | -      |
| L-threonine¹²        | 1.7    | 1.8    | 1.9    | 2.0    | 2.1    |
| DL-methionine¹³      | 1.9    | 1.6    | 1.5    | 1.3    | 1.1    |
| Vitamin-mineral premix¹⁴ | 4.0   | 4.0    | 4.0    | 4.0    | 4.0    |
| Butyl hydroxy toluene¹⁵ | 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 protein¹⁶ | 300.0  | 300.0  | 300.0  | 300.0  | 300.0  |
| Crude protein¹⁶      | 334.0  | 331.0  | 335.0  | 337.0  | 341.0  |
| Digestible methionine¹⁶ | 6.3   | 6.2    | 6.2    | 6.2    | 6.2    |
| Digestible threonine¹⁶ | 11.8  | 11.8   | 11.8   | 11.8   | 11.8   |
| Digestible lysine¹⁶  | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   |
| Ether extract¹⁷      | 67.0   | 60.6   | 55.7   | 57.0   | 55.0   |
| Crude fiber¹²        | 64.6   | 62.6   | 62.3   | 65.8   | 69.8   |
| Calcium¹²            | 11.3   | 10.8   | 10.5   | 12.1   | 10.6   |
| Available phosphorus¹⁶ | 7.1    | 7.1    | 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 BRFNova- Trouw Nutrition, Campinas, SP
¹¹, ¹⁵ Éxodo Científica, Sumaré, SP, Brazil
¹², ¹³ Ajinomoto Biotatina, Valparaiso, 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: 1,000 mg, vitamin B12: 2,000 mg, niacin: 2,500, folic acid: 500 mg, biotin: 10 mg, vitamin C, 10,000 mg, choline: 100,000 mg, 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: 200 mg
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)
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 a 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 (5,000 W), supplementary aeration (746 W) and 12 h light:12 h 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 consumed feed.

An initial 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 CaCO₃) 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 occasionally performed 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 h 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, and ash. Calcium, phosphorus and chromium (III) oxide levels in the ingredients, diets and feces were analyzed in an atomic absorption spectrophotometer (Varian Spectra AA 200FS, 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...
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) | CM¹ | ADC² |
|---------------------------------|-----|------|
| 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

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
- 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)

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.

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

Survival rate was 100% in all treatments. The weight gain (WG) decreased linearly as CM was increased in the tilapia diets (Ŷ = -1.2552x + 116.84; R² = 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 | CM (%) | 0     | 6     | 12    | 18    | 24    | P-value |
|------------|--------|-------|-------|-------|-------|-------|---------|
| WG (g)     | 123.99 ± 16.29 | 100.65 ± 13.22 | 98.74 ± 12.97 | 97.72 ± 12.84 | 87.80 ± 11.53 | 0.0005 |
| FCR        | 1.31 ± 0.09 | 1.46 ± 0.10 | 1.53 ± 0.11 | 1.49 ± 0.19 | 1.57 ± 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 ± 0.18 | 2.04 ± 0.16 | 1.95 ± 0.15 | 1.99 ± 0.13 | 1.89 ± 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: Ŷ = -1.2552x + 116.84 (R² = 0.79), FCR: Ŷ = 0.0092x + 1.362 (R² = 0.76), PER: Ŷ = -0.0145x + 2.208 (R² = 0.75)

Different letters in the same line indicate significant difference (P < .05) among treatments by Tukey’s test ns, not significant (P > .05)
The feed conversion ratio (FCR) increased linearly with the levels of CM ($\hat{Y} = 0.0099x + 1.362; R^2 = 0.76$) (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).

**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).

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 seen an increase demand for animal feeding and in its price to 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. In addition, 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.

**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 cramble meal (CM) during 80 days (Based on natural matter)

| Parameters | CM (%) | 0      | 6      | 12     | 18     | 24     | 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 | 13.73±0.37 | 13.88±0.25 | 13.39±0.54 | 13.30±0.23 | ns      |

ns, not significant (P > .05)
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. Previous researches demonstrated that 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), improving its nutritional characteristics.

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. Furthermore, Burel et al. (2001) also reported a decrease in the growth of rainbow trout fed 30.0 g kg\(^{-1}\) of RM (eruciferous) 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

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

| Parameters            | CM (%)                       | P-value |
|-----------------------|------------------------------|---------|
|                       | 0    | 6     | 12    | 18    | 24    |         |
| 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±1.06 | 9.79±1.65 | 9.43±1.59 | 9.86±1.66 | 8.61±1.45 | 0.001  |
| RBC (10\(^4\) µL\(^{-1}\)) | 1.57±0.19 | 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±3.21 | 32.09±4.89 | 29.77±4.53 | 29.12±3.78 | 26.22±4.57 | 0.004  |
| Biochemical and enzymatic |     |       |       |       |       |         |
| TPP (g dL\(^{-1}\))   | 5.2±0.34 | 5.3±0.27 | 5.3±0.44 | 6.0±0.38 | 5.6±0.21 | 0.012  |
| Gluc (mg dL\(^{-1}\)) | 29.40±5.82 | 30.80±6.10 | 41.80±8.28 | 44.20±8.75 | 45.20±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±8.21 | 20.07±5.90 | 41.87±7.37 | 42.00±12.39 | 42.12±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 ns, not significant (*P* > .05).
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 that 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 considered the major toxic compound, which limits 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 myrosinase 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 for decreased growth and feed efficiency of tilapia fed with highest levels of CM.

