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The Effect of Dehulling and Extrusion of Jackfruit *Artocarpus heterophyllus* Seeds on Digestibility and Antinutrients, in Tilapia (*Oreochromis niloticus*) Diets

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Keywords: Jackfruit seeds; antinutrients, dehulling; extrusion; apparent digestibility

Abstract

In the present study, the effect of dehulling and extrusion of Jackfruit seeds *Artocarpus heterophyllus,* and apparent digestibility of dry matter and protein was evaluated in feeds for Nile tilapia *Oreochromis niloticus*. For the digestibility bioassay, organisms with an average weight of 12.6 ± 3.1 g were used. The evaluation of the digestibility coefficient in vivo was determined by using the indirect method of chromium oxide as a marker.

The dehulled Jackfruit seeds caused a significant increase in protein and lipids, and a significant decrease in fiber and ash respectively. The extrusion process did not significantly affect protein, fiber, ash, and carbohydrate content of the extruded flour, but caused a significant decrease in the lipid content of Jackfruit seeds.

The results indicate that the extrusion process caused a significant decrease in the content of phytic acid, tannins, trypsin inhibitors, and saponins. In relation to trypsin inhibitors, dehulling did not have a significant effect. On the other hand, the combination of the extrusion and dehulling process allowed a significant decrease of phytic acid and tannins, while the dehulled of seeds did not have a significant effect on the phytic acid content. The combination of the dehulled and extrusion process caused a significant decrease in trypsin inhibitors and saponins with respect to raw Jackfruit seeds.

The combination of the extrusion and dehulling process caused a significant increase in ADM of diets prepared with 30% of Jackfruit flours. The combination of the process of extrusion and dehulling of Jackfruit seeds allowed a significant increase in ADP compared to the unprocessed seeds. We conclude that dehulled and extruded Jackfruit flours represent a potential low cost, highly available alternative to replace fishmeal in the production of food for tilapia *Oreochromis niloticus*.

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Introduction

Traditionally, fishmeal has been the main source of protein used in food processing for aquaculture species, making it the most expensive raw material (FAO, 2014). For this reason and with the objective of reducing food costs, numerous investigations have been carried out using protein sources of vegetable origin to substitute fishmeal in tilapia fish feed formulae (Montoya-Camacho et al., 2018).

Fishmeal can be substituted in different percentages depending on the source of vegetable protein, considering mainly the protein content, digestibility and amino acid balance (Ogunji & Wirth, 2001; Drew et al., 2007).

A limitation in the use of vegetable sources for fish is the presence of antinutrients (Valdez-González et al., 2018). The effect of plant antinutritional factors has been studied less in fish than in higher vertebrates (Guillaume et al., 2004).

Therefore, it is necessary to implement processes for the improvement and use of large amounts of legumes and seeds (Drew et al., 2007). An interesting proposal is the elimination of hull in legumes and seeds to reduce the content of fiber and tannins and increase protein digestibility.

Dehulling is a process in which the hull present in grains and seeds is removed. With this method it is possible to reduce the content of crude fiber and tannins, it also improves the appearance, texture, and increases the protein digestibility (Nikmaram et al., 2017; Valdez-González et al., 2017).

Extrusion is considered an efficient and versatile technological process that combines thermo-mechanical treatments such as humidity, pressure, particle cutting, high temperatures (150-200ºC), for short periods of time, and adaptation of screw speed of the extruder. The extrusion process improves the digestibility of vegetable proteins and increases the nutritional values (Milán-Carrillo et al., 2002; Cheng and Hardy, 2003). This technology is also used for the production of breakfast cereals and aquafeeds.

The objective of the present study is to test the effect of dehulling and extrusion on the chemical composition of Jackfruit (*Artocarpus heterophyllus*) seeds, digestibility of ingredients, including flours of processed and non-processed seed Jackfruit, and antinutrients (phytic acid, tannins, trypsin inhibitors and saponins) in diets for Nile tilapia *Oreochromis niloticus*. There are no antecedents concerning the use of Jackfruit for aquaculture in the literature.

Materials and Methods

Preparation of Jackfruit seed flours

Jackfruit *Artocarpus heterophyllus* seeds were used in their natural, dehulled, and extruded forms. The dehulled Jackfruit seed flour was prepared by milling the seeds in a 0.5 hp electric mill (Molino del Rey®, Mexico) until approximately four fragments per seed were obtained. The hull fragments were then removed by an electric fan and the seed fragments were milled to obtain 80 mesh meal (0.180 mm).

