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Effect of Jackfruit (*Artocarpus heterophyllus*) Seed Processing on the Diets of Nile Tilapia (*Oreochromis niloticus*): Growth, Antinutrients, and Blood Parameters

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ABSTRACT

Background: Jackfruit seeds have been studied in the pharmaceutical industry, one way of exploiting the potential of this ingredient could be as a protein source in the elaboration of fish food. Jackfruit seeds were subjected to different processes to obtain meals to be added to the diet of Nile tilapia (*Oreochromis niloticus*). The effects of this addition on growth, elimination of antinutritional factors, and repercussion on the health of tilapia was evaluated.

Method: For the bioassay of the productive yield, three experimental diets were prepared, consisting of a control diet of fishmeal and four treatments: raw jackfruit seeds (RJS), hulled jackfruit seeds (HJS), extruded jackfruit seeds (EJS) and hulled and extruded jackfruit seeds (HEJS). For the growth study, 1600-L experimental units were used. Three replicates per treatment were used, initial weight of tilapias was of 1.8±0.2 g. At the end of the feeding study, a blood sample was taken from the caudal vein, using a hypodermic syringe and EDTA as anticoagulant for the hematology (hemoglobin/hematocrit).

Results: Significant differences among treatments using jackfruit seeds, subjected to hulling and extrusion (CEJS) processes, were observed in weight gain (24.3±1.1 g), whereas blood parameters, the red blood cells (RBC) count and the hematocrit (Hct) were significantly higher in the jackfruit seeds subjected to only one treatment (39.8±6.49) respect to the control diet (27.4±3.18). Regarding the MCH and MCHC variables, lower values were observed with the diets using EDJS, showing a significant diminution in the RBC concentration (13.2±2.5) compared to the control diet (19.3±4.65). The processes used for the jackfruit seeds allowed formulating diets with a higher protein quality, which resulted in an increment in weight gain, without observing any effect on the health indicators and nutritional status of fish.

Key words: Blood parameters, Growth, Jackfruit seeds, Processing, Tilapia.

INTRODUCTION

According to FAO, aquaculture is the animal-derived food productive sector experiencing the highest growth (FAO, 2022). In 2018, worldwide aquaculture production reached a historical record of 114,5 million tons, equivalent to 263,600 million US-dollars (FAO, 2020). Fish farming is the most important activity in many countries, providing almost half of all the fish destined to human consumption as a protein source (Deng *et al.,* 2015). Tilapia is a freshwater fish cultivated in many tropical and subtropical countries because of its fast growth, meat quality, tolerance to a wide range of environmental conditions and acceptance of artificial foods immediately after the absorption of the vitelline sac (El. Sayed, 2019).

The Nile tilapia (*Oreochromis niloticus*) is of high value, placed as one of the most important aquaculture products worldwide and it is the second species of the highest productive interest in tropical aquaculture (Deng *et al.,* 2015). Different physiological attributes have allowed for the success of this species; they are organisms that tolerate a variety of environmental conditions and can adapt to wide salinity ranges, standing out their resistance, feeding habits and adaptation to diverse conditions in captivity (Van Doan *et al.,* 2019; Magouz *et al.,* 2020). They present high survival indices, fast growth and their filets are of high protein quality for human consumption (Khalifa *et al.,* 2016). However, as

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aquaculture activities have increased, relevant needs have arisen that limit the profitability of this activity; among these are those concerning their diet (Collins *et al.,* 2012), which represents up to 70% of the total costs of production in intensive and super-intensive aquaculture (Esmaeili *et al.,* 2016). A very high percentage of this cost corresponds to the cost of fish meal, a fundamental constituent in the formulations of balanced foods for any aquatic species (Kandathil *et al.,* 2020; Kaushik and Troell, 2010; Al-Thobaitib *et al.,* 2018).

From the nutritional point of view, fish meals have a high protein content and appropriate profiles of amino acids and unsaturated fatty acids, it is highly palatable and digestible (El-Saidy and Saad, 2011; Hassaan *et al*., 2016). These characteristics explain their predominance with respect to other protein sources in aquaculture diets. Notwithstanding, their cost has increased and their availability has been limited in recent years (Bowzer *et al.,* 2015; Simon *et al.,* 2019).