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 did show 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 suggests that the decrease in the ADC of lipids is caused 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. An increase in 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 feeding. 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 antinutritional factors in CM, the present study selected the use of untreated meal because it can be directly used and is less costly. Furthermore, it is also important to evaluate different processing techniques that detoxify or reduce the antinutrient 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. Although CM decreases the growth and feed efficiency of Nile tilapia, a partial replacement of SM protein can potentially be considered, based on the results of the hematological, biochemical and enzymatic parameters in this study. However, a rigorous cost-benefit evaluation is necessary, because the reduction on the growth and feed efficiency caused by increasing CM in diets needs to be offset by the lower dietary cost expected from the CM inclusion.

Conclusions

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

Acknowledgements This research was funded by Empresa Brasileira de Pesquisa Agropecuária - Embrapa (Project nº 03.10.06.015.00). The authors are grateful to Fundação MS for crambe meal donation, Piscicultura Sgarbi for fish donation, to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brazil) for scholarship to second author, and to J.L.Pilecco, J.S.Santos, A.Lopes, G.Possani and M.A.Della-Flora for their help during sampling.

Author contributions Conceptualization: Hamilton Hisano, Arielle Cristina Arena; Formal analysis: Hamilton Hisano, Pamela Souza de Pietro, Márcia Mayumi Ishikawa, Alex Júnio da Silva Cardoso, Arielle Cristina Arena; Methodology: Hamilton Hisano, Arielle Cristina Arena, Márcia Mayumi Ishikawa; Resources: Hamilton Hisano, Arielle Cristina Arena; Writing - review and editing: Hamilton Hisano, Pamela Souza de Pietro, Márcia Mayumi Ishikawa, Alex Júnio da Silva Cardoso, Arielle Cristina Arena.

Funding This research was supported by Empresa Brasileira de Pesquisa Agropecuária - Embrapa (Project nº 03.10.06.015.00).

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

Declarations

Competing Interests The authors declare no conflict of interest.

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, Brasilia, Brazil.

Consent to publish All authors agree to the content of paper for publication.

References

Ackman RG, Barlow SM, Duthie IF, Smit GL (1983) Two methods for determining erucic acid in edible fats and oils: results from a collaborative study on a rapid, open-tubular (capillary) GLC method and comparison with an isolation TLC procedure. J Chromatogr Sci 21:87–93. https://doi.org/10.1093/chromsci/21.2.87

AOAC (2000) Official methods of analysis of the association of analytical chemists. Association of Official Analytical Chemists, International 17th edition, Gaithersburg, MD, USA

Araújo SAC, Bicalho GP, Rocha NS, Bento CBP, Ortêncio MO (2018) Sorghum silage supplemented with crambe meal improves dry matter intake and milk production in crossbred Holstein cows. Trop Anim Health Prod 50:143–148. https://doi.org/10.1007/s11250-017-1414-5

Asztalos B, Nemcsók J, Benedeczky I, Gabriel R, Szabó A (1988) Comparison of effects of parquat and methidation on enzyme activity and tissue necrosis of carp, following exposure to the pesticides singly or in combination. Environ Pollut 55:123–135. https://doi.org/10.1016/0269-7491(88)90123-6

Baker EC, Mustakas GC, Gumbmann MR, Gould DH (1977) Biologic evaluation of crambe meals detoxified by water extraction on matter intake and milk production in crossbred Holstein cows. Trop Anim Health Prod 15:36–50. https://doi.org/10.1007/BF02671018

Barbosa KA, Pinheiro SRF, Vieira DJ, Carvalho DCO, Durrado LRB, Bonaře CM, Neto GLO (2017) Desempenho e características de carcaça de codornas de corte alimentadas com farelo de crambe. Rev Bras Saúde Prod Anim 18:282–292. https://doi.org/10.1590/S1519-99402017000200007

Barros GSAC, Silva AP, Ponchio LA, Alves LRA, Osaki M, Cenamo M (2006) Custos de produção de biodiesel no Brasil. Rev Polit Agric 15:36–50

Berzuini S, Zanetti F, Christou M, Alexopoulou E, Krzyzaniak M, Stolarski MJ, Fierioli F, Monti A (2021) Optimization of agricultural practices for crambe in Europe. Ind Crops Prod 171:113880. https://doi.org/10.1016/j.indcrop.2021.113880

Boscolo WR, Hayashi C, Meurer F (2002) Digestibilidade aparente da energia e nutrientes de alimentos convencionais e alternativos para a tilápia do Nilo (Oreochromis niloticus, L.). Rev Bras de Zootec 31:539–545. https://doi.org/10.1590/S1516-35982002000300001
Ictalurus punctatus

Crambe

Choe WM (2010) Tabelas brasileiras para a nutrição de tilápias. Aquaculture 87:145–154.