Preparation of diets

A reference diet and four experimental diets were prepared. The experimental diets contained 700 g/kg of the reference diet and 300 g/kg of the tested ingredients, which were: WJS= Whole Jackfruit seed, DJS= Dehulled Jackfruit seed, EWJS= Extruded whole Jackfruit seed, EDJS= Extruded dehulled Jackfruit seed (Table 1). The ingredients were ground and sieved through a 40 mesh (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).

Table 1. Composition of reference and experimental diets; Jackfruit flour varied, depending on the treatment (g/kg)

Ingredient	*Reference diet*	*Experimental diet*
Fishmeal	403	
Wheat flour	436	
Fish oil	20	
Soybean lecithin	20	
Starch	60	
Grenetina	40	
Minerals[1]	10	
Vitamins[2]	1	
Chrome oxide	10	
Reference diet		700
Tested ingredients		300
Total	1000	1000

[1]Mineral mixture (g/kg diet): KCl (0.5); $MgSO_4 \bullet 7H_2O$ (0.5); $ZnSO_4 \bullet 7H_2O$ (0.09); $MnCl_2 \bullet 4H_2O$ (0.00234); $CuSO_4 \bullet 5H2O$ (0.005); KI (0.005); $CoCl_2 \bullet 2H2O$ (0.00025); Na_2HPO_4 (2.37).

[2]Vitamins mixture (units in mg/kg: 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); and retinol (5000 IU) and cholecalciferol (4000 IU)

Bioassays of digestibility

Nile tilapia were held in 25 rectangular plastic tanks (300 L each) at a stocking density of 10 fish (12.6 ± 3.1 g) per tank. Every experimental unit received continuous aeration, maintaining dissolved oxygen at 6.01 ± 0.5 mg/L, and water temperature at 28 ± 2°C. Diets were tested in triplicate. Feed was offered to apparent satiation twice a day (08:00 and 16:00 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) was collected daily over 35 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 = [(100 × ADC of tested diet)
(100 − % tested ingredient × ADC of reference diet)] / (% tested ingredient)

ADP = {(100 × APD of tested diet × % protein in reference diet)
[(100 − % tested ingredient) × APD of reference diet × % protein of reference diet]}
/(% tested ingredient × % protein in tested ingredient)

Where ADC is the apparent dry matter digestibility (100 – 100 × (% Cr2O3 in diet) / (% Cr2O3 in feces)) and ADP is the apparent digestibility of protein ((100 – 100 × (% Cr2O3 in diet) / (% protein in diet) × (% protein in feces/% Cr2O3 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 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 and Tsukuhara (1966).

Table 2. Mean (±SD) content of chemical components (g/kg) of the reference and experimental diets (*n*=3)

Nutrients	*Diets*				
	Reference diet	*WJS*	*DJS*	*EWJS*	*EDJS*
Protein	353±0.06	30.1±0.4	30.4±0.4	29.9±0.2	30.1±0.3
Lipids	101±0.04	7.2±0.2	7.3±0.3	7.2±0.3	7.2±0.2
Ash	99±0.03	8.8±0.3	8.7±0.2	8.8±0.5	7.8±0.4
Fiber	13±0.01	2.7±0.08	1.1±0.05	2.6±0.06	1.0±0.03
NFE	434	51.2	52.5	51.5	53.9
Energy[a]	40.5±5.6	39.0±4.7	39.7±4.5	39.0±4.3	40.0±4.8

WJS: Whole Jackfruit seed flour, DJS: Dehulled Jackfruit seed flour, EWJS: Extruded whole Jackfruit seed flour, EDJS: Extruded dehulled Jackfruit seed flour, NFE= Nitrogen-free extract
[a]Energy (kcal/g).

Determination of antinutrients

Phytic acid

Phytic acid was determined following the procedure of Latta and Eskin (1980). The extraction was performed by shaking (400 rpm at 25°C for 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 was passed through the column, 15 mL of 0.1 M NaCl was added and the eluate was discarded. A 25 mL vessel was placed under the column and 15 mL 0.7 M NaCl was 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) was added, and shaken thoroughly. The tubes were centrifuged (5000 × g at 25°C for 10 min) and the supernatant was separated; following this, color 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 continuously shaken for 40 min at room temperature and centrifuged (20,000 × g, 30ºC, 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.

