Effect of extrusion in the elaboration of an animal feed based on *Moringa oleifera Lam* and *Zea mays* as a partial substitute of fishmeal in the diet of the adult stage of *Oreochromis niloticus*

Efecto de la extrusión en la elaboración de un pienso a base de *Moringa oleifera Lam* y *Zea mays* como un sustituto parcial de la harina de pescado en la dieta de la etapa adulta de *Oreochromis niloticus*

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Abstract

The rising demand for fishmeal, as well as the search for sustainable active food, has led to the development of food-based on by-products and the use of vegetable flours, leading to the elimination of poorly consumed and poorly assimilated food from aquaculture. These are the main economic, nutritional and environmental problems in the intensive cultivation of Nile tilapia (*Oreochromis niloticus*). The amount of fish protein in fish diets is above nutritional requirements. And tilapia does not ingest the food that does not float, leading to over-rationing. This study explores the effect of extrusion on the elaboration of a feed based on Moringa leaf flour (*Moringa oleifera* Lam) and *Zea mays* as a partial substitute of fishmeal in the diet of the adult stage of Oreochromis niloticus. The analysis revealed that the combination provides desirable physical properties for the feeding of the fish, and their optimal growth of tilapia because they present a content ideal of protein for the adult stage and characteristics such as the velocity of sinking, density, and hardness.

Keywords: Fishmeal; *Moringa oleifera* Lam; *Oreochromis niloticus*; *Zea mays*.

Resumen

La creciente demanda de harina de pescado, así como la búsqueda de alimentos sostenibles, ha llevado al desarrollo de alimentos basados en subproductos y el uso de harinas vegetales, promoviendo la eliminación de alimentos poco asimilados y/o mal consumidos en la acuicultura. Estas son las principales fuentes de los problemas económicos, nutricionales y ambientales en el cultivo intensivo de tilapia del Nilo (*Oreochromis niloticus*). La cantidad de proteína procedente de la harina de pescado en la dieta de los peces está por encima de los requisitos nutricionales y la tilapia no consume los alimentos que no flotan, lo que provoca un exceso de racionamiento. Este estudio explora el efecto de la extrusión en la elaboración de un pienso a base de harina de hoja de Moringa (*Moringa oleifera* Lam) y *Zea mays* como un sustituto parcial de la harina de pescado en la dieta de la etapa adulta de *Oreochromis niloticus*. El análisis reveló que la dieta propuesta proporciona propiedades físicas deseables para la alimentación de los peces y su crecimiento óptimo de tilapia, debido a que presentan un contenido ideal de proteína para la etapa adulta y características como la velocidad de hundimiento, densidad y dureza.

Palabras clave: Harina de pescado; *Moringa oleifera* Lam; *Oreochromis niloticus*; *Zea mays*.

Introduction

The ability for easy reproduction and low cost of maintenance of the Nile tilapia (*Oreochromis niloticus*) have made this species of white meal an important choice for aquaculture production (El-Sayed, 2006; Food and Agriculture Organization of the United Nations [FAO], 2016). Consequently, the exact cover of dietary nutrients, as proteins, lipids, carbohydrates, vitamins, and minerals, is critical to gain the right weigh in any growth stage. In this way, if the requirement is uncovered, the effect will be observed in the diminution of growth rates and chemical composition of fish meat (Ayisi, Zhao & Rupia, 2017). The nutritional requirement of Nile tilapia for growth, at all stages, has been distinguished and divided into four (larvae, fingerlings, juveniles, and adults) (FAO, 2017; Thongrod, 2007). The specificity of the nutritional requirements makes two stages critical, the larvae and adult stage due to the necessary amount and quality of proteins and carbohydrates (Silva-Soares, Nogueira-Alves, Beldade & Mirth, 2017).

The current commercial foods diets are based on the requirements of protein on the use of fishmeal so that it can be an economic source. This ingredient maintains a fluctuating and rising cost year after year because of its increasing request (FAO, 2016); additionally, fishmeal protein does not have a digestibility coefficient of 100% (El-Sayed, 2006), becoming the primary contaminant of discharge waters in the aquaculture industry (Draganovic, van der Goot, Boom & Jonkers, 2011; Schwitzguébel & Wang, 2007), where it is poorly assimilated by the fish. Moreover, the tilapia eats only on the surface of the bodies of water or at a medium altitude, this compromises the efficient utilization of the feed and leads to a need of well-designed feeds that float at the middle bottom or surface for more than two minutes (El-Sayed, 2006). Feeds that do not float is not consumed and becomes pollution and a waste of money (Schwitzguébel & Wang, 2007; Thongrod, 2007). Recently, the research and development of products have sought to satisfy the nutrition requirements, with a controlled and selective use of fishmeal and efficient designs of products (*i.e.* extruded pellets), because the nutrition in the intensive aquaculture industry is the most expensive component; it represents between 50%-80% of operating cost (El-Sayed, 2006).

