Research Article

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Bioprocessing of common beans in diets for tilapia: *in vivo* digestibility and antinutritional factors

Francisco Valdez-González,[a,b] Roberto Gutiérrez-Dorado,[b] Alfredo Hernández-Llamas,[c] Manuel García-Ulloa,[d] Luís Sánchez-Magaña,[b] Breidy Cuevas-Rodríguez[a] and Hervey Rodríguez-González[d*]

Abstract

BACKGROUND: Bioprocessing of ingredients by solid-state fermentation is a low-cost technique for preparing diets. It is performed by adding microorganisms such as *Rhizopus oligosporus* to bean grains, achieving minimal degradation of nutrients and a significant improvement in digestibility. In particular, fermentation induces favorable changes in beans by reducing enzyme inhibitors, such as phytates and tannins.

RESULTS: Fermentation significantly ($P < 0.05$) increased the protein content and digestibility of dry matter and protein compared with whole bean grains, and decreased the content of lipids, ash and phytic acid. Hardening did not have a significant ($P > 0.05$) effect on the chemical content of beans and digestibility of diets. The dehulled bean meal significantly ($P < 0.05$) increased protein and lipid content and digestibility of dry matter and protein of beans, and decreased fiber, ash and tannin content. The chemical content of beans and digestibility of ingredients compare favorably with those reported by other authors, indicating the benefits of fermentation and dehulling.

CONCLUSION: We concluded that bean meal obtained from fermentation or dehulling represents a low-cost alternative for diets for tilapia.

Keywords: bioprocessing; dehulling; fermented; hardened; phytic acid; tannin

INTRODUCTION

Tilapia fish is the most widely cultured species in the world. Its production, conducted in almost 140 countries and regions,[1] exceeded 3.77 million tons in 2015 and is expected to increase rapidly in the coming years.[2]

Since feed represents about 70% of production costs in tilapia cultivation, a priority area of research is substituting low-cost agro-industry by-products for traditional high-cost feed ingredient.[3] Typical feed sources for tilapia include fish and poultry meals,[4–6] soybean meal[7] and other plant sources.[8] Processing grains by fermentation or dehulling is needed to make nutrients digestible to fish. The seeds of several bean species, particularly *Phaseolus*, are a rich source of protein in diets for monogastric animals.[9] The inclusion of hardened beans in fish diets presents an economic advantage over the use of other plant sources such as soybeans.[10]

Bioprocessing of ingredients by solid-state fermentation is a low-cost technique for preparing diets. *Rhizopus oligosporus* has been used as producer of fermented foods in solid form and the bioconversion of agro-industrial by-products.[11] The genus *Rhizopus* is especially important for the production of proteins with high digestibility, preventing the formation of toxic substances and being considered safe for application in the food industry.[12]

Dehulling of seeds and grains is another process to improve digestibility and availability of nutrients to fish. Reduction in dry matter and carbohydrate digestibility occurs when feeds contain whole grains.[13,14] In contrast, when grains are dehulled, fish weight gain and feed efficiency improve.[15]

Hardening occurs when legumes are stored at high temperature and high relative humidity,[16] leading to longer cooking time and lower nutritional content.[17] The causes of bean hardening

* *Correspondence to: H Rodríguez-González, Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa, Blvd Juan de Dios Batiz Paredes 250, 81101 Guasave, Sinaloa, Mexico. E-mail: hrodriguezg@ipn.mx*

a *Unidad Académica Escuela Nacional de Ingeniería Pesquera, Universidad Autónoma de Nayarit, San Blas, Nayarit, Mexico*

b *Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, Mexico*

c *Centro de Investigaciones Biológicas del Noroeste, Instituto Politécnico Nacional, La Paz, BCS, Mexico*

d *Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Guasave, Sinaloa, Mexico*

are lipid oxidation and/or polymerization, formation of insoluble pectates, lignification of middle lamella and multiple other mechanisms.[18,19] Other limiting factors for digestibility are antinutritional compounds in legumes.[18,20] Oligosaccharides, phytates and tannins reduce protein use and palatability, resulting in poor growth in cultivated fish.[21]

The objective of this study was to test common bean (*Phaseolus vulgaris*) meals as substitutes for fish meal in diets for tilapia (*Oreochromis niloticus*). We determined the effect of dehulling and fermentation on apparent digestibility of dry matter (ADDM) and apparent digestibility of protein (ADP) of fresh and hard-to-cook common beans, comparing the results with control diet. This kind of investigation had not been previously conducted.