Saponins

The extraction was performed on 0.5 g of flour in 10 mL of 80% v / v methanol for 16 h in an orbital shaker. The tubes were centrifuged at 3800 rpm / 10 min and the supernatant was collected in 25 mL glass tubes. 200 µL of the extract was placed, 50 µL at 80% at room temperature. The tubes were transferred to an ice bath where 250 µL of vanillin reagent (1.6 g of vanillin in 20 mL of absolute methanol) was added, the tubes were taken out of the ice bath and 2.5 mL of 72% v/v sulfuric acid, was added then vortexed, the mixture was heated in a water bath at 60°C for 10 min. The tubes were cooled in an ice bath and the absorbance was measured at 520 nm against a blank of reagents. A diosgenin curve (0 µg/ mL-125 µg/mL) was used. The results were expressed in mg equivalents of diosgenin per 100 g of sample (Hiai et al., 1976).

Trypsin inhibitors

Trypsin inhibitory activity was determined by the method of the AACC (1983), using benzoyl-DL-arginine-p-nitroaniline (BAPNA) as a substrate. The extraction was carried out with 1 g of flour in 50 mL of 0.01 N NaOH for 3 h with continuous mixing, before the determination, the pH was adjusted to 8.2 with 0.1 N HCl. Aliquots of the extract (0.0, 0.3, 0.5, 0.7 and 1 mL) were pipetted into test tubes and adjusted to 1 mL with distilled water, then 1 mL of trypsin solution and 2.5 mL of BAPNA solution were added to the tubes. The tubes were placed rapidly in a water bath with stirring at 37°C for 10 min. The reaction was stopped with 0.5 mL of 30% acetic acid, filtered on Whatman paper #2 and the absorbance measured at 410 nm. One unit of inhibited trypsin (UTI) is defined as the decrease of 001 units of the absorbance of the samples with respect to the concentration 0 of the extract (1 mL of distilled water, 1 mL of trypsin and 2.5 mL of BAPNA). The results are expressed as ICU/mg of sample.

Statistical analysis

The values obtained were analyzed with a test of normality and homogeneity. To determine if the data obtained were significantly different, the computer software STATISTICA 7.0 (StatSoft, Tulsa, OK) was used, the data were subjected to a one-way analysis of variance (ANOVA, $\alpha<0.05$), later the test was applied of Tukey's multiple ranges, to classify the treatments.

Results

Table 3 shows the chemical composition of the processed and unprocessed Jackfruit seeds. Dehulling and extrusion significantly ($P<0.05$) affected the chemical composition of the Jackfruit seeds. The dehulled Jackfruit seeds caused a significant increase in protein and lipids (6.6% and 32.5%, respectively), and a significant decrease in fiber and ash (92.3 and 60.7%, respectively).

Table 3. Mean (±SD) content of proximate composition (g/kg) of ingredients used in diets (*n*=3)

Nutrients	*FM*	*WJS*	*DJS*	*EWJS*	*EDJS*
Protein	65.5±0.06	17.6±0.4^{a}	18.77±0.4^{a}	17.45±0.2^{b}	18.45±0.3^{a}
Lipids	12.17±0.04	0.4±0.2^{a}	0.53±0.3^{a}	0.35±0.3^{a}	0.43±0.2^{a}
Ash	16.73±0.03	6.1±0.3^{a}	2.4±0.2^{b}	5.9±0.5^{a}	2.9±0.4^{b}
Fiber	0.03±0.01	5.87±0.08^{b}	0.45±0.05^{a}	5.67±0.06^{b}	0.67±0.03^{a}
NFE	5.57	70.03	77.85	70.63	77.55

WJS: Whole Jackfruit seed, DJS: Dehulled Jackfruit seed, EWJS: Extruded whole Jackfruit seed, EDJS: Extruded dehulled Jackfruit seed, NFE= Nitrogen-free extract

The extrusion process did not significantly affect the protein, fiber, ash, and carbohydrate content of the extruded flour, but caused a significant (12.5%) decrease in the lipid content of Jackfruit seeds.