For these reasons, it became interesting to consider replacing partially or totally fish meals with less costly protein sources, more available and fulfilling the nutritional requirements, without affecting the productive performance of the species (Al-Thobait *et al.,* 2018). The latter has led to diverse investigations and the search for new protein alternatives for the formulation of tilapia diets. Hence, the most viable option to replace the fish meal is the use of vegetable proteins, given that their production is only limited by the availability of land and can be extended to the whole year if environmental conditions allow it (Cruz *et al.,* 2018; Magouz *et al.,* 2020; Hodar *et al.,* 2020); besides, these vegetable proteins are more abundant and less costly (Shamna *et al.*, 2015).

Based on the aforementioned, many studies have been performed using different vegetable sources. Among the raw materials analyzed in diets for tilapia is soybean derivates (Vidal *et al*., 2017), cotton seeds (El-Saidy and Saad, 2011), canola paste (Plaipetch and Yakupitiyage, 2013) jatropha seeds (Shamna *et al*., 2015), rubber seeds (Deng *et al*., 2015), corn protein concentrate (Khalifa *et al*., 2016), among others. Another possible source of alternative protein could be jackfruit (*Artocarpus heterophyllus*) seeds.

The jackfruit (*A. heterophyllus*) is a plant from the Moracea family, native to India and, currently, its cultivation has extended to diverse tropical zones of the world (Canto-Herrera, 2015). In Mexico, jackfruit is produced in approximately 1500 ha of land and the state of Nayarit represents 91% of the production (SIAP, 2021). The pulp is the eatable portion of this fruit, whereas its seeds are considered an agricultural by-product representing 8 to 16% of the total weight of the fruit (Baliga *et al*., 2011). Seeds of *A. heterophyllus* contain 120 g/kg of starch and from 170 to 220 g/kg of crude protein (Madrigal-Aldana *et al.,* 2011).

Jackfruit seeds have been studied in the pharmaceutical industry (Omale and Friday, 2010); one way of exploiting the potential of this ingredient could be as a protein source in the elaboration of fish food. However, the nutritional richness of this ingredient is affected by the presence of antinutrients (Swami *et al.,* 2012). Antinutrients are compounds or natural substances found in vegetable sources, which, when ingested crude or without any processing, delay or inhibit the catalytic action of digestive enzymes, produce low palatability, reduce the digestibility coefficients, induce adverse effects on growth and retain certain nutrients (Francis *et al*., 2001; Phumee *et al.,* 2010; Nikmaram *et al.,* 2017). However, several processes exist that can improve the nutritional leverage of vegetal sources, among them are hulling and extrusion (Milán-Carrillo *et al*., 2002; Pastor-Cavada *et al*., 2011; Valdez-González *et al.,* 2017). The present work is aimed at evaluating different processes of jackfruit (*A. heterophyllus*) seeds to be used as feeding ingredient for tilapia (*O. niloticus*) and their effect on growth, elimination of antinutritional factors and influence on the tilapia health.

MATERIALS AND METHODS

This research work was carried out at the National School of Fisheries Engineering, Bahía Matanchén, San Blas, Nayarit, Mexico. In summer of 2021.

Procurement of raw materials

Seeds from jackfruit (*A. heterophyllus*) in their natural form, hulled and extruded were used. Seed meal from the hulled fruit was prepared by grinding the seeds in an electric grinder of 0.5 hp (Molino del Rey®, Mexico) until obtaining four fragments per seed. Thereafter, the hull fragments were removed with an electric ventilator and the seed fragments were ground until obtaining a #80 (0.180 mm)-mesh meal.

Extruded meals

Extrusion of seeds was performed according to Milán-Carrillo *et al*. (2002). A temperature of 164 °C and screw velocity of 188 rpm were used. The extruded seeds were dried, ground and sieved through a #80 (0.180 mm)-mesh to obtain the extruded meal. This process was performed in a single screw extruder Mod. 20DN (CW Brabender Instruments, Inc, South Hackensack, NJ, USA).

