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RESEARCH ARTICLE

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Effect of fermented, hardened, and dehulled of chickpea (*Cicer arietinum*) 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 arietinum*) 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).

Authors' contributions: Conceived and designed the experiments: RGD and HRG. Performed the experiments: FJVG, RGD and BLCR. Analyzed the data: FJVG, MGU and HRG. All authors wrote and approved the final manuscript.

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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-Mejía *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* NRRL2710 (1×10^6 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 ºC during 51 h. Subsequently, samples were dried in an oven with forced air circulation (50 ºC, 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):

$$\text{ADM} = [(100 \times \text{ADC of the tested diet}) - (100 - \%\text{ tested ingredient}) \times \text{ADC of the reference diet})] / \%\text{ tested ingredient} \quad [1]$$

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

where ADC is the apparent dry matter digestibility (100 – 100 × ((% Cr_2O_3 in diet) / (% Cr_2O_3 in feces)) and ADP is the apparent digestibility of protein (100 – 100 × ((% Cr_2O_3 in diet) × (% protein in diet) $^{-1}$) × (% protein in feces/% Cr_2O_3 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 Kjeltec 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 H_2SO_4 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).

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	
Minerals[1]	1	
Vitamins[2]	0.1	
Chrome oxide	1	
Reference diet		70
Tested ingredients		30
Total	100	100

[1]Mineral mixture (g/kg diet): KCl (0.5); $MgSO_4 \cdot 7H_2O$ (0.5); $ZnSO_4 \cdot 7H_2O$ (0.09); $MnCl_2 \cdot 4H_2O$ (0.00234); $CuSO_4 \cdot 5H_2O$ (0.005); KI (0.005); $CoCl_2 \cdot 2H_2O$ (0.00025); Na_2HPO_4 (2.37). [2]Vitamins 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).

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 × 27 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 $FeCl_3.6H_2O$ + 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 2. Mean (± SD) content of chemical components (%) of the reference and experimental diets[1]

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[2]	44.52	46.72	46.72	44.36	46.12	46.71	46.71	46.73	46.62

[1]FWC: 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. [2]NFE: nitrogen-free extract.

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 $p<0.05$.

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

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

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

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
		Dehulled	2.14	0.45
	Hardened	Whole grain	2.14	2.71
		Dehulled	2.13	0.41
Fermented	Fresh	Whole grain	1.19	2.72
		Dehulled	1.17	0.43
	Hardened	Whole grain	1.18	2.70
		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
		Dehulled	63.30 ± 3.43	68.90 ± 1.84
	Hardened	Whole grain	53.85 ± 2.65	60.27 ± 1.37
		Dehulled	64.39 ± 9.20	71.49 ± 4.94
Fermented	Fresh	Whole grain	69.73 ± 4.19	90.64 ± 1.76
		Dehulled	75.26 ± 2.39	95.63 ± 1.04
	Hardened	Whole grain	69.33 ± 4.96	88.86 ± 2.18
		Dehulled	73.88 ± 9.70	94.50 ± 4.10
Factorial Anova				
Non-fermented-Fermented (1)			$p<0.001$	$p<0.001$
Fresh-hardened (2)			$p>0.05$	$p>0.05$
Whole grain-dehulled (3)			$p<0.001$	$p<0.001$
1×2			$p=0.154$	$p=0.233$
1×3			$p=0.001$	$p=0.607$
2×3			$p=0.721$	$p=0.551$
1×2×3			$p=0.760$	$p=0.744$

fermenting. Mean values of ADM for diets using whole and dehulled chickpea were respectively 61.3 ± 9.5% and 69.2 ± 6.2%, indicating an increase of 12.8% associated with dehulling.

Fermentation and dehulling effects on ADP were significant ($p<0.001$) (Table 5). No significant interactions among the three factors were detected. Values of ADP in non-fermented and fermented chickpea grains averaged 65.1 ± 5.9% and 92.4 ± 3.1%, respectively, showing an increment of 41.9% caused by fermentation. Mean values of ADP for whole and dehulled chickpea were 74.9 ± 17.1% and 82.7 ± 14.5% respectively, increasing 10.4% by dehulling. The combination of fermentation and dehulling in grains of chickpea caused an increase of 40.27% for the ADM, and 58.20% for the ADP with respect to non-fermented and not dehulled treatments.

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

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 (Ruíz-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 (*Bydanus bydanus*) 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 (*Oncorhynchus mykiss*).

Various studies indicate that grains with high phytate content may decrease protein digestibility (Vielma *et al.*,

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 & Stom, 1990; Lanna *et al.*, 2004). In our study, dehulling allowed to reduce the levels of fiber below that level, which could contribute to increase ADP.

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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