Effect of fermented, hardened, and dehulled of chickpea (Cicer arrietinum) meals in digestibility and antinutrients in diets for tilapia (Oreochromis niloticus)

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Abstract

Among the most typical feed sources for tilapia, plants represent a low-cost source in substituting for traditional high-cost feed ingredients. Fermentation, hardening and dehulling are common grains processing techniques to make plant nutrients available and more digestible to fish. Apparent digestibility coefficients (ADC) of dry matter and protein, and antinutrients (phytic acid and tannins) in fermented, hardened and dehulled chickpea (Cicer arrietinum) meals were determined for juvenile Nile tilapia (Oreochromis niloticus). The highest ADC was obtained with processed (fermented, hardened and dehulled) chickpea meals compared with non-processed. Results indicated that fermentation increased the protein content by 13.1%, decreased the content of ash and phytic acid (47.5 and 45%, respectively), and increased the ingredient apparent digestibility of dry matter (ADM) by 23.2%, and the ingredient apparent digestibility of protein (ADP) by 41.9%. Dehulling meal increased the protein (5.7%) and lipid (6.4%) content of chickpea grains; decreased fiber, ash and tannin content (75.3%, 19.1%, and 84.5%, respectively); and increased ADM by 12.8%, and ADP by 10.4%. We conclude that fermented, hardened and dehulled chickpea meals represent a potential alternative in diets for juvenile O. niloticus.

Additional key words: aquafeeds; plant-based feed ingredients; bioprocessing; antinutritional compounds; tilapia.

Abbreviations used: ADC (apparent digestibility coefficients); ADM (apparent digestibility of dry matter); ADP (apparent digestibility of protein); SSF (solid-state fermentation).

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Introduction

Since feed represents up to 70% of production costs in tilapia cultivation, a priority area of research is substituting traditional high cost ingredients such as fish meal by the incorporation of low-cost agro-industry by-products (Tacon & Metian, 2008; Montoya-Mejia et al., 2016). Typical feed sources for tilapia include fish and poultry meals (Abdel-Warith et al., 2001; Gonzales et al., 2007), soybean meal (Azaza et al., 2009) and other plant sources (El-Saidy & Gaber, 2003; Guimarães et al., 2008). To more accurately formulate diets, the nutritional value of new ingredients should be validated to make them more available to fish. In the case of vegetable by-products, different processing techniques are applied on seeds or grains to promote better nutrient and energy digestibility (Valdez-González et al., 2016), and to reduce or avoid the presence of antinutritional components (Valdez-González et al., 2013). Fermentation (Cuevas-Rodríguez et al., 2004), dehulling (Booth et al., 2001), and hardening (Reyes-Moreno et al., 2000), are the most representing processing techniques commonly used on plant-based ingredients. Then, an important prerequisite to validate the nutritional value of new ingredients is the determination of apparent digestibility
coefficients (ADC) (Davies et al., 2011) of dry matter (ADM) and protein (ADP), among others, with different bioprocessing techniques.

The biotechnological processing of ingredients by solid-state fermentation (SSF) represents a technological alternative for processing a great variety of legumes and/or cereals to improve their nutritional and nutraceutical values (Angulo-Bejarano et al., 2008). In general, SSF can be performed with Rhizopus sp. fungi since an important function of the fungus during fermentation is the synthesis of enzymes, which can hydrolyze some of the substrate constituents and contribute to the development of a desirable texture, flavor and aroma of the product (Reyes-Moreno et al., 2004; Sánchez-Magaña et al., 2014).

SSF is a low-cost alternative processing technique for preparing aquaculture diets for aquatic species. It yields high quality products with a minimal degradation of nutrients, and has a significant improvement in digestibility and biological value of proteins (Cuevas-Rodríguez et al., 2004; Guzmán-Uriarte et al., 2013). It has also been proved that fermentation induce favorable changes in the legumes antinutrients, such as reduction in the activity of enzymatic inhibitors (i.e. phytates and tannins; Reyes-Moreno et al., 2004).

On the other hand, a factor limiting the use of legumes as ingredients in diets is hardening, which occurs when legumes are stored under adverse conditions of high temperature and relative humidity (Reyes-Moreno et al., 2000; Medina-Godoy et al., 2011). Longer periods of cooking and lower nutritional value characterize legumes with this deficiency (Cuevas-Rodríguez et al., 2004; Medina-Godoy et al., 2011). Hardening affects a high percentage of grain during storage, causing hardened grain being considered a by-product that is sold at low prices. Another limiting factor for using legumes in diets is their content of antinutrients (Guillaume et al., 2004; Glencross et al., 2004, 2007). The effect of vegetal antinutritional factors has been less studied in fish than in higher vertebrates (Guillaume et al., 2004; Valdez-González et al., 2013).

To overcome those limitations, processes need being implemented for, on one hand, improvement and utilization of large quantities of legumes and cereals stored for a long time (Drew et al., 2007; Adamidou et al., 2011; Valdez-González et al., 2013) and, on the other hand, reducing the content of fiber, tannins and increase protein digestibility by, for example, the elimination of the hull or dehulling (Reyes-Moreno et al., 2004). The objective of the present study was to test the effect of the processes of fermentation, hardening and dehulling on the chemical composition of chickpea (*Cicer arietinum*) grains, in vivo digestibility of ingredients, and antinutrients (phytic acid and tannins) in diets for Nile tilapia *Oreochromis niloticus* including flours of processed and non-processed grain chickpea. There are no antecedents in the literature of investigations like the one conducted in this study.