Chemical composition

Chemical analysis of the ingredients, diets and feces were performed according to standard methods by AOAC (1999). 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 $600^{\frac{238}{92}}C$ for 5 hours and the energy content was determined by an adiabatic calorimeter (Table 2).

Antinutrients

Phytic acid

Was determined following the procedure of Latta and 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 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°C, 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

The 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. The solution was filtered on Whatman paper # 2 and the absorbance was 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 were expressed as ICU/mg of sample.

Elaboration of diets

Five diets were prepared, one control diet based on fish meal and four experimental treatments: whole jackfruit seeds (WJS), hulled jackfruit seed (HJS), extruded jackfruit seeds (EJS) and hulled and extruded jackfruit seeds (HEJS). The diets used in the growth bioassay were formulated at 30% protein 10% lipids. In the experimental diets, 47% of fish meal was replaced, compared to the control diet. Ingredients were milled until passing a #40 (0.425 mm) mesh. Thereafter, ingredients were mixed and homogenized, food was prepared in a Torrey® meat grinder (Monterrey, NL, Mexico).

Experimental design of the growth bioassay

For the growth study, 1600-L capacity experimental units were used, with three replicates and experimental organism's weight of 1.8±0.2 g. Each experimental unit was provided with continuous aeration, an oxygen level of 5±0.5 mg/L and a temperature of 28±3°C. Biometric measures were performed every 120 days to determine the weight in grams of all organisms of each unit. At the beginning of the growth assay, organisms were fed at a rate of 6% of their total biomass. Growth assays lasted 65 days. Afterward, according to the biomass calculated for each experimental unit, the feeding ratios were applied per tank for each treatment.

Body weight (BW) and total length (TL) of the tilapia were measured every 10 days using a digital precision balance ± 0.01 g (Ohaus®, Parsippany, NJ, USA) and a caliper gauge (0.00 mm), respectively. The SGR was calculated using:

$$SGR = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Days of culture}}$$

After 60 days, all surviving organisms in each replicated tank were used to calculate food efficiency, somatic indices and to perform the blood analyses.

Food efficiency was calculated using the standard formula:

Weight gain (WG) = (Final weight - Initial weight)

Food conversion rate (FCR) =

$$\frac{\text{Food consumption (g in dry weight)}}{\text{Body weight gain (g, wet weight)}}$$

Food efficiency rate (FER) =

$$\frac{WG}{\text{Food consumption (g in dry weight)}} \times 100$$

Protein efficiency rate (PER) =

$$\frac{\text{Net weight gain (g, wet weight base)}}{\text{Fed protein (g, dry matter base)}}$$

Twenty fish per diet were analyzed to determine the hepatosomatic (HSI) and intestinal somatic (ISI) indices using the following formulas:

$$HSI\ (\%) = \frac{\text{Liver wet weight (g)}}{\text{Fish wet weight (g)}} \times 100$$

$$ISI\ (\%) = \frac{\text{Intestine wet weight (g)}}{\text{Fish wet weight (g)}} \times 100$$

Hematological parameters

At the end of the feeding test, fish were fasted for 24 h immediately before blood sampling. A blood sample was taken with a hypodermic syringe from the caudal vein. Each syringe contained 0.5 mL EDTA, used as anticoagulant for hematology determinations (hemoglobin/hematocrit). Hemoglobin (Hb) was determined colorimetrically, measuring the formation of cyanmethemoglobin according to Van Kampen and Zijlstra (1961). Hematocrit (Hct) values were determined immediately after the sampling, placing the fresh blood in glass capillary tubes and centrifuging for 5 min in a microhematocrit centrifuge.

Hematological indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH] and mean corpuscular hemoglobin concentration [MCHC]) were calculated through conventional formulas:

$$MCV = Hct \times 10/CSR \times 106/mm^3$$

$$MCH = Hb \times 100/CSR \times 106/mm^3$$

$$MCHC\ \%) = Hb \times 100/CSR \times 106/mm^3$$

Total content of proteins was determined colorimetrically according to (Henry,1964).