Table 4 shows the content of phytic acid, tannins, trypsin inhibitors, and saponins in Jackfruit seeds subjected to dehulling and extrusion treatments. The results indicate that the extrusion process caused a significant decrease (P> 0.05) in the content of phytic acid, tannins, trypsin inhibitors and saponins. Dehulling had no significant effect (P> 0.05) on the decrease of trypsin inhibitors. However, the extruded and dehulled (EDJS) treatment allowed for a significant decrease of phytic acid and tannins (10.1 and 80.2%, respectively), while the dehulled and non-extruded seeds had no significant effect (P> 0.05) on the phytic acid content. The treatment that combined the dehulling and extrusion processes (EDJS) caused a significant decrease in trypsin and saponin inhibitors (59.1 and 58.4%, respectively) when compared to raw Jackfruit seeds.

Table 4. Mean (± SD) content of tannins, saponins, trypsin inhibitors and phytic acid in ingredients used in diets (n=5)

Ingredients	*Tannins (mg EC/100 g)[1]*	*Saponins (mg ED/g)[2]*	*Trypsin I. (UIT/g)[3]*	*Phytic acid (mg/g)*
WJS	65.3^{d}±6.44	8.9^{d}±1.8	2494^{b}±132	187.6^{b}±53.4
DJS	26.5^{b}±7.60	5.2^{b}±1.1	956.1^{a}±154	173.9^{a}±52.7
EWJS	54.7^{c}±5.86	6.4^{c}±1.3	2466^{b}±128	185.4^{b}±55.2
EDJS	12.9^{a}±3.82	3.7^{a}±0.9	1020^{a}±143	168.7^{a}±45.6

WJS: Whole Jackfruit seed flour, DJS: Dehulled Jackfruit seed flour, EWJS: Extruded whole Jackfruit seed flour, EDJS: Extruded dehulled Jackfruit seed flour, [1]mg EC/100g = mg equivalents of catechin g sample. [2] mg equivalents of diosgenin/g sample. [3]Units of inhibited trypsin/g of sample.

The coefficients of apparent dry matter digestibility (ADM) in tilapia *Oreochromis niloticus* of the ingredients shown in Table 5 range between 61.3 and 78.5%. Individually, extrusion and dehulling promoted a significant increase in ADM (22.7 and 20.1%, respectively) but the treatment combining extrusion and dehulling processes (EDJS) caused a significant increase (28.1%) in ADM of diets prepared with 30% of Jackfruit flours. The protein digestibilities (ADP) in tilapia *Oreochromis niloticus* of the ingredients based on Jackfruit varied from 68.6 to 92.6%. However, the treatment that combines the process of extrusion and dehulling of the Jackfruit seeds allowed a significant increase (35%) in ADP compared to the unprocessed seeds.

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

Ingredients	*ADM*	*ADP*
	Percentage %	
WJS	61.3±0.3^{d}	68.6±0.8^{d}
DJS	73.6±0.2^{c}	89.2±0.7^{b}
EWJS	75.8±0.1^{b}	85.7±0.2^{c}
EDJS	78.5±0.4^{a}	92.6±0.4^{a}

ADM= apparent digestibility coefficient of dry matter; ADP, apparent digestibility coefficient of protein.
WJS: Whole Jackfruit seed flour, DJS: Dehulled Jackfruit seed flour, EWJS: Extruded whole Jackfruit seed flour, EDJS: Extruded dehulled Jackfruit seed flour.
*Values in a column with different superscripts are significantly different (P<0.05).

Discussion

The elimination of the hull of the Jackfruit seed reduced the content of ash and fiber mainly, as a result of the husk that contains certain minerals (calcium, phosphorus, magnesium, iron, potassium) and a high concentration of fiber (Williams and Singh, 1987). An increase in protein and lipid content in dehulled Jackfruit seeds may reflect the loss of ash and fiber (Hardy and Barrows, 2000; Valdez-González et al., 2017).

Extrusion caused a decrease in lipids due to the volatilization of these components during the process as a result of the high temperatures inside the extruder (Marzo et al., 2002). The extrusion process did not affect the fiber and ash content, which include the minerals present in the grain.

The presence of antinutritional factors is a common characteristic of plant sources and affects the growth of fish (Mbahinzireki et al., 2001). The interaction of phytate with proteins, vitamins and several minerals is one of the factors limiting the nutritional value of vegetable meals (Deshpande & Cheryan, 1984). High levels of phytic acid in the diet decreased food efficiency and availability of protein in rainbow trout and salmon (Sklan et al., 2004).