### Material and methods

#### Preparation of chickpea flours

Fresh and hardened chickpea variety ‘Blanco Sinaloa 92’, were used. Hardening of chickpea was applied according the procedure of Reyes-Moreno & Paredes-López (1993) under laboratory conditions, with slight modifications. The hardening of seeds was produced using accelerated storage (37 ± 1 °C, relative humidity of 100%, 15 d). Flours of fresh and hardened chickpea were prepared utilizing SSF. Accordingly, whole grains were soaked for 16 hours in a 0.4% glacial acetic acid solution (pH = 3.1), drained and manually dehulled. The hull grains were added at the end of the fermentation process and drying of the samples. Cotyledons were cooked in distilled water at 90 °C for 30 min and then cooled at 25 °C for 4 h. The substrate was inoculated with a suspension of *Rhizopus oligosporus* NRRL 2710 (1 × 10⁶ spores/mL) in 15 × 25 cm polyethylene bags with small holes (3 cm separation). Lots of bags with cotyledons were incubated (Riossa, mod EC-33, Mexico) for fermentation at 34.9 °C during 51 h. Subsequently, samples were dried in an oven with forced air circulation (50 °C, 24 h). Finally, samples were milled (Tecator Mill, mod 1083, Sweden) to obtain a flour mesh #80 (0.180 mm).

The fresh and hardened non-fermented chickpea flours were prepared by milling the grains in a ½ HP electric mill until approximately four fragments per chickpea were obtained. After this, the hulls were removed using an electric fan and the fragments were milled to obtain a flour mesh #80 (0.180 mm). Dehulled chickpea was fermented separately, and finally, milled with the hulls.

#### Preparation of diets

A reference diet and eight experimental diets were prepared. In each experimental diet, 30% of the ingredients of the reference diet were replaced by the corresponding chickpea flour (Table 1). The experimental diets were: Fresh whole chickpea, fresh hulled chickpea, hardened whole chickpea, hardened hulled chickpea, fresh whole fermented chickpea, fresh hulled fermented chickpea, hardened whole fermented chickpea, hardened hulled fermented chickpea.

The ingredients were ground through a mesh #40 (0.425 mm), and subsequently mixed and homogenized.
Chromium oxide (1%) was added as an inert marker to determine feed digestibility. Feed was prepared in a meat mill Torrey® Mexico (Monterrey, Mexico).

**Digestibility assays**

Nile tilapias were confined in 27 rectangular plastic tanks (270 L each) using a stocking density of 6 fish (25.0 ± 2.6 g) per experimental tank. Every experimental unit received continuous aeration, keeping dissolved oxygen at 7.25 ± 0.7 mg/L and water temperature at 26 ± 2 °C. Diets were tested with three replicates. Feed was offered to apparent satiation twice a day (08:00 and 15:00 h). Two hours after each feeding, feces were collected with a plastic siphon, washed with distilled water, and placed at -40 °C. One gram of feces (dry weight) were recollected during 30 days. Subsequently, feces were lyophilized and analyzed to determine the content of chromium oxide and proteins.

For ingredients, the apparent digestibility of dry matter (ADM) and protein (ADP) were calculated using the equations of Maynard et al. (1981):

\[
ADM = \left[ (100 \times \text{ADC of the tested diet}) - \left( (100 - \% \text{ tested ingredient}) \times \text{ADC of the reference diet} \right) \right] / \% \text{ tested ingredient}
\]

\[
ADP = \left[ (100 \times \text{ADP of the tested diet}) - \left( (100 - \% \text{ protein in the reference diet}) \times \% \text{ protein in the reference diet} \right) \right] / \% \text{ protein in the tested ingredient}
\]

where ADC is the apparent dry matter digestibility \((100 - 100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in diet}) / \% \text{ Cr}_2\text{O}_3 \text{ in feces})\) and ADP is the apparent digestibility of protein \((100 - 100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in diet}) \times (\% \text{ protein in diet}) \times (\% \text{ protein in feces})\).

**Chemical analysis**

Chemical analysis of the ingredients, diets and feces were performed according to standard methods by AOAC (1995). MicroKjeldahl method was used to determine protein, and determination of nitrogen was conducted in a Kjelted system (Mod 1009 and 1002, Tecator, Sweden). For determination of lipids, extraction with petroleum ether in a Soxtec system (Mod 1043, Tecator, Sweden) was utilized. Fiber was determined by drying and burning of the sample after extraction using 0.5 M H₂SO₄ and 0.5 M NaOH. Ash content was determined by calcination of the sample in a Muffle furnace (Thermolyne 6000) at 600 °C for 5 h, and the energy content was determined by an adiabatic calorimeter (Table 2). Chromic oxide in the feces and diets were evaluated by the method of Bolin et al. (1952) and using the equation proposed by Furukawa & Tsukuhara (1966).

**Determination of antinutrients**

**Phytic acid**

Phytic acid was determined following the procedure of Latta & Eskin (1980). The extraction was performed by shaking (400 rpm at 25 °C during 1 h) 1 g of flour, adding 20 mL of HCl at 2.4%. After this, the suspension was centrifuged (20,000 × g at 25 °C for 5 min) and the supernatant was kept in a freezer. Subsequently, a glass column \((0.7 \times 27 \text{ cm})\) packed with glass fiber and 0.5 g of ion exchange resin (Bio-Rad) was used. The column was washed with 15 mL 5% HCl and then with 20 mL of deionized water. The supernatant was diluted 1:25 and 10 mL were added in the column. Once the fluid went through the column, 15 mL of 0.1 M NaCl were added and the eluate was discarded. A 25 mL vessel was placed under the column and 15 mL 0.7 M NaCl were added to collect the eluate. After this, deionized water was added to complete a volume of 25 mL. Three milliliters were taken from this solution, and 3 mL of deionized water + 1 mL reagent Wade (0.15 g FeCl₃·6H₂O + 1.5 g of sulfosalicylic acid in 500 mL deionized water) were added, shaking thoroughly. The tubes were centrifuged (5000 × g at 25°C for 10 min) and the supernatant was separated; following this, color

| **Table 1.** Composition (%) of reference and experimental diets. Chickpea flour varied depending on the treatment |
| **Ingredient** | **Reference diet** | **Experimental diets** |
|----------------|-------------------|------------------------|
| Fishmeal       | 34                |                        |
| Wheat flour    | 45.3              |                        |
| Fish oil       | 2.3               |                        |
| Soybean lecithin | 2.3             |                        |
| Starch         | 10                |                        |
| Grenetina      | 4                 |                        |
| Minerals¹      | 1                 |                        |
| Vitamins²      | 0.1               |                        |
| Chrome oxide   | 1                 |                        |