EXPERIMENTAL

Preparation of bean meals

Meals of hardened, fermented and hulled bean (Azufrado Higuera variety, Sinaloa, Mexico) were prepared. Hardening was done under laboratory conditions, following Farhangi and Carter,[15] with slight modifications, namely storing at high temperature (37 ± 1 °C) and 100% relative humidity for 15 days. For that, samples of beans (250 g) were placed in 1 L plastic bottles with perforations at the top. Then, bottles were introduced into 20 L plastic buckets containing 5 L distillated water and a rack where the bottles were placed. Finally, buckets were hermetically closed and kept in an oven (37 ± 1 °C) for 2 weeks. Bottles with beans were opened every 2 days to provide fresh air and to review humidity degree.

Solid-state fermentation of fresh and hardened bean meal was accomplished by soaking beans for 16 h in a 0.06 mol L^{-1} glacial acetic acid solution (pH 3.1); beans were then drained, rinsed and manually dehulled. For treatments using hulls, these were added to the fermented and non-fermented diets 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 stored at 25 °C for 4 h. The substrate was placed in 15×25 cm polyethylene bags with small holes and inoculated with a suspension of *R. oligosporus* NRRL2710 (1×10^6 spores m L^{-1}). Bags with cotyledons were incubated in an oven for fermentation at 34.9 °C for 51 h. The samples were then dried in an oven with forced-air circulation (50 °C, 24 h).

Finally, the samples were milled to obtain 0.180 mm particulate size (#80 mesh). Meal obtained from fresh beans was prepared by milling the beans in a 0.5 hp electric mill (Molino del Rey®, Mexico) until approximately four fragments per bean were obtained. The hull fragments were then removed using an electric fan and the bean fragments were milled to obtain a #80 mesh meal (0.180 mm). For meals that included hulls, these were added and mixed after they were milled to the same size.

Preparation and composition of diets

A reference diet and eight experimental diets were prepared and assessed for their nutritional value and their effect on growth. The experimental diets contained 700 g kg^{-1} of the reference diet and 300 g kg^{-1} of the tested ingredients, which were: unfermented/fresh/whole (NFW), unfermented/fresh/dehulled (NFD), unfermented/hardened/whole (NHW), unfermented/hardened/dehulled (NHD), fermented/fresh/whole (FFW), fermented/fresh/dehulled (FFD), fermented/hardened/whole (FHW) and fermented/hardened/dehulled (FHD).

Table 1. Composition of reference and experimental diets; bean flour varied, depending on the treatment (g kg^{-1})

Ingredient	Reference diet	Experimental diet
Fish meal	340	
Wheat flour	453	
Fish oil	23	
Soybean lecithin	23	
Starch	100	
Grenetina	40	
Minerals[a]	10	
Vitamins[b]	1.0	
Chrome oxide	10	
Reference diet		700
Experimental ingredients		300

[a] Mineral mixture (g kg^{-1} diet): KCl (0.5); $MgSO_4.7H_2O$ (0.5); $ZnSO_4.7H_2O$ (0.09); $MnCl_2.4H_2O$ (0.00234); $CuSO_4.5H_2O$ (0.005); KI (0.005); $CoCl_2.2H_2O$ (0.00025); Na_2HPO_4 (2.37).
[b] Vitamin mixture (units in mg kg^{-1}: 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); retinol (5000 IU); cholecalciferol (4000 IU).

The ingredients were ground to 0.425 mm and homogenized, along with 10 g kg^{-1} chromic oxide as an inert marker to assess digestibility of the experimental ingredient. Diets were prepared with an extruder and dried at 45 °C until their moisture content was 8–10%. Afterwards, pellets were ground to a size appropriate for the size of fish, and stored at −20 °C until required. The composition of the diets is shown in Table 1.