Some nutritional designs have focused on the substitution of fishmeal, by fishery by-products, terrestrial animal by-products, single-cell proteins or vegetable protein sources. With greater sustainability, the utilization of vegetable flour is proposed, it is also more difficult to standardize due to the presence of several antinutrients (Ayisi *et al.*, 2017; El-Sayed, 2006). These substitutions have been documented in the diet of *O. niloticus, O. mossambicus, T. guineensis, T. rendalli,* and *O. aureus*, with promising results in the use of seaweed (Pereira, Valente, Sousa-Pinto & Rema, 2012), a mixture like soybean with corn (Hassaan, Soltan & Abdel-Moez, 2015; Herath, Haga & Satoh, 2016), beans (Azaza *et al.*, 2009), and also, recently, jojoba has been tested (Labib, Zaki, & Mabrouk, 2012). However, these studies propose substitutions that result deficient in the content of essential amino acids, needing a step up in the content of lysine, methionine, and tryptophan. Even if the content of vegetable protein is in little amounts, during the substitution, it naturally contains anti-nutrients and is related to adverse effects on growth. Whether they are reinforcing a new diet or supplementing a commercial food or starting from a fermented mixture, a proper heat treatment is indispensable (Carter & Hauler, 2000; Davis, 2015).

Under this idea, the processing of the feed has to be ideal for nutrition and pollution control (feed well consumed and well assimilated), this lead two manufacture possibilities: the formation of submersible pellets or floating pellets. The best alternative is the preparation of floating extruded pellets, because among its advantages is to improve a better digestibility and stability of its components, a complete gelatinization of starches. Besides, many anti-nutrient factors are eliminated with the heat treatment. Also, the feed does not disintegrate nor diffuse, allowing the water of the pond to remain transparent, and the fish can be observed feeding, despite the operating cost that this could represent (Davis, 2015; De Cruz, Kamarudin, Saad & Ramesani-Fard, 2015). However, the presence of vegetable flours, such as leaves, fruits or vegetables in mixtures for extrusion, can hinder the process, due to the presence of sugar, fiber or protein can complicate the transformation to the extrusion (Liu, Alavi & Abughoush, 2011).

The aim of this study is the generation of an extruded pellet to feed the Nile tilapia with the partial replacement of fishmeal through the utilization of vegetable flours as corn and moringa leaves, flours previously described in their amino acid content (Richter, Siddhuraju & Becker, 2003). Also, corn is the best source of starch in the extrusion process (Liu *et al.*, 2011;

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Richter *et al.*, 2003). Moringa leaves and corn, together, present an amino acid content that can cover the requirements of the Tilapia, even more than other vegetable flours reported.

Materials and methods

Preparation of samples

Proximate chemical composition

The official methods (Association of Official Analytical Chemists [AOAC], 1990) were used to determine the proximate composition of all ingredients (Yellow corn grown in Sinaloa-México, sardine fish meal provided by a fishmeal merchant, moringa grown in Sinaloa-México, starch and unflavored gelatin): moisture (method 925.09) and in the extruded product, ashes (method 923.03), lipids (method 923.05), protein (method 979.09) and fiber (method 962.09). Carbohydrate content was obtained by subtraction, 100 % (crude protein% + crude fat% + crude fiber% + ash %). All measurements were performed in triplicate, and the results were expressed in percentage (%) on dry weight. The chemical analysis of ingredients as moringa, corn, fish meal, starch, and unflavored gelatin is presented in %, with means of three repetitions in table 1.

Table 1. Chemical composition of the ingredients. Data on mean values with their standard deviation with 95% of confidence.

Component (%)	Ingredient				
	Moringa oleifera Lam. (Leaves)	Fish meal	Corn	Starch	Unflavored gelatin
Moisture	8.12±0.02	8.66±0.17	12.43±0.01	0.66±0.00	0.85±0.12
Crude protein (Nx6.25)	26.55±0.15	44.54±0.24	5.11±0.09	1.17±0.00	2.62±0.29
Crude lipid	8.57±0.03	12.60±0.29	0.16±0.01	2.12±0.53	1.85±0.03
Ash	10.53±0.02	20.50±0.15	0.51±0.02	0.35±0.15	1.35±0.19
Fiber	1.54±0.00	1.26±0.17	0.03±0.00	0.00±0.00	0.00±0.00
Nitrogen-free extract	60.93±0.01	29.76±0.07	94.19±0.07	96.36±0.37	94.18±0.63

Source: Author's own elaboration.