¹Mineral mixture (g/kg diet): KCl (0.5); MgSO₄·7H₂O (0.5); ZnSO₄·7H₂O (0.09); MnCl₂·4H₂O (0.00234); CuSO₄·5H₂O (0.005); KI (0.005); CoCl₂·2H₂O (0.00025); Na₂HPO₄ (2.37).
²Vitamins mixture (units in mg/kg, except): retinol (5000 IU); cholecalciferol (4000 IU); α-tocopherol acetate (100); menadione (5); thiamine (60); riboflavin (25); pyridoxine HCl (50); pantothenic acid (75); niacin (40); biotin (1); inositol (400); cyanocobalamin (0.2); folic acid (10).
Tukey's multiple-range test were used to compare mean values of digestibility of diet ingredients. The factors and levels analysed corresponded to the different conditions of the chickpea grains: fermentation (fermented/non-fermented); hardening (levels: hardened/fresh); and dehulling (dehulled/whole grain). Statistica 7.0 software (StatSoft, Tulsa, OK, USA) was used for the analysis, setting significance at $p<0.05$.

**Results**

Table 3 shows that fermentation increased protein (13.1%) and ash (47.5%) contents of chickpea meal. Dehulling increased the protein (5.7%) and lipid (6.4%) contents; and decreased fiber and ash contents (75.3 and 19.1%, respectively) in chickpea grains.

In the case of antinutrients, fermentation decreased the content phytic acid (45%); meanwhile, dehulling decreased tannins by 84.5% (Table 4).

The effect of fermentation and dehulling on ADM were significant ($p<0.001$) (Table 5). There were no significant interactions among the three factors. Average values of ADM in non-fermented and fermented chickpea diets were 58.5 ± 6.2% and 72.1 ± 2.9%, respectively, improving ADM by 23.2% by

was measured in a spectrophotometer (Spectronic 21D mod, Milton Roy, USA) at 500 nm.

**Tannins**

The content of tannin was determined by the method of vanillin proposed by Price *et al.* (1978) with modifications. Extraction was carried out within 24 h after milling using approximately 1 g of sample and 10 mL of a 1% HCl solution in methanol. The suspension was kept on shaking for 40 min at room temperature and centrifuged (20,000 × g, 30 ºC, 20 min). Five milliliters of reagent of vanillin (50:50 v/v 1% vanillin in methanol and 8% HCl in methanol) were added to 1 mL of supernatant at a rate of 1 mL/min. After this, the suspension was kept in the dark for 20 min and read in a spectrophotometer (Spectronic 21 mod D Milton Roy, USA) at 500 nm. A blank solution, zero absorbance, was prepared with 1 mL methanol by adding 5 mL of 4% HCl at a rate of 1 mL/min. A standard curve of catechin was plotted and the results were reported as equivalents of catechin.

**Statistical analysis**

Values of digestibility were tested for normality and variance homogeneity. A multifactorial ANOVA and Tukey's multiple-range test were used to compare mean values of digestibility of diet ingredients. The factors and levels analysed corresponded to the different conditions of the chickpea grains: fermentation (fermented/non-fermented); hardening (levels: hardened/fresh); and dehulling (dehulled/whole grain). Statistica 7.0 software (StatSoft, Tulsa, OK, USA) was used for the analysis, setting significance at $p<0.05$.

| Nutrient | Reference diet | FWC | HWC | FDC | HDC | FWFC | HWFC | FDFC | HDFC |
|----------|----------------|-----|-----|-----|-----|------|------|------|------|
| Protein  | 31.96±0.06     | 28.07±0.03 | 27.68±0.08 | 28.51±0.11 | 28.44±0.15 | 28.87±0.08 | 28.74±0.11 | 29.27±0.07 | 29.29±0.1 |
| Lipids   | 9.86±0.04      | 8.89±0.08 | 8.82±0.1 | 8.99±0.1 | 8.97±0.04 | 8.85±0.05 | 8.81±0.11 | 8.94±0.15 | 8.95±0.08 |
| Fiber    | 4.01±0.03      | 6.87±0.08 | 5.91±0.05 | 3.07±0.03 | 3.20±0.07 | 5.87±0.13 | 5.30±0.05 | 3.10±0.09 | 4.33±0.06 |
| Ash      | 9.20±0.03      | 7.37±0.08 | 7.37±0.03 | 7.33±0.12 | 7.33±0.07 | 7.03±0.05 | 6.92±0.11 | 6.82±0.06 | 6.81±0.05 |
| Energy   | 41.0±7.8       | 45.9±3.7 | 45.4±7.9 | 45.8±4.8 | 45.6±8.5 | 46.1±7.6 | 46.2±3.8 | 46.1±6.5 | 45.9±7.2 |
| NFE      | 44.52          | 46.72 | 46.72 | 44.36 | 46.12 | 46.71 | 46.71 | 46.73 | 46.62 |

1FWC: Fresh whole grain chickpea flour, HWC: Hardened whole grain chickpea flour, FDC: Fresh dehulled chickpea flour, HDC: Hardened dehulled chickpea flour, FWFC: Fresh whole grain fermented chickpea flour, HWFC: Hardened whole grain fermented chickpea flour, FDFC: Fresh dehulled fermented chickpea flour, HDFC: Hardened dehulled fermented chickpea flour. 2NFE: nitrogen-free extract.

**Table 3.** Mean (± SD) content of proximate chemical components (%) of ingredients used in the diets

| Chickpea flour | Protein | Lipids | Fiber | Ash    |
|----------------|---------|--------|-------|--------|
| Non-fermented  |         |        |       |        |
| Fresh          | 21.38   | 6.30   | 2.8   | 3.10   |
| Dehulled       | 22.85   | 6.63   | 0.7   | 2.96   |
| Hardened       | 21.09   | 6.06   | 2.4   | 3.08   |
| Whole          | 22.60   | 6.57   | 0.5   | 2.97   |
| Fermented      |         |        |       |        |
| Fresh          | 24.37   | 6.18   | 2.3   | 1.97   |
| Dehulled       | 25.38   | 6.48   | 0.6   | 1.27   |
| Hardened       | 24.27   | 6.05   | 2.2   | 1.89   |
| Whole          | 25.45   | 6.49   | 0.6   | 1.23   |
Discussion

The increase of protein in chickpea during the fermentation process is related to the protein synthesis caused by proliferation and increase in biomass of R. oligosporus (Paredes-López et al., 1991; Reyes-Moreno et al., 2000; Cuevas-Rodríguez et al., 2004). In this sense, Sánchez-Magaña et al. (2014) reported that the increase in protein content is associated with the decrease of other constituents, which might have been lost by leaching during the initial steps of fermentation or might have been consumed by the fungus for its own growth. There are similar reports when using R. oligosporus with various substrates, such as fresh and hardened chickpea (Reyes-Moreno et al., 2000; Angulo-Bejarano et al., 2008).