Bioassays of digestibility

For bioassays, 27 tanks (270 L) were used. Every diet was tested in triplicate, using a stocking density of six fish (23.0 ± 2.4 g) per tank. Continuous aeration maintained dissolved oxygen above 7.0 mg L^{-1}, and water temperature was maintained at 26 ± 2 °C with electric heaters. Feed was provided twice daily (08:00 h and 15:00 h) to apparent satiation. Two hours after each feeding, fecal samples were collected with a plastic Pasteur pipette. Feces were washed with distilled water and stored at −40 °C. Feces were lyophilized (Labconco FreeZone®, model 7750020, 4.5 L) and, together with the experimental diets, were analyzed to determine the chromic oxide and protein content.

Apparent digestibility of dry matter (ADDM) and apparent digestibility of protein (ADP) of the ingredients were calculated as follows: [22,23]

$$\mathrm{ADDM} = \left[\left(100 \times \mathrm{ADC\ of\ tested\ diet}\right) - \left(100 - \%\ \mathrm{tested\ ingredient} \times \mathrm{ADC\ of\ reference\ diet}\right)\right] \times \left(\%\ \mathrm{tested\ ingredient}\right)^{-1}$$

$$\mathrm{ADP} = \{(100 \times \mathrm{APD\ of\ tested\ diet} \times \%\ \mathrm{protein\ in\ reference\ diet}) - [(100 - \%\ \mathrm{tested\ ingredient}) \times \mathrm{APD\ of\ reference\ diet} \times \%\ \mathrm{protein\ of\ reference\ diet}]\} \times (\%\ \mathrm{tested\ ingredient} \times \%\ \mathrm{protein\ in\ tested\ ingredient})^{-1}$$

where ADC is the apparent dry matter digestibility [100 - 100 (% Cr_2O_3 in diet × % Cr_2O_3 in feces^{-1})] and APD is the apparent protein digestibility [100 - 100 ((% Cr_2O_3 in diet) % protein in diet^{-1}) (% protein in feces % Cr_2O_3 in feces^{-1}))].

Table 2. Mean (±SD) content of chemical components (g kg^{-1}) of the reference and experimental diets ($n = 3$)

Nutrient	Reference diet	NFW	NFD	NHW	NHD	FFW	FFD	FHW	FHD
Dry matter	925.7 ± 1.5	913 ± 0.5	925.6 ± 2.0	914.9 ± 2.0	905.7 ± 1.5	902 ± 2.0	902.4 ± 1.5	90.11 ± 0.2	90.66 ± 0.26
Protein	319.6 ± 0.6	284.4 ± 0.3	303.0 ± 1.1	287.3 ± 0.8	304.8 ± 1.5	302.6 ± 0.8	318.2 ± 0.7	306.5 ± 1.1	319.0 ± 1.0
Lipids	98.6 ± 0.4	82.9 ± 0.8	88.9 ± 1.0	83.6 ± 1.0	87.6 ± 0.4	76.0 ± 0.5	77.3 ± 1.5	76.5 ± 1.1	78.0 ± 0.8
Fiber	40.1 ± 0.3	38.5 ± 0.8	13.5 ± 0.3	38.0 ± 0.5	14.6 ± 0.7	36.5 ± 1.3	12.5 ± 0.9	34.8 ± 0.5	11.7 ± 0.6
Ash	92.0 ± 0.3	73.3 ± 0.8	72.6 ± 1.2	71.9 ± 0.3	75.7 ± 0.7	65.0 ± 0.5	63.8 ± 0.6	63.0 ± 1.1	64.7 ± 0.5
NFE	445.2	513.2	515.6	517.1	484.6	443.2	483.2	484.6	484.2
Energy[a]	41.0 ± 7.8	43.9 ± 3.7	43.8 ± 4.8	43.4 ± 7.9	43.6 ± 8.5	44.1 ± 7.6	44.1 ± 6.5	44.2 ± 3.8	43.9 ± 7.2

NFW, unfermented/fresh/whole; NFD, unfermented/fresh/dehulled; NHW, unfermented/hardened/whole; NHD, unfermented/hardened/dehulled; FFW, fermented/fresh/whole; FFD, fermented/fresh/dehulled; FHW, fermented/hardened/whole; FHD, fermented/hardened/dehulled; NFE, nitrogen-free extract.
[a] Energy (kcal g^{-1}).