The mineral and vitamin premix composition were formulated by Aqua Florida Farms Inc. and are presented in table 2. The mineral and vitamin premix represent the necessary requirements for the development of *Oreochromis niloticus* at any stage. The premix dose is only adjusted according to the stage of development.

Table 2. Concentration of the minerals and vitamins present in the premix.

Mineral	Amount (ppm kg-1)	Vitamin	Amount (g kg^{-1})
Manganese	13.25	Vitamin A	0.2152
Zinc	11.04	Vitamin D3	0.0036
Iron	8.83	Vitamin E	64.5695
Copper	0.88	Vitamin K	1.752
Cobalt	0.11	Vitamin B12	0.0223
Iodine	8.83	Riboflavin	0.0072
Selenium	0.09	p-Pantothenic acid	34.4371
		Niacin	0.043
		Choline	5.7395
		Thiamine	5.7395
		Pyridoxine	5.7395
		Folic acid	1.7219
		Ascorbic acid	192.2737
		Biotin	0.0883

Source: Author's own elaboration.

Elaboration of feed by extrusion cooking

Blends were prepared by mixing yellow corn, fishmeal, and moringa leaf powder in the ratio of a dry-to-dry weight basis. The blends rations were chosen according to the preliminary test without clogging the extruder and for acceptable product characteristics like expansion, continuity of the extruded pellet, and structural stability after drying. From the preliminary extrusion tests, eight diets were generated in which the presence of fishmeal was sought to be reduced and to cover the nutritional requirements of *Oreochromis niloticus*, which is present in table 3 (where D diets with unflavored gelatin and Ds represent the diets that are not contained unflavored gelatin). The moisture content of the blended samples for extruder feed was adjusted at 30% (w.b) by mixing it continuously at medium speed with a calculated amount of water sprayed onto each blend. After that, the sample was put in a plastic bag and stored at 4 ºC all night to reach homogeneous moisture distribution. Before extrusion, the blends were allowed to come up to an ambient temperature of 25 ºC and was mixed again.

Table 3. Formulation of dietary diets for O. niloticus during the adult stage (g 100 g^{-1}).

Ingredient	D1	D2	D3	D4	Ds1	Ds2	Ds3	Ds4
Fishmeal	3.5	10.5	11.5	42.5	5	12	12	42
Moringa leaf meal	30.5	22.5	11.5	1.5	30	22	12	3
Corn	50	60	70	40	50	60	70	40
Starch	9	-	-	9	9	-	-	9
Unflavored gelatin	1	1	1	1	-	-	-	-
Vitamin Premix	3	3	3	3	3	3	3	3
Mineral Premix	3	3	3	3	3	3	3	3

Source: Author's own elaboration (D, Diet with unflavored gelatin; Ds, Diet without unflavored gelatin).

Extrusion conditions

Feeds were obtained by extrusion, cooking using a single-screw laboratory extruder model 20DN/8-235-00C (CW Brabender Instrument, Inc., Nj, USA), under the following conditions:

- Temperature of feed zone at 80 °C.

- Temperature of cooking zone at 100 °C.

- Temperature output die at 125 °C.

- S crew with a compression force 2:1.

- 0.3 mm exit die internal diameter.

- Screw speed of 100 rpm to 160 rpm.

Physicochemical characterization

Expansion Index (EI) and Bulk density (BD)

The EI was calculated according to Gujska & Khan (1990) by dividing extrudate diameter by exit die opening diameter. Bulk density was calculated according to Wang, Klopfenstein & Ponte (1993). Each determination was done ten times using randomly 10 samples of each product. Each extrudate was weighed (Pm), density was calculated using the following equation, where d = diameter and l = longitude. The results were expressed in g/cm^3.

$$Density = \frac{Pm}{\pi(d/2)^2 l} \tag{1}$$

Water absorption index (WAI) and water solubility index (WSI)

WAI and WSI of extrudates were determined using the technique developed (Anderson, Conway & Peplinski, 1970). One gram of ground product was sieved at 0.420 mm and dispersed in 10 ml of distilled water at 25 °C ± 1 °C. The resulting suspension was gently stirred for 30 min and then centrifuged at 3000x g for 15 minutes (Hermle Z 323K, Labnet, Woodbring, USA). The supernatant was decanted into a tared evaporating dish. The WAI is the weight of gel obtained after removing the supernatant per unit weight of original dry solids. WAI was calculated using:

$$WAI = \frac{(Weight\ gain\ in\ gel)}{(Dry\ weight\ of\ extrudate)} = \frac{gH_2O}{g} \tag{2}$$

The WSI was the weight of dry solids in the supernatant expressed as a percentage of original sample weight on a dry basis. All the determination was made in triplicate.