The decrease of lipid content in fermented chickpea can be explained as a consequence of the oxidation and

Table 4. Mean (± SD) content of phytic acid (mg/100 g dry weight of sample) and tannins (mg/g) in ingredients utilized in diets

| Chickpea flour | Phytic acid | Tannins |
|----------------|------------|---------|
| Non-fermented | Fresh Whole grain | 2.15 | 2.74 |
|                | Fresh Dehulled   | 2.14 | 0.45 |
|                | Hardened Whole grain | 2.14 | 2.71 |
|                | Hardened Dehulled | 2.13 | 0.41 |
| Fermented      | Fresh Whole grain | 1.19 | 2.72 |
|                | Fresh Dehulled   | 1.17 | 0.43 |
|                | Hardened Whole grain | 1.18 | 2.70 |
|                | Hardened Dehulled | 1.17 | 0.40 |

Table 5. Mean (± SD) content of apparent digestibility of dry matter (ADM) and apparent digestibility of protein (ADP) of tested ingredients

| Chickpea flour | ADM          | ADP          |
|----------------|--------------|--------------|
| Non-fermented | Fresh Whole grain | 52.47 ± 7.08 | 59.91 ± 3.46 |
|                | Fresh Dehulled   | 63.30 ± 3.43 | 68.90 ± 1.84 |
|                | Hardened Whole grain | 53.85 ± 2.65 | 60.27 ± 1.37 |
|                | Hardened Dehulled | 64.39 ± 9.20 | 71.49 ± 4.94 |
| Fermented      | Fresh Whole grain | 69.73 ± 4.19 | 90.64 ± 1.76 |
|                | Fresh Dehulled   | 76.26 ± 2.39 | 95.63 ± 1.04 |
|                | Hardened Whole grain | 69.33 ± 4.96 | 88.86 ± 2.18 |
|                | Hardened Dehulled | 73.88 ± 9.70 | 94.50 ± 4.10 |

Factorial Anova

|                      | p-value | p-value |
|----------------------|---------|---------|
| Non-fermented-Fermented (1) | <0.001  | <0.001  |
| Fresh-hardened (2)      | >0.05   | >0.05   |
| Whole grain-dehulled (3) | <0.001  | <0.001  |
| 1×2                   | =0.154  | =0.233  |
| 1×3                   | =0.001  | =0.607  |
| 2×3                   | =0.721  | =0.551  |
| 1×2×3                 | =0.760  | =0.744  |
utilization of fatty acids as main source of energy by the fungus (Ruiz-Terán & Owens, 1996). There are similar reports when using *R. oligosporus* and various substrates: mix corn/soybean (Mugula & Lyimo, 2000); soybean (Ruiz-Terán & Owens, 1996), quality protein maize (Cuevas-Rodríguez et al., 2004) and common bean (Guzmán-Uriarte et al., 2013).

Dehulling chickpea decrease the content of ash and fiber mainly as a consequence of hulls containing certain minerals (calcium, phosphorus, magnesium, iron, potassium) and a high concentration of fiber (Laurena et al., 1986; Williams & Singh, 1987). The increase in lipid content in dehulled chickpea may be an effect of concentration, considering the loss of other components such as ash and fiber.

Antinutritional factors are chemical elements contained in vegetables affecting the digestibility and the metabolism of energy sources (proteins and lipids) in artificial diets used for animal nutrition (Allan et al., 1999; Valdez-González et al., 2013). Deshpande & Cheryan (1984) considered that the interaction of phytate with proteins, vitamins and several minerals is one of the factors limiting the nutritional value of vegetable meals. In this study, we found that both fermentation and dehulling significantly decreased the content of phytic acid and tannins in chickpea. The content of phytic acid observed in this study was similar to those reported by Jukanti et al. (2012) for chickpea (1.0 mg/100 g dw of sample). Fermentation decreased phytic acid content most likely as a consequence of an increase in phytase activity (an enzyme that synthesizes *R. oligosporus*), and the soaking-cooking-leaching process used for fermentation (Laurena et al., 1986). Similar results were reported by Sánchez-Magaña et al. (2014) using *R. oligosporus* and chickpea as a substrate.

It is known that most of the tannin content is found in the hull of legume grains (Egounlety & Aworh, 2003; Guillaume et al., 2004). Adewusi & Osuntogun (1991) mentioned that dehulling reduces 95% of the tannins content in legumes. In close agreement with those authors, in this investigation the tannin content in chickpea grains was substantially reduced (85%) by removing hulls.

The use of highly digestible feed ingredients for cultivable aquatic species is highly recommended (Silva et al., 2013; Yu et al., 2013). There are studies affirming that fermentation causes an increased availability of the protein content in legumes (Cuevas-Rodríguez et al., 2004; Angulo-Bejarano et al., 2008; Sánchez-Magaña et al., 2014). Higher digestibility associated with fermentation is a consequence of improved nutritional balance of the ingredients and proteolytic activity of fungus, releasing peptides of the substrate and increasing the susceptibility of the protein to enzymatic activity (Cuevas-Hernández et al., 1999). Our study shows that diets based on fermented-dehulled chickpea were easily digested by juvenile Nile tilapia, mainly as a consequence of the protein quality produced by *R. oligosporus*, in combination with a reduction and blocking of antinutrients activity.