Analytical methods

Proximal chemical analysis of the ingredients, diets and feces was performed using standard AOAC methods.[24] The micro-Kjeldahl method was used to determine crude protein; determination of nitrogen was conducted in a Kjeltec system (model 1009, Foss Tecator). For determining lipids, extraction with petroleum ether in a Soxtec system (model 1043, Foss Tecator) was used. Fiber was determined by drying and burning the sample after extraction with 0.5 mol L^{-1} H_2SO_4 and 0.5 mol L^{-1} NaOH. Ash content was determined by calcination of the sample in a muffle furnace at 600 °C for 5 h. The energy content was determined with a bomb calorimeter (Parr Instrument Co.) (Table 2). Analyses were performed in triplicate.

Determination of antinutrients

Phytic acid content was determined.[25] Extraction was performed by shaking (25 °C for 1 h) 1 g meal with 20 mL of 0.65 mol L^{-1} HCl. The suspension was centrifuged (20 000 × *g* at 25 °C for 5 min). The supernatant was diluted (1:25) with deionized water and inserted into a glass column for anion-exchange chromatography. The glass column (0.7 × 27 cm) was packed with glass fiber and 0.5 g ion exchange resin (Bio-Rad Laboratories, Hercules, CA, USA). The column was washed with 15 mL of 1.36 mol L – 1 HCl, followed by 20 mL deionized water. Once the fluid passed through the column, 15 mL of 0.1 mol L^{-1} NaCl was added and the eluant was discarded. A 25 mL vessel was placed under the column and 15 mL of 0.7 mol L^{-1} NaCl was added to collect the eluant. After this, deionized water was added to make up to 25 mL. Then 3 mL were taken from this container and added to 3 mL deionized water and 1 mL Wade reagent (0.15 g $FeCl_3.6H_2O$ + 1.5 g sulfosalicylic acid) in 500 mL deionized water). The solution was shaken vigorously, centrifuged (5000 × *g* at 25 °C for 10 min), and the supernatant was removed. The supernatant was measured in a spectrophotometer (Spectronic 21D, Thermo Scientific, Waltham, MA, USA) at 500 nm.

Tannin content was determined by the vanillin method,[26] with modifications. Extraction was carried out within 24 h after milling, using approximately 1 g of the sample and 10 mL of 0.27 mol L – 1 HCl in methanol. The suspension was shaken for 40 min at room temperature and centrifuged (20 000 × *g*, 30 °C, 20 min). Five milliliters of vanillin reagent (1 mL vanillin 0.52 mol L^{-1} in methanol and 2.19 mol L^{-1} HCl in methanol, 50:50, v/v,) was added to 1 mL supernatant at a rate of 1 mL min^{-1}. The suspension was kept in the dark for 20 min and assayed by spectrophotometry (Spectronic 21D model 1146, Milton Roy, Ivyland, PA, USA) at 500 nm. A blank solution, zero absorbance, was prepared with 1 mL methanol by adding 5 mL of 1.09 mol L^{-1} HCl at a rate of 1 mL min^{-1}. A standard curve for catechin was plotted, and the results were reported as equivalents of catechin.

Statistical analyses

Values of digestibility were tested for normality for Lilliefors' method and homoscedasticity tested for Bartlett's method. A multifactorial analysis of variance (ANOVA) and Tukey's multiple-range test were used to compare mean values of chemical composition and antinutritional factors of beans, and digestibility of diet ingredients. The factors and levels analyzed corresponded to the different conditions of the beans: fermentation (fermented/non-fermented); hardening (fresh/hardened) and dehulling (dehulled/whole pod). Statistica 7.0 software (StatSoft, Tulsa, OK, USA) was used for all analyses, with significance set at $P < 0.05$.

RESULTS

The results from ANOVA indicated that the main effects of fermentation and dehulling on increasing protein content of beans were significant, whereas the main effect of hardening was not significant. The interactions of these factors were not significant (Table 3). Mean protein values of treatments when the beans were not fermented (274 ± 10 g kg^{-1}) and those that were fermented (312 ± 17 g kg^{-1}) showed an increase of 14.2%. Mean protein values of treatments when whole grain beans were used (282 ± 18 g kg^{-1}) and those that were dehulled (304 ± 27 g kg^{-1}) showed that dehulling increased protein content of beans by 7.9%.