Sinking Velocity (SV)

Sinking velocity was measured according to (Himadri, Hattula, Myllymäki & Mälkki, 1993). It was measured by recording the time required for an extruded pellet to sink from water surface to the bottom of 425 mm in a test tube of 2000 ml.

Hardness (H)

Texture characteristics of selected extrudates were measured using a texture analyzer (Model TA-XTplus, Stable Micro Systems, Ltd., Surrey, UK). Hardness was determined by measuring the maximum force required to the rupture of the extrudate. Twenty randomly selected samples from each treatment were measured, and the values averaged. The results were expressed in Newton (N).

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectroscopy analyses were performed in all the raw blends and extruded products to qualitatively characterize the organic compounds of the solids by this technique transmission. The samples were prepared by grinding the diets and sieved

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at 0.420 mm. The FTIR spectrum of the powder complexes was measured at room temperature with 32 scans in the FTIR spectrometer (Agilent, Carry 630), in a spectral range of 4000 cm^{-1} to 650 cm^{-1}. The transmittance results of each diet in its raw state and processed as pellets were plotted with respect to the sweep wavelength.

Structural characterization

Viscosity analysis

The viscosity properties were measured in the Rapid Visco Analyser (RVA 4500, Perten Instruments, Australia). Samples were prepared with 3.24 g of powder and 25.26 g of distilled water; the measurements were performed with three repetitions each. The speed of the paddle was set at 160 rpm, then heated for five minutes from 50 ºC to 90 °C, for four minutes at 90 °C, and cooled at 50 °C in five minutes.

Scanning electron microscopy (SEM)

The raw blend and extrudate pellet were examined in a high vacuum scanning electron microscope using JEOL, JSM-6010LA, Akishima, Tokyo, Japan. Micrographs were taken at 3 kV and 60 magnifications,100 magnifications and 1000 magnifications.

Statistical analysis

The statistical analysis was performed for all data on the software Number Cruncher Statistical System (NCSS) version 7.1, using one-way Analysis of Variance (Anova) and the Tukey-Kramer test for multiple comparisons, all tests performed at 95% confidence.

Results

Proximate composition

The chemical-proximal analysis of raw materials allows to balance each component of the diets which is presented in table 1. The protein content of moringa leaf flour with 26% and fishmeal with 44% being relevant as well as the values in the ash content, being 10% moringa leaf flour and flour of fish of 20%. The nitrogen-free extract allowed to balance the amount of carbohydrates in each diet.

The proximal chemical analysis of the raw components and the processed diets is shown in table 4 to observe that the diets indicate a protein content of 14% to 25% for the feed of Nile tilapia in an adult stage, as an alternative for its composition, having substitutions of fishmeal for vegetable flour of up to 96.5%.

Table 4. Proximal chemical analysis of all the diets. Data on mean values with their standard deviation with 95% of confidence.

Diet	Component (%)					
	Moisture	Crude protein (Nx6.25)	Crude lipid	Ash	Fiber	Nitrogen-free extract
D1	5.33±0.00	14.37±0.00	5.48±0.01	7.67±0.11	0.57±0.01	71.91±0.09
D2	6.55±0.05	24.59±0.03	5.11±0.03	8.38±0.14	0.55±0.01	61.37±0.05
D3	6.99±0.01	25.69±0.04	4.57±0.01	7.58±0.05	0.39±0.03	61.77±0.07
D4	5.12±0.03	20.30±0.07	6.76±0.04	9.64±0.07	0.60±0.04	62.70±0.02
Ds1	5.56±0.01	14.69±0.04	4.57±0.01	7.10±0.05	0.61±0.03	73.03±0.07
Ds2	6.77±0.03	17.50±0.03	7.96±0.04	7.81±0.03	0.59±0.04	66.14±0.04
Ds3	7.21±0.03	20.30±0.07	4.29±0.02	7.01±0.04	0.44±0.04	67.96±0.02
Ds4	5.35±0.07	21.10±0.07	3.80±0.04	10.18±0.06	0.65±0.05	64.27±0.03

Source: Author's own elaboration (D, Diet with unflavored gelatin; Ds, Diet without unflavored gelatin).