Apparent digestibility of dry matter and protein in the tested ingredients depended on the type of ingredient. The differences in ADM may be explained by differences in chemical composition, which in turn is determined by the origin and processing of the feed ingredients (Köprücü & Özdemir, 2005). Nile tilapia is apparently able to assimilate a wide variety of feedstuffs (Davies et al., 2011) and digestibility data in this study compare favorably with those obtained by studies with other freshwater tropical fish species. Yigit & Olmez (2011) showed that low digestibility of canola meal in tilapia might be a consequence of the presence of large amounts of fiber and antinutritional factors in these by-products. In our study, fermentation and dehulling of grain chickpea improved digestibility of dry matter and protein, most likely as a consequence of reduction in the content of phytic acid and tannins.

Bairagi et al. (2004) reported that the increase in protein digestibility in fermented diets is related to the reduction of antinutrients, such as tannins and phytic acid. Yuan et al. (2013) reported that fermented soybean flour in diets for juvenile Chinese sucker *Myxocyprinus asiaticus* showed high digestibility without causing adverse effects on growth and body composition. On the other hand, Ramachandran & Ray (2004) reported that the fermented seed flour of the legume *Phaseolus mungo*, using the bacterium *Bacillus* sp. in diets for *Labeo rohita*, showed values of ADP of 86.76%. In contrast, previous studies have shown that ingredients derived from legumes without any process show low levels of digestibility (Bureau et al., 1999; Lara-Flores et al., 2007; Azaza et al., 2009; Phumee et al., 2011; Collins et al., 2012).

In this study, higher protein digestibility coefficients were obtained in comparison to those reported by Adamidou et al. (2011) for *Sparus aurata* fed with diets prepared with chickpea (*Cicer arietinum* L.). They reported ADP of 80% for chickpea flour, while in the present investigation, using dehulled chickpea flour, resulted in ADP as high as 94.5 and 95.6%. Booth et al. (2001) reported ADP of 81.1% in diets for Australian silver perch (*Bydaenus byd anus*) using chickpea, which is lower than that obtained in the present study. Tiril et al. (2009) reported a coefficient of 80.6% digestibility of protein using extruded chickpea in rainbow trout (*Oncorhyncus mykiss*).

Various studies indicate that grains with high phytate content may decrease protein digestibility (Vielma et al., 2007; Azaza et al., 2009; Phumee et al., 2011; Collins et al., 2012).
1998; Guillaume et al., 2004; Chan et al., 2008). Levels higher than 0.5 mg/100 g dw of sample of phytic acid in diets for rainbow trout and salmonids have been shown to decrease protein digestibility (Reddy et al., 1989). In this investigation, fermentation caused a reduction in the content of phytic acid in chickpea and an increase in protein digestibility of diets for Nile tilapia.

We observed that dehulling reduced the content of tannins in grains of chickpea. Several reports indicate that enzymatic inhibition caused by tannins, decrease the digestibility of nitrogenous nutrients (Allan & Rowland, 1994; Booth et al., 2001), thus causing a low protein digestibility (Reichert et al., 1980). Pinto et al. (2000) mentioned that levels of tannins higher than 0.63 mg/g significantly affect the digestibility of dry matter, protein, and lipids in Nile tilapia. In addition, the removal of hull may cause a decrease in endogenous antinutrients of the hull (Booth et al., 2001), so as in structural polysaccharides, which in high concentrations are known to reduce dry matter digestibility in fish diets (Wilson, 1994; McGoogan & Reigh, 1996). There are reports that levels of fiber lower than 3 g/100 g, improve the protein digestibility in Nile tilapia (Dioundick et al., 2000) mentioned that levels of tannins higher than 0.63 mg/g significantly affect the digestibility of dry matter, protein, and lipids in Nile tilapia. In addition, the removal of hull may cause a decrease in endogenous antinutrients of the hull (Booth et al., 2001), so as in structural polysaccharides, which in high concentrations are known to reduce dry matter digestibility in fish diets (Wilson, 1994; McGoogan & Reigh, 1996). There are reports that levels of fiber lower than 3 g/100 g, improve the protein digestibility in Nile tilapia (Dioundick et al., 2000) mentioned that levels of tannins higher than 0.63 mg/g significantly affect the digestibility of dry matter, protein, and lipids in Nile tilapia. In addition, the removal of hull may cause a decrease in endogenous antinutrients of the hull (Booth et al., 2001), so as in structural polysaccharides, which in high concentrations are known to reduce dry matter digestibility in fish diets (Wilson, 1994; McGoogan & Reigh, 1996). There are reports that levels of fiber lower than 3 g/100 g, improve the protein digestibility in Nile tilapia (Dioundick et al., 2000) mentioned that levels of tannins higher than 0.63 mg/g significantly affect the digestibility of dry matter, protein, and lipids in Nile tilapia. In addition, the removal of hull may cause a decrease in endogenous antinutrients of the hull (Booth et al., 2001), so as in structural polysaccharides, which in high concentrations are known to reduce dry matter digestibility in fish diets (Wilson, 1994; McGoogan & Reigh, 1996). There are reports that levels of fiber lower than 3 g/100 g, improve the protein digestibility in Nile tilapia (Dioundick et al., 2000) mentioned that levels of tannins higher than 0.63 mg/g significantly affect the digestibility of dry matter, protein, and lipids in Nile tilapia. In addition, the removal of hull may cause a decrease in endogenous antinutrients of the hull (Booth et al., 2001), so as in structural polysaccharides, which in high concentrations are known to reduce dry matter digestibility in fish diets (Wilson, 1994; McGoogan & Reigh, 1996). There are reports that levels of fiber lower than 3 g/100 g, improve the protein digestibility in Nile tilapia (Dioundick et al., 2000) mentioned that levels of tannins higher than 0.63 mg/g significantly affect the digestibility of dry matter, protein, and lipids in Nile tilapia.

We found that fermented and dehulled chickpea (C. arietinum) meals represent a potential alternative feed ingredient for preparation of diets for juvenile Nile tilapia O. niloticus. The combination of fermentation and dehulling increases the digestibility coefficients of dry matter and protein in chickpea meals. Hardening did not affect chemical composition or digestibility of the diets, indicating that low priced hardened chickpea could be used in diets. The results obtained in this study showed that fermentation and the removal of chickpea hull, increased protein content, decreased antinutrients, and favored the digestibility of dry matter and protein ingredients in diets for Nile tilapia.