Statistical analysis

Obtained values were analyzed with a normality and homogeneity test. To establish statistically significant differences, the STATISTICA 7.0 (StatSoft, Tulsa, OK, USA) was used and data were subjected to a one-way variance analysis (ANOVA, $\alpha < 0.05$) (Sokal and Rohlf, 1981). The Tukey multiple comparison test was used to classify treatments.

RESULTS AND DISCUSSION

Table 1 depicts the proximal chemical composition of the processed and non-processed jackfruit seed's ingredients. The main effect of extrusion on the chemical composition was a significant diminution ($P \leq 0.05$) in ashes (2.9 g kg^{-1}). Whereas the proximal chemical composition of the experimental diets elaborated with jackfruit seed meal is depicted on Table 2. The diets used in the growth bioassay were formulated at a ratio of 30% protein and 7% lipids. The use of vegetal sources as less costly alternatives and more easily available products to replace fish meal in the diets used in aquaculture is becoming a common practice in this industry (Brinker and Reiter, 2011). In the last years, many studies have been performed in which the use of these alternative sources has been assessed and found to not exert a negative effect on the organisms performance (Olude *et al*., 2016; Al-Thobaiti *et al.,* 2018; Meng *et al.*, 2020; Ergenton *et al.,* 2020). The development of diets where fish meal can be replaced either partially or completely by other vegetal protein sources will allow sustaining aquaculture in the next generations, yielding adequate and less costly vegetal protein alternatives without affecting the reproductive performance of animals (Alhazzaa *et al.,* 2019).

Productive performance, like growth, in one of the main factors to be considered in aquaculture; several authors have mentioned that different variables affect the growth of the Nile tilapia, such as protein requirements, feeding rate and water temperature, among others (Yue and Zhou, 2008; Hernández *et al.,* 2010; Akinleye *et al.,* 2012). Hua (2019) mentions that it is necessary to consider the quality of proteins, the energy content and the digestibility of

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

Nutrients	FM	WJS	DJS	EWJA	EDJS
Protein	65.5±0.06	17.6±0.4b	18.77±0.4a	17.45±0.2b	18.45±0.3a
Lipids	12.17±0.04	0.4±0.2a	0.53±0.3a	0.35±0.3a	0.43±0.2a
Ash	16.73±0.03	6.1±0.3a	2.4±0.2b	5.9±0.5a	2.9±0.4b
Fiber	0.03±0.01	5.87±0.08a	0.45±0.05b	5.67±0.06a	0.67±0.03b
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.

ingredients. Including adequate processes like hulling and extrusion allow obtaining products with better nutritional and sensorial properties for the designed diets; thereby, warranting a protein of high biological value (Milan Carrillo *et al*., 2000) with an adequate availability of essential amino acids, fatty acids and high digestibility (Gasco *et al.,* 2020; Salh, 2020; Weththasinghe *et al.,* 2021).

Some processes were evaluated in the present investigation such as the analysis of tannins, saponins, trypsin inhibitors and phytic acid contents of the jackfruit seeds subjected to hulling and extruding (HEJS), Table 3 depicts a significant diminution (P≤0.5) in tannins and saponins (12.9% and 3.7%, respectively) as well as in trypsin inhibitors and phytic acid [P≤0.5; 1020 (UIT/g) and 168.7 (mg/g), respectively]. Hulling (HJS) induced a significant effect (P≤0.5) on trypsin inhibitors and phytic acid [956.1 (UIT/g) and 173.9 (mg/g), respectively] compared to raw jackfruit seeds. Antinutrients impact the digestive system and affect other metabolic systems in the body (Li *et al*., 2023). In the present study, the hulling and extrusion process decreased the content of trypsin and phytic acid inhibitors; these enzymatic inhibitors influence the bioaccessibility and bioavailability of nutritional and functional phytochemical components (Biswas *et al*., 2022). Kaur *et al*. (2014), report that extrusion temperatures of 140°C cause the inactivation of protease inhibitors in rice and wheat. Nikmaram *et al*. (2017), mention that the high temperatures used in the extrusion process reduce the content of thermolabile substances.