Physicochemical characterization of extruded products

Expansion index (EI) and bulk density (BD)

The expansion index (EI) is related to the bulk density (BD) and the sinking velocity of the products obtained. It was observed that the processing variables and the formulation of the diets had a statistically significant effect ($p < 0.05$) on EI, obtaining higher expansion rates in the diets without unflavored gelatin (treatments Ds1, Ds2, Ds3, and Ds4) (table 5). It was observed that the IE values were higher in the diets whose concentration of fishmeal was 5% or lower.

In general, the BD values are related to the degree of expansion, as well as the possible effect on the buoyancy of the food. The proposed diets have statistically significant differences ($p < 0.05$). The lowest BD was obtained in the diets with lower fishmeal content; these diets were D1 and with 3.5% and 5%, respectively. The highest BD was obtained in D4 (1.24 g/cm^3 ± 0.018 g/cm^3), the diet with the lowest content of moringa leaf meal (table 5).

Water absorption index (WAI) and water solubility index (WSI)

Water absorption and water solubility indexes are shown in table 5. The lowest WAI values were found in the D4 and Ds4 diets, with values of 3.96 g H_2O/g and 3.37 g H_2O/ g per sample, respectively, because the WAI is related to the amount of water absorbed by the starch and proteins. The latter influenced by the hydrophilic balance of the proteins present in each diet, which is related to the fact that the lower the WAI values, the more compact the food will be.

The water solubility index (WSI) is related to the degree of gelatinization of the starches and the degree of denaturation of the proteins, produced by the extrusion process. The highest values of WSI were found in the D4 and Ds4 diets, with values of 14.49% ± 0.10% and 14.53% ± 0.05%, respectively, which correspond to the diets with the lowest concentration of corn flour and the highest concentration of fishmeal, which can be explained by the fact that both the starch and the proteins of the fishmeal had a greater degradation during the extrusion process. WAI and WSI reflect the interaction between different protein sources and starch, and the results show that the differences between the observed interactions were statistically significant ($p < 0.05$), being the D4 and Ds4 diets the ones with the greatest interaction.

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Table 5. Data on mean values of all characteristics of extrudates along with their standard deviation with 95% of confidence.

Diet	EI (mm/mm)	Bulk density (g/ml)	WAI (g H2O/g)	WSI (%)	Hardness (N)	SV (cm/s)
D1	0.82 ± 0.01	0.91 ± 0.02	4.64 ± 0.30	12.78 ± 0.11	16.40 ± 5.47	0.0029 ± 0.00
D2	0.66 ± 0.00	1.20 ± 0.01	4.48 ± 0.03	13.17 ± 0.09	13.72 ± 6.19	4.1821 ± 0.05
D3	0.71 ± 0.01	0.98 ± 0.02	4.45 ± 0.04	12.06 ± 0.12	13.69 ± 7.16	0.0088 ± 0.00
D4	0.56 ± 0.00	1.24 ± 0.02	3.96 ± 0.03	14.49 ± 0.10	9.53 ± 3.56	6.7398 ± 0.08
Ds1	0.83 ± 0.01	0.85 ± 0.01	4.79 ± 0.01	12.17 ± 0.12	15.17 ± 5.56	0.0016 ± 0.00
Ds2	0.74 ± 0.01	0.97 ± 0.02	4.84 ± 0.01	13.07 ± 0.10	15.70 ± 4.39	0.0109 ± 0.00
Ds3	0.72 ± 0.00	1.12 ± 0.01	4.67 ± 0.07	10.49 ± 0.10	15.58 ± 6.00	0.0270 ± 0.00
Ds4	0.59 ± 0.01	1.17 ± 0.28	3.36 ± 0.10	14.53 ± 0.05	10.18 ± 2.66	5.9016 ± 0.08

Source: Author's own elaboration (EI: Expansion Index; WAI: Water Absorption Index; WSI: Water Solubility Index; SV: Sinking velocity).

Sinking velocity (SV)

The lowest SV values were found in the D3 diet, which showed a minimum sinking velocity of 0.0088 cm/s, while the maximum SV was obtained in the D4 diet with 6.7398 cm/s (table 5). The matrix generated by the ingredients presented a statistically significant effect ($p < 0.05$). One of the objectives in the design of a diet for Tilapia is to obtain a food with low sink rates; this was obtained in all the proposed diets; however, they were selected as the best diets, according to this parameter, the lowest VH values (D1, D3, Ds1, Ds2, and Ds3).