Lipid content of beans was significantly lowered by fermentation and dehulling, but no significant effect was detected for hardening. No significant interactions of these factors were found (Table 3). Mean values of lipid content in treatments where fermentation was not used (22 ± 6 g kg^{-1}) and where it was used (13 ± 7 g kg^{-1}) showed a reduction of 39.1% by fermentation. Mean lipid values of treatments where grains were not dehulled (12 ± 6 g kg^{-1}) and those that were dehulled (23 ± 4 g kg^{-1}) showed 97.9% more lipids from dehulling.

The fiber content of beans was significantly reduced by dehulling. There were no significant interactions of the factors (Table 3). Mean values of fiber in treatments not using dehulling (37 ± 1 g kg^{-1}) and those that used it (19 ± 1 g kg^{-1}) showed a decrease of 49.0%. Fermentation and dehulling significantly reduced ash content, but hardening did not. There were no significant interactions of the factors (Table 3). Mean values of ash in treatments where beans were not fermented (42 ± 1 g kg^{-1}) and those that used fermentation (22 ± 3 g kg^{-1}) showed reduction by 47.3% from fermentation. Mean values of ash in treatments that

Table 3. Mean (±SD) content of proximate chemical components (g kg^{-1}) of ingredients used in the diets ($n = 3$)

Bean flour			Dry matter	Protein	Lipids	Fiber	Ash
Not fermented	Fresh	Whole	917.3 ± 1.1	263 ± 11	17 ± 0.3	36 ± 2.0	43 ± 1.0
		Dehulled	910.6 ± 1.5	287 ± 11	27 ± 3.0	17 ± 2.0	41 ± 0.4
	Hardened	Whole	919.0 ± 2.0	269 ± 1.0	16 ± 3.0	37 ± 3.0	42 ± 0.4
		Dehulled	907.0 ± 1.0	275 ± 6.0	28 ± 2.0	19 ± 2.0	41 ± 0.4
Fermented	Fresh	Whole	929.6 ± 2.0	299 ± 9.0	6.0 ± 0.3	36 ± 0.3	25 ± 0.3
		Dehulled	918.0 ± 2.6	327 ± 7.0	19 ± 0.3	19 ± 0.3	20 ± 0.2
	Hardened	Whole	937.3 ± 2.5	296 ± 5.0	8.0 ± 1.0	38 ± 1.0	24 ± 1.0
		Dehulled	926.0 ± 1.7	327 ± 9.0	20 ± 0.3	20 ± 0.1	19 ± 1.0
Factorial ANOVA							
Not fermented/fermented (1)			n.s.	*	*	n.s.	*
Fresh/hardened (2)			n.s.	n.s.	n.s.	n.s.	n.s.
Whole bean/dehulled (3)			n.s.	*	*	*	*
1 × 2			n.s.	n.s.	n.s.	n.s.	*
1 × 3			n.s.	n.s.	n.s.	n.s.	*
2 × 3			n.s.	n.s.	n.s.	n.s.	n.s.
1 × 2 × 3			n.s.	n.s.	n.s.	n.s.	n.s.

* significant different ($P < 0.05$); n.s. = no significant different ($P > 0.05$).

did not use dehulled beans (34 ± 10 g kg^{-1}) and those that used them (30 ± 12 g kg^{-1}) showed a reduction of 9.7% for dehulling.

The main effect of fermentation was significantly influenced by phytic acid content, whereas the main effects of hardening and dehulling were not significant (Table 4). No significant interactions were found for these factors. Phytic acid content averaged 15.7 ± 0.4 mg g^{-1} phytic acid in treatments using non-fermented beans, and 2.5 ± 0.4 mg g^{-1} phytic acid in treatments that used fermented beans; that is, fermentation reduced phytic acid content by 84.2%. Tannin content was significantly affected by dehulling, but fermentation and hardening did not significantly affect tannin content (Table 4). There were no significant interactions of the three factors. Tannin content averaged 0.8 ± 0.1 mg g^{-1} in treatments where beans were not dehulled and 0.1 ± 0.03 mg g^{-1} in treatments using dehulled beans; that is, dehulling reduced tannin content by 90.7%.