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References

Abdel-Warith AA, Russel PM, Davies SJ, 2001. Inclusion of a commercial poultry by-product meal as a protein replacement of fish meal in practical diets for African catfish Clarias gariepinus (Burchell, 1822). Aquac Res 32: 296-305. https://doi.org/10.1046/j.1355-557x.2001.00053.x

Adamidou S, Nengs I, Henry M, Ioakeu-Midoy N, Rigos G, Bell GJ, Jauncey K, 2011. Effects of dietary inclusion of peas, chickpeas and faba beans on growth, feed utilization and health of gilthead seabream (Sparus aurata). Aquacult Nutr 17: 288-296. https://doi.org/10.1111/j.1365-2095.2010.00762.x

Adewusi SRA, Osuntogun BA, 1991. The effects of cooking on tannin content, trypsin inhibitor activity and in vitro digestibility of some legume seeds in Nigeria. Niger Food J 9: 139-145.

Allan GL, Rowland SJ, 1994. The use of Australian oilseeds and grain legumes in aquaculture diets. Proc 3rd Asian Fisheries Forum; Chou LM et al. (eds.). pp: 667-670, Asian Fisheries Society Publication, Philippines.

Allan G, Rowland S, Parkinson S, Stone D, Jantrarotai W, 1999. Nutrient digestibility for juvenile silver perch Bidyanus bidyanus: development of methods. Aquaculture 170: 131-145. https://doi.org/10.1016/S0044-8486(98)00397-4

Angulo-Bejarano P, Verdugo-Montoya N, Cuevas-Rodriguez E, Milán-Carrillo J, Mora-Escobedo R, López-Velanzuela J, Garzón-Tiznado J, Reyes-Moreno C, 2008. Tempeh flour from chickpea (Cicer arietinum L.). Nutr Physicochem Prop Food Chem 106: 106-112. https://doi.org/10.1016/j.foodchem.2007.05.049

AOAC, 1995. Official methods of analysis, Official Analytical Chemists International, 16th ed. AOAC, Arlington, VA, USA.

Azaza MS, Wassim K, Mensi F, Abdelmouleh A, Brini B, Kbraim M, 2009. Evaluation of faba beans (Vicia faba L. var. minuta) as a replacement for soybean meal in practical diets of juvenile Nile tilapia Oreochromis niloticus. Aquaculture 287: 174-179. https://doi.org/10.1016/j.aquaculture.2008.10.007

Bairagi A, Sarkar-Ghosh K, Sen K, Ray A, 2004. Evaluation of nutritive value of Leucaena leucocephala leaf meal inoculated with fish intestinal bacteria Bacillus subtilis and Bacillus circulans in formulated diets for rohu, Labeo rohita (Hamilton) fingerlings. Aquac Res 35: 436-446. https://doi.org/10.1111/j.1365-2109.2004.01028.x

Bolin DW, King RP, Klosterman EW, 1952. A simplified method for the determination of chromic oxide (Cr₂O₃) when used as an index substance. Science 116: 634-634. https://doi.org/10.1126/science.116.3023.634

Booth M, Allan G, Frances J, Parkinson S, 2001. Replacement of fish meal in diets for Australian silver perch, Bidyanus bidyanus IV. Effects of dehulling and protein concentration on digestibility of grain legumes. Aquaculture 196: 67-85. https://doi.org/10.1016/S0044-8486(00)00578-0

Bureau DP, Harris AM, Cho CY, 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). Aquaculture 180: 345-358. https://doi.org/10.1016/S0044-8486(99)00210-0
Chan CR, Lee DN, Cheng YH, Hsieh DJY, Weng CF. 2008. Feed deprivation and re-feeding on alterations of proteases in tilapia Oreochromis mossambicus. Zool Stud 47: 207-215.

Collins SA, Desai AR, Mansfield GS, Hill JE, Kessel van AG, Drew MD. 2012. The effect of increasing inclusion rates of soybean, pea and canola meals and their protein concentrates on the growth of rainbow trout: Concepts in diet formulation and experimental design for ingredient evaluation. Aquaculture 212: 2-18. https://doi.org/10.1016/j.aquaculture.2012.02.018

Cuevas-Hernández B, Perez-Quilantán JM, Galan-Wong LJ, Alanis-Guzmán MG, Maiti RK. 1999. Fermentation with Rhizopus oligosporus increases nutritional value of pearl millet Pennisetum glaucum grains. Phyton Buenos Aires 65: 91-95.

Cuevas-Rodriguez E, Milán-Carrillo J, Mora-Escobedo R, Cárdenas-Valenzuela O, Reyes-Moreno C. 2004. Quality protein maize (Zea mays L) tempc grain though solid state fermentation process. Lebensm Wiss Technol 37: 59-67. https://doi.org/10.1016/S0023-6438(03)00134-8

Davies SJ, Abdel-Warith AA, Gouveia A. 2011. Digestibility characteristics of selected feed ingredients for developing bespoke diets for Nile tilapia culture in Europe and North America. J World Aquacult Soc 42: 388-398. https://doi.org/10.1111/j.1749-7345.2011.00478.x

Deshpande S, Cheryan M. 1984. Changes in soybean lipids during tempc fermentation. Food Chem 50: 171-175.