Our results indicate that the proximal chemical composition of the tested ingredients were determinant for the productive variables like final weight gain (FWG) and protein efficiency rate (PER). Results of the growth bioassays, performed during 65 days, are shown on Table 4. The diets with hulled and extruded jackfruit seeds (HEJS) showed significant differences (P≤0.5) regarding more weight gain as compared to the other treatments. The diet with the HJS showed the least weight gain and was significantly different (P≤0.05), with respect to the other diets. Enzyme inhibitors cause a reduction in protein digestion, growth and survival of some fish species (Asare *et al*., 2022). Phytic acid causes chelation of minerals and proteins, altering the digestion and absorption of essential nutrients. There fore, it limits the nutritional value and quality of plant sources (Chen and Xu, 2023). The decrease enzyme inhibitors and phytic acid in the diets, allowed an increase in productive performance compared to the other treatments. Likewise, we confirmed that both processes, hulling and extrusion, impact positively the nutritional performance of vegetal sources, as shown also by (Milán-Carrillo *et al*., 2002). On the one side, hulling allows removing the fibrous envelopes, the glucosinolates, the phytic acid, the phenolic compounds and the oligosaccharides found in the hulls, thereby, increasing the protein proportion (Carré, 2021). Likewise, hulling allows eliminating tannins, phytates and enzyme inhibitors as described by (Nikmaram *et al*., 2017). Another study coinciding with the previous one is that of Li *et al.* (2020), who demonstrated that the use of hulled soymeal in diets for the largemouth bass (*Micropterus salmoides*) leads to significant improvements in growth variables. Shao *et al.* (2021) assessed the effects of the levels of dietary fiber on growth and the digestive and absorptive abilities in the grass carp (*Ctenopharyngodon idella*), observing that high levels of dietary fiber in the

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

Nutrients	Diets				
	Control 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^{-1}).

Table 3: 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±6.38[d]	8.9±1.7[d]	2494±135[b]	187.6±53.2[b]
DJS	26.5±7.52[b]	5.2±1.2[b]	956.1±158[a]	173.9±52.6[a]
EWJS	54.7±5.74[c]	6.4±1.1[c]	2466±126[b]	185.4±55.4[b]
EDJS	12.9±3.76[a]	3.7±0.8[a]	1020±141[a]	168.7±45.4[a]

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/100 g= mg equivalents of catechin g sample. [2]mg equivalents of diosgenin/g sample. [3]Units of inhibited trypsin/g of sample.

designed diets did not favor the digestion and absorption of nutrients, leading to a diminution in growth performance. Thus, hulling is a simple method to improve the nutritional quality of fish diets based on vegetal proteins, demonstrating a better ingestion of grains and seeds, in turn, improving the growth and digestibility indices (Bandara, 2018). On the other side, the extrusion process used in the present work induced positive effects on the nutritional composition of the tested ingredients and the effect on growth with the designed diets; the extrusion process is an alternative used to improve the nutritional quality of ingredients and reduce the undesirable compounds of vegetal-origin foods (Simawan *et al.,* 2023). These effects are directly reflected in the extruded treatments when compared with the non-extruded jackfruit seeds (Table 5). The present study agrees with that reported by Salh and Jaza (2020), who performed growth assays with extruded barley and soybean meals in the rainbow trout *(Oncorhynchus mykiss)* and found that up to 20% can be included in the diet without any significant effect on growth and health of the organisms. Another study by Barrieto-Curiel *et al.* (2018) evaluated the effect of the extrusion process in aquaculture feed for totoaba (*Totoaba macdonaldi*) juveniles on the productive yield, observing that the extrusion process improved clearly protein efficiency index and demonstrating the advantage of using the extrusion technology. Vidal *et al.* (2017) stated that the extrusion process improves significantly the apparent and dry matter digestibility coefficients, raw energy and essential and non-essential amino acids of the wheat-based diet in the Nile tilapia. Flora *et al.* (2023) demonstrated that feeding the Nile tilapia with diets containing extruded jatropha leads to final weight gain. Also, Meng *et al.* (2020), in a similar study, demonstrated that the extrusion process improves the nutritional values for *Salvelinus malma,* being able to substitute up to 50% of extruded soybean without affecting growth of organisms.