Hardness (H)

The hardness values obtained in the diets allow inferring the possible interactions between the components of the diets, which are influenced by the starch and the different proteins (table 5). There are statistically significant differences ($p < 0.05$) among hardness of the diets. The differences are a consequence of the cutting effort applied, which is directly proportional to the degree of degradation of the starch and the level of denaturation of the proteins. The applied forces allow the components of the diet to interact in the extruded matrix. The lowest values were found in the diets D4 and Ds4 (9.248 N and 10.180 N, respectively), and the highest were found in the remaining diets (between 15.584 N and 16.406 N), being necessary to apply a greater cutting effort in the D1 diet, whose content of moringa leaf meal was the highest among the proposed diets.

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectra of the blends and the pellets verify the presence of many functional groups, which indicates the complex nature of the material. The band centered at 3311 cm^{-1} correspond to the stretching of -OH bonds present in proteins, fatty acids, carbohydrates, and lignin. Due to the high content of protein contained in the diets, there is also a contribution in this region from the N-H stretching of the amide bond. The band observed at 2998 cm^{-1} corresponds to the stretching vibration of the hydrogen with respect to the bonds (C-H) associated with the glucopyranose ring; the peaks present at 2923 cm^{-1} and 2852 cm^{-1}, respectively, correspond to asymmetric and symmetric stretching of the C-H bonds. Due to the high intensity of these bands, it is possible to assign them to the lipid component of the moringa leaf, which is present in a portion similar to that of the protein. The peak observed at 1485 cm^{-1} can be attributed to the stretching connecting C-N and also to the deformation of the N-H bond present in the proteins. In the region known as the fingerprint, characteristic peaks of starch of 1200 cm^{-1} to 800 cm^{-1} are observed, corresponding to the vibrations of glucose C-O-C bonds, and peaks at 1131 cm-1, 1090 cm-1, 881 cm-1, and 818 cm^{-1} can be attributed to a pyranose ring (figure 1).
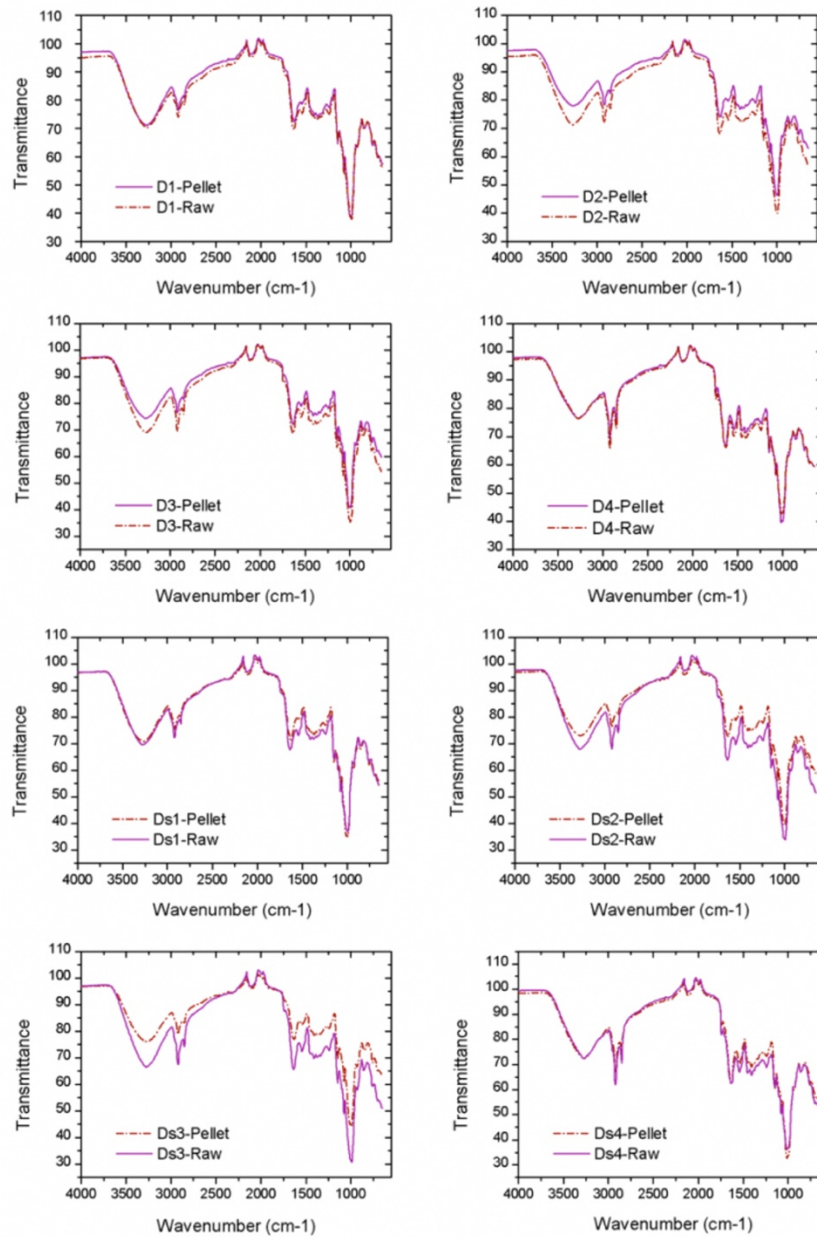
Figure 1. FTIR of all the blends pre-processed (Raw) and processed (Pellet).
Source: Author's own elaboration.