Dioundick QB, Stom D. 1990. Effects of dietary á-cellulose on the juvenile tilapia, Oreochromis mossambicus (Peters). Aquaculture 91: 311-315. https://doi.org/10.1016/0044-8486(90)90196-T

Drew MD, Borgeson TL, Thiessen DL. 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Anim Feed Sci Tech 138: 118-136. https://doi.org/10.1016/j.anifeedsci.2007.06.019

Egounlety M, Aworth OC. 2003. Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna unguiculata L. Walp) and groundbean (Macrottyloma geocarpa Harms). J Food Eng 56: 249-254. https://doi.org/10.1016/S0260-8774(02)00262-5

El-Saidy DM, Gaber MM. 2003. Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia Oreochromis niloticus (L.) diets. Aquac Res 34: 1119-1127. https://doi.org/10.1046/j.1365-2109.2003.00914.x

Furukawa A, Tsukahara H. 1966. On the acid digestion method for the determination of chrome oxide as an index substance in the study of digestibility ok fish fed. Bull Jap Soc Fish Sci 32(6): 502-508. https://doi.org/10.2331/suisan.32.502

Glencross BD, Booth MA, Allan GL. 2007. A feed is only as good as its ingredients a review of ingredient evaluation strategies for aquaculture feeds. Aquacult Nutr 13: 17-34. https://doi.org/10.1111/j.1365-2095.2007.00450.x

Gonzales JM, Ruston AH, Rosinski ME, Wu YV, Powlss TF, Brown PB. 2007. Evaluation of fish meal-free diets for first feeding Nile tilapia, Oreochromis niloticus. J Appl Aquac 19: 89-99. https://doi.org/10.1300/J028v19n03_06

Guillaume J, Kauschik S, Bergot P, Metailler R. 2004. Nutrición y alimentación de peces y crustáceos. Mundo Prensa, Madrid, España. pp: 353-365.

Guimarães IG, Pezzato LE, Barros MM, Tachibana L. 2008. Nutrient digestibility of cereal grain products and by-products in extruded diets for Nile tilapia. J World Aquacult Soc 39: 781-789. https://doi.org/10.1111/j.1749-7345.2008.00214.x

Guzmán-Uriarte L, Sánchez-Maíga L, Angulo-Meza E, Cuevas-Rodríguez EO, Gutiérrez-Dorado R, Mora-Rochín S, Milán-Carrillo J, Valdez-Ortiz A, Reyes-Moreno C. 2013. Solid state bioconversion for producing common bean (Phaseolus vulgaris L.) functional flour with high antioxidant activity and antihypertensive potential. Food Nutr Sci 4: 480-490. https://doi.org/10.4236/fns.2013.44061

Jukanti A, Gaur P, Gowda C, Chibbar R. 2012. Nutritional quality and health benefits of chickpea (Pennisetum glaucum) L.: A review. Brit J Nutr 108: 11-26. https://doi.org/10.1016/S0007114512000797

Köprücü K, Özdemir Y. 2005. Apparent digestibility of selected feed ingredients for Nile tilapia (Oreochromis niloticus). Aquaculture 250: 308-316. https://doi.org/10.1016/j.aquaculture.2004.12.003

Lanna EA, Pezzato LU, Furuya WM, Vicentini CA, Cecon PR, Barros MM. 2004. Fibra bruta e óleo em dietas práticas para alevinos de tilápia do Nilo (Oreochromis niloticus). Rev Bras Zootec 33: 2177-2185. https://doi.org/10.1590/S1516-35982004000900001

Lara-Flores M, Granados-Puerto SG, Olivera-Castillo L, Novoa MA. 2007. Nutritional evaluation of treated X’pelon Phaseolus vulgaris L.) tempeh flour though solid state fermentation process. Lebensm Wiss Technol 37: 59-67. https://doi.org/10.1016/j.lwt.2006.11.020

Latta M, Eskin M. 1980. A simple and rapid colorimetric method for phytate evaluation. J Agric Food Chem 28: 7345.2008.00214.x

Lettre TF, Brown PB. 2007. Evaluation of fish meal-free diets for Nile tilapia, Oreochromis niloticus. Anim Feed Sci Tech 138: 178-188. https://doi.org/10.1016/j.anifeedsci.2007.06.023

Latta M, Eskin M. 1980. A simple and rapid colorimetric method for phytate evaluation. J Agric Food Chem 28: 1313-1315. https://doi.org/10.1021/jf00232a049

La Luna AC, Garcia VV, Mendoza EM, 1986. Effects of soaking in aqueous acidic and alkali solutions on removal of polyphenols and in vitro digestibility of cowpea. Plant Foods Hum Nutr 6: 107-118. https://doi.org/10.1007/BF01092138

Glencross BD, Booth MA, Allian GL. 2007. A feed is only as good as its ingredients a review of ingredient evaluation strategies for aquaculture feeds. Aquacult Nutr 13: 17-34. https://doi.org/10.1111/j.1365-2095.2007.00450.x

Furukawa A, Tsukahara H. 1966. On the acid digestion method for the determination of chrome oxide as an index substance in the study of digestibility ok fish fed. Bull Jap Soc Fish Sci 32(6): 502-508. https://doi.org/10.2331/suisan.32.502

Glencross B, Evans D, Hawkins W, Jones B. 2004. Evaluation of dietary inclusion of yellow lupin (Lupinus luteus) kernel meal on the growth, feed utilization and tissue histology of rainbow trout (Oncorhynchus mykiss). Aquaculture 235: 411-422. https://doi.org/10.1016/j.aquaculture.2003.09.022

La Luna AC, Garcia VV, Mendoza EM, 1986. Effects of soaking in aqueous acidic and alkali solutions on removal of polyphenols and in vitro digestibility of cowpea. Plant Foods Hum Nutr 6: 107-118. https://doi.org/10.1007/BF01092138
Maynard LA, Loosli JK, Hintz HF, Warner RG 1981. Animal nutrition. McGraw-Hill Book Company, NY, 289 pp.

McGoogan B.B., Reigh RC 1996: Apparent digestibility of selected ingredients in red drum (Sciaenops ocellatus) diets. Aquaculture 141: 233-244. https://doi.org/10.1016/0044-8486(95)01217-6

Medina-Godoy S, Ambriz-Perez DL, Fuentes-Gutiérrez CI, Germán-Báez LJ, Gutiérrez-Dorado R, Reyes-Moreno C, Valdez-Ortiz A, 2011. Angiotensin-converting enzyme inhibitory and antioxidant activities and characterization of protein hydrolysates of hard-to-cook chickpeas. J Food Sci Agr 9: 1974-81.