Blood parameters

Hematological parameters are useful indicators of the health and nutritional status of fish (Nakharuthai *et al.,* 2020). Table 5 shows the effect of the experimental diets on the blood parameters of the Nile tilapia. No significant differences ($P \leq 0.05$) were observed among the values obtained for the variables *RBC, WBC, Hb, MCV,* whereas total albumin, globulin and protein concentrations showed no significant differences ($P \leq 0.05$) among treatments. Regarding mean corpuscular hemoglobin (MCH and MCHC) values, these were lower in the diet supplemented with hulled and extruded jackfruit seeds (HEJS) with significant differences ($P \leq 0.05$), revealing a diminution of red blood cells (13.2±2.5) compared to the control diet (19.3±4.65). Values like hemoglobin, hematocrit and differential leukocyte count and blood biochemical tests can be used to monitor physiological conditions of fish and diagnose pathological states and stress situations in all species of aquaculture interest, because they are fast indicators of physiological or environmental alterations (Fazio, 2019). In this study, the red blood count (RBC) and white blood count (WBC), the

Table 4: Growth parameters, feed efficiency, and somatic indexes of tilapia (*Oreochromis niloticus*) fed diets containing jackfruit seeds (*Artocarpus heterophyllus*).

Diet	IBW (g)	FBW (g)	TL (cm)	SGR	WG (g)	FCR	FER	PER	HSI	ISI
Control	1.8±0.2^{a}	23.2±3.7^{ab}	11.7±1.4^{a}	5.0±0.2^{a}	107.2±17.9^{a}	1.7±0.1^{a}	58.9±4.6^{a}	1.7±0.1^{ab}	2.9±0.7^{a}	5.5±0.3^{a}
WJS	2.1±0.1^{a}	20.5±3.9^{bc}	11.5±0.8^{a}	4.3±0.4^{a}	91.9±19.4^{a}	1.9±0.1^{a}	52.2±3.6^{a}	1.5±0.1^{c}	2.4±0.4^{a}	5.1±1.5^{a}
DJS	1.9±0.2^{a}	22.2±2.2^{b}	11.7±0.2^{a}	4.6±0.2^{a}	89.7±10.5^{a}	1.9±0.1^{a}	51.8±3.4^{a}	1.7±0.1^{ab}	2.1±0.4^{a}	4.4±0.6^{a}
EWJA	1.8±0.1^{a}	20.3±4.0^{bc}	12.4±1.1^{a}	4.8±03^{a}	92.3±19.7^{a}	1.6±0.0^{a}	61.2±0.8^{a}	1.9±0.0^{a}	2.4±0.2^{a}	5.4±0.5^{a}
EDJS	1.9±0.3^{a}	24.3±1.1^{a}	12.7±0.3^{a}	4.8±0.4^{a}	111.6±5.6^{a}	1.8±0.2^{a}	57.2±6.7^{a}	1.9±0.2^{a}	2.1±0.1^{a}	4.6±0.5^{a}

IBW- Initial body weight; FBW- Final body weight; TL- Total length; SGR- Specific growth rate; WG- Weight gain; FCR- Feed conversion ratio; FER- Feed efficiency ratio; PER- Protein efficiency ratio; HIS- Hepatosomatic index; ISI- Intestinal somatic index. Means in the same column with different letters are significantly different.

Table 5: Effects of experimental diets on the blood parameters [RBC (10^{6} cells/mm^{3}), WBC (10^{3} cells/mm), Hb (g/dl), Hct (%), MCV (fL), MCH (pg), MCHC (g/dl)] of tilapia (*Oreochromis niloticus*).