Structural characterization

Viscosity analysis

In the viscosity results shown in figure 2, the characterization of the raw materials (figure 2a), the mixtures (figure 2b), and the pellets of the diets is presented (figure 2c). In the case of raw materials, starch showed the maximum viscosity at 8900 cp, followed by yellow corn flour, with a peak of 1980 cp, and moringa flour, with a peak of 108 cp. The result of fishmeal and unflavored gelatin was close to 0 cp. With respect to the pellets, the highest peak was found in the Ds1 and D1 diets, with values of 399.5 cp and 387.5 cp, respectively, and the lowest values were found in the D4 and Ds4 diets. Similarly, in the mixtures, the highest peaks were found in the Ds1 and D1 diets, with values of 1315.50 cp and 1271.50 cp, respectively, and the lowest peaks were found in the D4 and Ds4 diets.
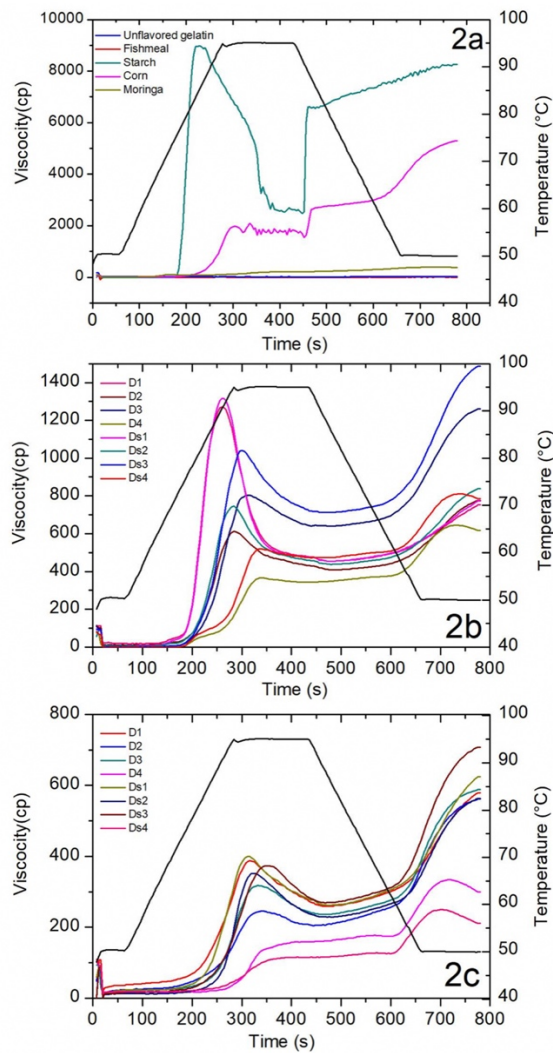

Figure 2. Viscosity profile of the raw material in 2a, viscosity profile of the blends pre-processed in 2b and viscosity profile of the blend processed by extrusion in the 2c.
Source: Author's own elaboration.