Helianthus annuus

Oreochromis niloticus

Furuya WM (2010) Tabelas brasileiras para a nutrição de tilápias. Aquaculture 87:145–154.

Francis G, Makkar HPS, Becker K (2001) Antinutritional factors

Feldman BF, Zinkl JG, Jain CN (2006) Schalm’s Veterinary Hematology, 5th edn. Lippincott Williams and Wilkins, Philadelphia

Cremonez PA, Feroldi M, Nadaleti WC, de Rossi E, Feiden A, de Costa E, Almeida MF, Alvim-Ferraz C, Dias JM (2019) Cultivation of Nile tilapia, (Oreochromis niloticus) and channel catfish (Ictalurus punctatus). Aquaculture 199:197–227.

Cho CY, Cowey CB, Watanabe T (1985) Finfish nutrition in Asia: methodological approaches to research and development. Oita. IDRCC, pp. 154

Cho CY, Cowey CB, Watanabe T (1985) Finfish nutrition in Asia: methodological approaches to research and development. Oita. IDRCC, pp. 154

Chou RL, Her BY, Su MS, Hwang G, Wu YH, Chen HY (2004) Substituting fish meal with soybean meal in diets of juvenile cobia Rachycentron canadum. Aquaculture 229:325–333. https://doi.org/10.1016/S0044-8486(03)00395-8

Clements S, Lovell RT (1994) Comparison of processing yields and nutrient composition of culture Nile tilapia (Oreochromis niloticus) and channel catfish (Ictalurus punctatus). Aquaculture 119:299–310. https://doi.org/10.1016/0044-8486(94)90184-8

Collier HB (1944) The standardization of blood haemoglobin determinations. Can Med Assoc J 50:550–552

Costa E, Almeida MF, Alvim-Ferraz C, Dias JM (2019) Cultivation of Crabe abyssinica non-food crop in Portugal for bioenergy purposes: Agronomic and environmental assessment. Ind Crops Prod 139:111501. https://doi.org/10.1016/j.indcrop.2019.111501

Cremonze PA, Feroldi M, Nadaleti WC, de Rossi E, Feiden A, de Camargo MP, Cremonez FE, Klajn FF (2015) Biodiesel production from crambe (Crambe abyssinica) and sunflower (Helianthus annuus) seeds: an agronomic and environmental assessment. Ind Crops Prod 139:111501. https://doi.org/10.1016/j.indcrop.2019.111501

Crambe meal as an alternative protein source in complete diets for tilapia, (Oreochromis niloticus) and channel catfish (Ictalurus punctatus). Plant Breed 122:517–520. https://doi.org/10.1016/S0377-8401(98)00225-9

Leoni O, Cinti S, Aliano N, Tittonel ED (2003) A rapid chromatographic method for determining the glucosinolate content in crambe seed. Plant Breed 122:517–520. https://doi.org/10.1111/j.1439-0523.2003.00839.x

Liu Y, Smits B, Steg A, Jongbloed R, Jensen SK, Eggum BO (1995) Crambe meal: digestibility in pigs and rats in comparison with rapeseed meal. Anim Feed Sci Technol 52:257–270. https://doi.org/10.1016/S0377-8401(94)00723-M

Lovato NM, Goulart FR, Loureiro BB, Speroni CS, Bender AB, Giacomini SJ, Neto JR, Silva LP (2017) Crambe (Crambe abyssinica) and sunflower (Helianthus annuus) protein concentrates: production methods and nutritional properties for use in fish feed. An Acad Bras Cienc 89:2495–2504. https://doi.org/10.1590/0001-3765201702140630

Lovato NM, Loureiro BB, Pianesso D, Adorjan TJ, Goulart FR, Speroni CS, Bender ABB, Mülner J, Silva LP (2018) Sunflower protein concentrate and crame protein concentrate in diets for silver catfish Rhamdia quelen (Quoy and Gaimard, 1824): use as sustainable ingredients. An Acad Bras Cienc 90:3781–3790. https://doi.org/10.1590/0001-3765201820170991

Mawson R, Heaney RK, Zdunczyk Z, Kozlowska H (1994) Rapeseed protein isolate as fish meal substitute for juvenile turbot (Psetta maxima L.) - Impact on growth performance, body composition, nutrient digestibility and blood physiology. Aquaculture 356:357–364. https://doi.org/10.1016/j.aquaculture.2012.04.045

Nagel F, Danowitz AV, Tursche K, Kroeckel S, Bussel CGJV, Schlachter M, Adem H, Tressel RP, Schulz C (2012) Nutritional evaluation of rapped protein isolate as fish meal substitute for juvenile turbot (Psetta maxima L.) - Impact on growth performance, body composition, nutrient digestibility and blood physiology. Aquaculture 356:357–364. https://doi.org/10.1016/j.aquaculture.2012.04.045

Naylor RL, Hardy RW, Bureau DP, Chiu A, Elliott M, Farrell AP, Foster I, Gatlin DM, Goldburg RJ, Hua K, Nichols PD (2009) Feeding