In the present study, the extrusion process caused a decrease in the content of tannins in Jackfruit seed. The results obtained coincide with those reported by other researchers (Marzo et al., 2002) with a 75% decrease in this antinutritional component. A study reported that the decrease of tannins in the extrusion process was due to the thermal degradation of its components (Alonso et al. (2000). The highest content of tannins is found in the seed hull of legume seeds (Egounlety and Aworh, 2003, Guillaume et al., 2004). Similar to other reports, in this research the content of tannins in Jackfruit seeds was considerably reduced (80.2%) by eliminating the hull.

In the present study the extrusion process caused a decrease of (59.1%) of the trypsin inhibitors. Extrusion uses high temperatures during the process that reduce or eliminate the content of thermolabile substances (Nikmaram et al., 2017). These compounds affect protein digestion and inhibit growth by inactivating trypsin (Nwabueze et al., 2007). However, it has been demonstrated that applying extrusion temperatures of 140ºC causes the inactivation of this antinutrient in wheat and rice (Kaur et al., 2014). Similar temperatures and conditions were those used in this study.

The properties of saponins are varied, but due to their flavor they can decrease the palatability of diets (Guillaume et al., 2004). In another study the authors concluded that diets with low amounts of raw soy can cause rupture of the intestinal membranes in Atlantic salmon *Salmo salar* L (Chikwati et al., 2012). In the present study the extrusion process allowed a decrease in the content of saponins (58.4%) with respect to the work reported by (Knudsen et al., 2006) they found that the extrusion had no effect on the decrease of this antinutrient.

Apparent digestibility of dry matter (ADM) and apparent digestibility protein (ADP) are two parameters indicative of the amount of dry matter and protein that are digested and absorbed by organisms (Bowzer et al., 2015). Therefore, it is very important to use highly digestible ingredients in the feed of cultivable aquaculture species (Valdez-González et al., 2016).

The high digestibility coefficients obtained in this study could be related to the elimination of tannins during dehulling and to the high temperatures of the extrusion process which also inactivate enzyme inhibitors and denaturalize the proteins, making them more vulnerable to enzymatic action (Pastor-Cavada et al., 2012).

The apparent digestibility of dry and protein matter of the tested ingredients depends on the type of ingredient. The differences in ADM can be explained by the processing of dietary ingredients (Köprücü and Özdemir, 2005).

Nile tilapia can digest various foods (Davies et al., 2011). The low digestibility of canola meal in tilapia could be a consequence of the presence of large amounts of fiber and anti-nutritional factors in these products (Yigit et al. 2011). In our study, the extrusion and dehulling of Jackfruit seeds improved the digestibility of dry matter and protein, probably as a consequence of the reduction in the content of antinutritional factors and fiber. Therefore, it can be affirmed that the dehulling process improves the indices of digestibility and nutrimental values of grains and seeds (Booth et al., 2001).

Several reports mention that fiber levels lower than 3% improve protein digestibility in Nile tilapia (Lanna et al., 2004). In this study, dehulling caused a reduction in fiber levels below 3%, which allowed the ADP to increase. However, despite being a simple and inexpensive processing method that improves some nutritional aspects of plant sources, it is not effective in reducing other antinutritional factors present in the ingredients (Nikmaram et al., 2017).

The results obtained in this study coincide with those reported by Cheng and Hardy (2003) which showed that the extrusion process significantly improved the coefficients of dry matter and protein in diets for rainbow trout *Oncorhynchus mykiss* based on plant sources.

These results can be explained by those reported by other authors where trypsin inhibitors are considered to be the main protease inhibitors found mainly in raw seeds. These substances decrease protein digestion and inhibit growth by inactivating trypsin (Nwabueze et al., 2007). However, it has been demonstrated that extrusion temperatures of 140° C cause the inactivation of this antinutrient in wheat and rice (Kaur et al., 2014). Similar temperatures and conditions were used in this study. In this way, the impact of extrusion on plant sources is beneficial (Nikmaram et al., 2017).

The results obtained in this study showed that the elimination of the hull and the extrusion process decrease the antinutrient content and increase the dry matter and protein digestibility coefficients in *Oreochromis niloticus* tilapia of Jackfruit seeds.

Conclusion

Dehulling and extrusion are low-cost and efficient technological processes that allow improving the nutritional use of plant sources. For this reason, extruded and dehulled Jackfruit flours represent a potential alternative to replace fishmeal in the preparation of food for tilapia *Oreochromis niloticus* because the seeds are inexpensive.

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