Montoya-Mejía M, Hernández-Llamas A, Garcia-Ulloa M, Nolasco-Soria H, Gutiérrez-Dorado R, Rodríguez-González H, 2016. Apparent digestibility coefficient of chickpea, maize, high-quality protein maize, and beans diets in juvenile and adult Nile tilapia (Oreochromis niloticus). Rev Bras Zootecn 48: 427-432. https://doi.org/10.1590/S1806-92902016000800001

Mugula JK, Lyyimo M, 2000. Evaluation of the nutritional and acceptability sorghum based tempeh as potential weaning food in Tanzania. Int J Food Sci Nutr 51: 269-277. https://doi.org/10.1080/09637480050077158

Paredes-López O, González-Castañeda J, Cárabos-Trejo A, 1991. Influence of solid substrate fermentation on the chemical composition of chickpea. J Ferment Bioeng 71: 58-62. https://doi.org/10.1016/0922-338X(91)90304-Y

Phume P, Wei WY, Ramachandran S, Hashim R, 2011. Inclusion of extruded chickpea, common bean and red lentil meals as protein source in diets for juvenile rainbow trout (Oncorhynchus mykiss). Acta Sci 22: 677-681

Price ML, Butler LG, Featherston WR, Rogler JC, 1978. Detoxification of high-tannin sorghum grain. Nutr Rep Int 17: 229-236.

Ramachandran S, Ray AK, 2004. Inclusion of extruded grass seed meal in compound diet for rohu (Labeo rohita) fingerlings. Acta Ichtyol Pisc 34: 205-208. https://doi.org/10.1080/10408399309527621

Reyes-Moreno C, Paredes-López O, 1993. Hard-to-cook phenomenon in common beans. Crit Rev Food Sci 33: 226-286. https://doi.org/10.1080/10408399309527621

Reyes-Moreno C, Romero-Urias C, Milán-Carrillo J, Valdez-Torres B, Zárate-Márquez E, 2000. Optimization of the solid state fermentation process to obtain tempeh from hardened chickpeas (Cicer arietinum). J Sci Food Agr 85: 219-228. https://doi.org/10.1023/A:1008192214018

Reyes-Moreno C, Cuevas-Rodriguez EO, Milán-Carrillo J, Cárdenas-Valenzuela OG, Barrón-Hoyos J, 2004. Solid state fermentation process for producing chickpea (Cicer arietinum) tempeh flour. J Sci Food Agr 84: 271-278. https://doi.org/10.1002/jsfa.1637

Ruiz-Terán F, Owens JD, 1996. Chemical and enzymic changes during the fermentation of bacteria-free soya bean Tempe. J Food Sci Agr 71: 523-530. https://doi.org/10.1002/(SICI)1097-0010(199608)74:5<523::AID-JSF613>3.0.CO;2-R

Sánchez-Magaña LM, Cuevas-Rodriguez EO, Gutiérrez-Dorado R, Ayala-Rodriguez AE, Valdez-Ortiz A, Milán-Carrillo J, Reyes-Moreno C, 2014. Solid-state bioconversion of chickpea (Cicer arietinum L.) by Rhizopus oligosporus to improve total phenolic content, antioxidant activity and hypoglycemic functionality. Int J Food Sci Nutr 65: 558-564. https://doi.org/10.3109/0963748X.2014.839234

Silva TS, Moro GV, Silva TB, Dairiki JK, Cyrino JE, 2013. Digestibility of feed ingredients for the striped surubim Pseudoplatystoma reticulatum. Aquaculture 19: 491-498. https://doi.org/10.1111/anu.12000

Tacon AGJ, Metian M, 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285: 146-158 https://doi.org/10.1016/j.aquaculture.2008.08.015

Tiril SU, Karayucel I, Alagil F, Dernekbası S, Yagci FB, 2009. Evaluation of extruded chickpea, common bean and red lentil meals as protein source in diets for juvenile rainbow trout (Oncorhynchus mykiss). J Anim Vet Adv 8: 2079-2086.

Valdez-González FJ, Gutiérrez-Dorado R, García-Ulloa M, Rodríguez-González H, 2013. Revisión del efecto de los antinutrientes y la fibra de leguminosas en la alimentación para peces. Ciencia Nicolaita 51: 21-40.

Valdez-González FJ, García-Ulloa M, Hernández-Llamas A, Rodríguez-Montes de Oca GA, Rodríguez-González H, 2016. Effect of shrimp head silage hydrolysate and distiller's dried corn grain on digestibility and growth of red tilapia (Oreochromis mossambicus). Anim Nutr Feed Tech 16: 51-60. https://doi.org/10.5958/0974-181X.2016.0005.6

Vielma J, Lall SP, Keskela J, Schöner FJ, Mattila P, 1998, Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (Oncorhynchus mykiss). Aquaculture 163: 309-323. https://doi.org/10.1016/S0044-8486(98)00240-3
Williams PC, Singh U, 1987. The chickpea-nutrimental quality and evaluation of quality in breeding programmes. In: The chickpea; Saxena MC & Singh KB, (eds). pp: 324-356. CAB Int, UK.

Wilson RP, 1994. Utilization of dietary carbohydrate by fish. Aquaculture 124: 67-80. https://doi.org/10.1016/0044-8486(94)90363-8

Yigit N, Olmez M, 2011. Effects of cellulase addition to canola meal in tilapia (Oreochromis niloticus L.) diets. Aquacult Nutr 17: 494-500. https://doi.org/10.1111/j.1365-2095.2010.00789.x

Yu HR, Zhang Q, Cao H, Wang XZ, Huang GQ, Zhang BR, Fan J, Liu SW, Li W, Cui Y, 2013. Apparent digestibility coefficients of selected feed ingredients for juvenile snakehead, Ophiocephalus argus. Aquacult Nutr 19: 139-147. https://doi.org/10.1111/j.1365-2095.2012.00947.x

Yuan YC, Lin YC, Yang HJ, Gong Y, Gong SY, Yu DH, 2013. Evaluation of fermented soybean meal in the practical diets for juvenile Chinese sucker, Myxocyprinus asiaticus. Aquacult Nutr 19: 74-83. https://doi.org/10.1111/j.1365-2095.2012.00939.x