The main effects of fermentation and dehulling on ADDM were significant, whereas the main effect of hardening was not (Table 5). There were no significant interactions of the three factors. Average values of ADDM in diets that used unfermented beans was 67.9% ± 8.3% and 74.2% ± 4.1% in diets where beans were fermented, meaning an improvement of 9.2% by fermenting. Mean values of ADDM for diets using whole and dehulled beans were 65.8% ± 5.8% and 76.3% ± 2.1%, indicating an increase of digestibility by 16.0% associated with dehulling.

The main effects of fermentation and dehulling on ADP were significant, but the main effect of hardening was not (Table 5). No significant interactions of the three factors were detected. Values of ADP in diets using unfermented beans averaged 82.5% ± 3.4% and 87.7% ± 3.3% in diets where beans were fermented, with an increase of 6.4% resulting from fermentation. Mean values of ADP for diets using whole and dehulled beans were 82.4% ± 3.3% and 87.9% ± 3.4%, respectively, indicating that dehulling led to an increase of 6.7%.

DISCUSSION

Our results showed that fermentation and removal of hulls of the common bean increased its protein content, decreased antinutrient content, and improved digestibility of dry matter and protein ingredients in diets for tilapia.

Increase in protein during fermentation of the common bean is related to protein synthesis caused by the increase in biomass of *R. oligosporus*.[27,28] The increase in protein occurs with the decrease in other constituents, which might be lost by leaching during the initial fermentation or might be consumed by the fungus.[29] There are similar reports of *R. oligosporus* used with fresh and hardened common beans.[30,31]

Lower lipid content in fermented beans is a consequence of oxidation and use of fatty acids as the main source of energy by a fungus.[17] There are similar reports using *R. oligosporus* and beans as a substrate.[30–32]

Dehulling beans decreases the content of ash and fiber because hulls contain calcium, phosphorus, magnesium, iron and potassium, and a high concentration of fiber.[33] An increase in lipid content in dehulled beans may reflect the loss of ash and fiber.

The phytate reduces protein, vitamin and mineral absorption of vegetable meals.[34] In our study, fermentation and dehulling significantly decreased phytic acid and tannins in beans. Phytic acid content of beans was similar to that reported by Alonso *et al.*[35] for raw beans (15.9 mg g^{-1}). It was proved that fermentation decreased phytic acid from phytase in cowpea when this seed was synthesized by *R. oligosporus,* and soaking–cooking–leaching treatment was used for fermentation.[36] Similar results were reported by[37] using *R. oligosporus* with chickpea as a substrate. Most of the tannin content is in the hull of legumes.[38] The tannins in beans are reduced by 95% by dehulling,[39] which is similar to our results (94.0%).

Higher digestibility from fermentation results from improved nutritional balance of the ingredients and proteolytic activity of fungi that releases peptides of the substrate and increasing susceptibility of protein to enzymes.[40] Our study showed that diets based on fermented–dehulled common bean were digested with ease by juvenile tilapia, mainly as a consequence of two factors: the quality of the proteins produced by *R. oligosporus,* and the combination of reduction and blocking of antinutrient activity.

Apparent digestibility of dry matter and protein in the tested ingredients depended on the type of ingredient. Differences

Table 4. Mean (±SD) content of antinutrient contents in ingredients used in diets ($n = 3$)

Bean flour			Phytic acid(mg g^{-1})	Tannins(mg g^{-1})
Not fermented	Fresh	Whole	16.2 ± 0.1	0.9 ± 0.1
		Dehulled	15.3 ± 0.2	0.1 ± 0.0
	Hardened	Whole	15.8 ± 0.4	0.6 ± 0.3
		Dehulled	15.3 ± 0.3	0.1 ± 0.0
Fermented	Fresh	Whole	2.6 ± 0.7	0.7 ± 0.1
		Dehulled	2.3 ± 0.2	0.04 ± 0.03
	Hardened	Whole	2.9 ± 0.1	0.8 ± 0.1
		Dehulled	2.1 ± 0.1	0.04 ± 0.02
Factorial ANOVA				
Not fermented/fermented (1)			*	n.s.
Fresh/hardened (2)			n.s.	n.s.
Whole bean/dehulled (3)			n.s.	*
1 × 2			n.s.	n.s.
1 × 3			n.s.	n.s.
2 × 3			n.s.	n.s.
1 × 2 × 3			n.s.	n.s.