Diet	RBC	WBC	Hb	Hct	MCV	MCH	MCHC	Albumin	Globulin	Total protein
Control	1.2±0.9^{a}	30.7± 1.47^{a}	5.0±0.03^{a}	27.4±8.02^{b}	224.5±59^{a}	41.3±3.18^{a}	19.3±4.65^{a}	1.6±0.48^{a}	2.2±0.27^{a}	3.8±0.54^{a}
WJS	1.4±0.87^{a}	32.5±1.95^{a}	5.0±0.09^{a}	38.8±6.50^{a}	212.3±55^{a}	35.8±2.38^{b}	17.7±4.40^{ab}	1.6±0.42^{a}	2.3±0.16^{a}	3.9±0.52^{a}
DJS	1.5±0.91^{a}	32.7±1.67^{a}	5.0±0.09^{a}	39.2±5.59^{a}	267.8±27^{a}	34.7±2.70^{b}	13.1±2.43^{b}	1.4±0.16^{a}	2.5±0.23^{a}	3.9±0.21^{a}
EWJA	1.5±0.76^{a}	33.5±1.84^{a}	5.0±0.08^{a}	39.8±6.49^{a}	213.2±54^{a}	36.8±1.27^{b}	18.7±3.30^{ab}	1.7±0.31^{a}	2.4±0.24^{a}	3.8±0.41^{a}
EDJS	1.4±0.80^{a}	33.6±1.58^{a}	5.0±0.07^{a}	39.1±5.48^{a}	267.6±28^{a}	34.6±2.71^{b}	13.2±2.52^{b}	1.4±0.15^{a}	2.5±0.33^{a}	3.8±0.32^{a}

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

Values are mean (n= 5) ± standard deviation.

MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC. mean corpuscular haemoglobin concentration.

Hb content and the MCV did not differ significantly among the five assessed treatments. Variables like albumin, globulins and total proteins did not show significant variations. The Hct values were higher in the groups fed the vegetal protein; it has been described that the Hct is related with the activity and habitat of fish, indicating that its values are higher in freshwater fish than in marine fish, the latter presenting a higher amount of red blood cells (Alaye-Rahy and Morales-Palacios, 2013). This increase in the number of erythrocytes would improve the gas exchange because of a greater surface/volume relation, improving the transport of the water-dissolved oxygen (Bosisio *et al.,* 2017; Elarabany *et al.,* 2017). In this work the Hct values were higher in the organisms fed the vegetal diet, showing a mean of 39.8±6.49. The obtained data suggest that the globular volume or hematocrit is independent from the growth stage of fish and is rather related with the amount and type of red blood cells and is, therefore, a good indicator of the health status of fish (Ayale- Rahy and Morales- Palacios, 2013). The results of the MCH assessment are similar to those obtained by (Akinleye *et al.,* 2012; Lourenco *et al.,* 2014), which oscillate between 34 and 51 pg in the Nile tilapia. The obtained mean corpuscular hemoglobin (MCH) values indicate that the experimental diets tested in this study did nor induce anemia or malnutrition in the Nile tilapia. No significant differences were observed in the mean corpuscular hemoglobin concentration (MCHC) values obtained in this study. The values oscillated between (21.22 and 29.85 g/dL. Protein values ranged between 4.30% and 5.32%. Similar results were reported by Abdel-Tawwab *et al.* (2010) in Nile tilapia. Mohamed *et al.* (2021) determined the influence exerted by the percentages of protein on the performance and health status of Nile tilapia and found an increase in the total protein when evaluating the effects of the extract of essential dietary oils from the sweet orange (*Citrus sinensis*) and lemon (*Citrus limon*) peels.

CONCLUSION

Hulling and extrusion are processes technological and efficient low-cost that increase the nutritional quality of plant sources, decrease the content of antinutrients and do not cause a negative effect on the hematological parameters of tilapia. This study shows that 47% of fish meal can be replaced by extruded and dehulled jackfruit meal without affecting the productive performance of tilapia. Extruded and hulling Jackfruit meals represent a potential alternative to replace fish meal in the preparation of feed for tilapia *Oreochromis niloticus*, because the seeds are inexpensive.

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