* significant different ($P < 0.05$); n.s. = no significant different ($P > 0.05$).

Table 5. Mean (±SD) content of apparent digestibility of dry matter and apparent digestibility of protein of tested ingredients ($n = 6$)

Bean flour			ADDM	ADP
Not fermented	Fresh	Whole	59.9 ± 6.6	81.0 ± 4.4
		Dehulled	76.4 ± 5.4	86.1 ± 3.5
	Hardened	Whole	61.8 ± 4.3	78.4 ± 2.4
		Dehulled	73.6 ± 3.5	84.3 ± 2.4
Fermented	Fresh	Whole	71.2 ± 12.7	85.6 ± 6.1
		Dehulled	78.6 ± 0.7	89.3 ± 0.2
	Hardened	Whole	70.2 ± 3.6	84.4 ± 1.4
		Dehulled	76.6 ± 7.1	91.6 ± 2.0
Factorial ANOVA				
Not fermented/fermented (1)			*	*
Fresh/hardened (2)			n.s.	n.s.
Whole bean/dehulled (3)			*	*
1 × 2			n.s.	n.s.
1 × 3			n.s.	n.s.
2 × 3			n.s.	n.s.
1 × 2 × 3			n.s.	n.s.

* significant different ($P < 0.05$); n.s. = no significant different ($P > 0.05$); ADDM, apparent digestibility coefficient of dry matter; ADP, apparent digestibility coefficient of protein.

in ADDM result from chemical composition, which in turn was determined by the origin and processing of feed ingredients.[41] Tilapia can assimilate a wide variety of feedstuffs.[42] Digestibility in this study compares favorably with values obtained by studies with other freshwater tropical fish species. A study conducted with cotton and cocoa bran in tilapia shown that the low digestibility comes from large amounts of fiber and antinutritional factors.[43] In our study, fermentation and dehulling of beans improved digestibility of dry matter and protein, most likely as a consequence of reduction in the content of phytic acid and tannins.

The increase in protein digestibility in fermented diets is related to the reduction of antinutrients, such as tannins and phytic acid.[44] The fermented soybean meal in diets for juvenile Chinese sucker (*Myxocyprinus asiaticus*) and Atlantic salmon (*Salmo salar*) showed high digestibility, without causing adverse effects on growth and body composition.[45,46] The use of the bacterium *Bacillus* sp. to ferment *Phaseolus mungo* meal improved ADP by 86.76% in diets for *Labeo rohita*.[47] Studies have shown that beans without processing have low digestibility.[48–51]

We obtained higher protein digestibility compared to results for *Clarias gariepinus* fed diets prepared with lima bean (*Phaseolus lunatus*).[52] The latter study reported ADP of 79.4% and 88.0% for toasted and cooked beans, whereas in the present investigation using dehulled bean meal resulted in ADP as high as 89.3% and 92.0%. A study shown to be similar to our results reported ADP of 90–91% in diets for tilapia using dehulled *Vigna unguiculata*.[53]

In our study, dehulling reduced tannins and fiber in beans. Several reports indicate that enzymatic inhibition caused by tannins decreases the digestibility of nitrogenous nutrients,[11,50] thus

causing low protein digestibility.[54] Levels of tannins higher than 0.63 mg g^{-1} significantly affect digestibility of dry matter, proteins and lipids in tilapia.[55] In addition, the removal of hull may cause a decrease in endogenous antinutrients of the hull,[13] structural polysaccharides, which in high concentrations are known to reduce dry matter digestibility in fish diets.[56,57] Levels of fiber lower than 30 g kg^{-1} improve protein digestibility in tilapia.[58] In our study, dehulling reduced fiber below that level, which contributed to greater ADP.

CONCLUSION

Since no differences in digestibility values were found compared with fresh bean, hardened bean may be included in tilapia diets due to its high availability, lower price as raw material and low cost of bioprocessing and dehulling.

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