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Scanning electron microscopy (SEM)

Figure 3 showed the micrographs taken from the blends and the extrudates of the D2 and D3 diets, because these diets have the best chemical composition and the best sinking characteristics. In figures 3a and 3b, intact starch granules can be observed, a polyhedral shape and approximately 15 μm in size; some starch granules are coated with protein, while the leaf meal particles of *M. oleifera* had a variable size and an irregular shape. There is interaction between leaf particles of *M. oleifera* and starch granules. Figures 3c and 3d show the cross-section of the extrudates, and figures 3e and 3f show the longitudinal. These figures show a complete gelatinization of the starch granules and a plasticization of the proteins. The cross-section of the pellets corresponding to the D2 and D3 diets show a compact structure with some holes; the holes are the product of water evaporation.
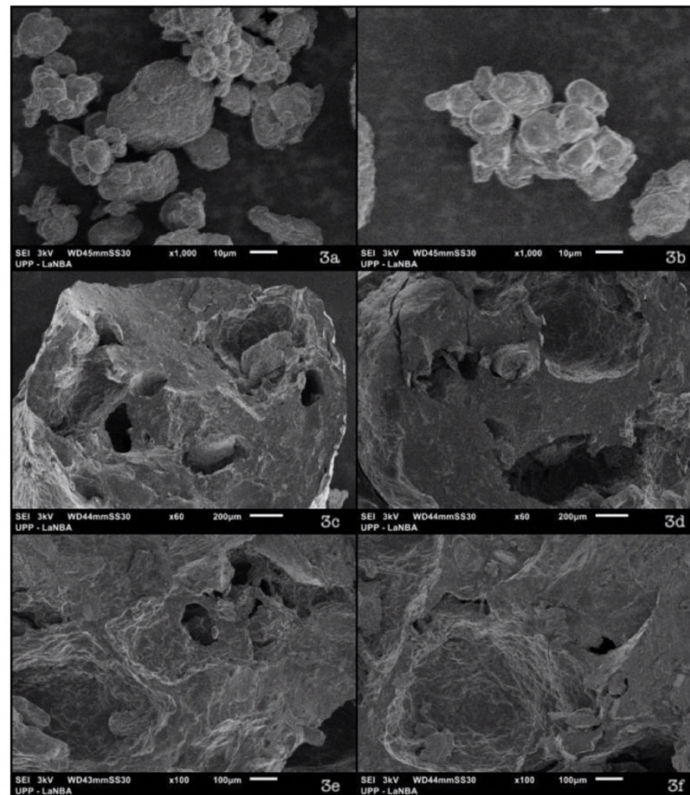

Figure 3. Micrographs of a 3a-3b-Raw blend, 3c-3d-Transverse profile of the pellet, and 3e-3f- longitudinal profile of the pellet. Figure 3a,3c and 3e are of diet D2 and figures 3b, 3d and 3f correspond to diet D3.
Source: Author's own elaboration.

Discussion

Proximate composition

Proximate chemical composition of the blends

Different sources of vegetable protein have been tested to obtain balanced feed for the fattening of fish, such as seaweed and mixtures such as soybeans with corn and jojoba (Azaza *et al*., 2009; Hassaan *et al*., 2015; Herath *et al*., 2016; Labib *et al*., 2012; Pereira *et al*., 2012).

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Proximate chemical composition of the pellet extruded

The case of Moringa has been proposed (Richter *et al*., 2003) due to its composition of amino acids, which is special because it meets the requirements of Tilapia in a specific way, coupled with the advantages of the use of proteins of vegetable origin compared to fishmeal, by not possessing biogenic amines. The results indicate that the diets D2 and D3 present the best options, with partial substitutions of 89.5% and 88.5% of fishmeal, apart from meeting the protein requirements of the Tilapia in adult stage, as well as the specific demand of amino acids in the adult stage (Sá, Sabry-Neto, Cordeiro-Júnior & Nunes, 2013; Thongrod, 2007).

Physicochemical characterization of extruded products

Expansion index (EI) and bulk density (BD)

The result of EI may be related to the protein composition of the materials used in the formulation of the diets and their solubility profiles, since the composition and type of protein present in fishmeal and moringa are completely different. However, due to the lack of a characterization of the solubility profiles of commercial fishmeal proteins, the EI values of the diets elaborated in this investigation ranged from 0.56 to 0.83, while the BD values varied from 0.85 g/cm^3 to 1.24 g/cm^3, which were lower than those reported (Kanmani *et al*.,2018), who used tapioca corn starches, pregelatinized or not, and reported EI values of 9.14 to 20.43 and BD values of 522.12 g/cm^3 to 633.75 g/cm^3. Similarly, in this study, the highest EI values were presented by the diets with the highest moringa content, despite the presence of higher starch content in other formulations, which may be related to possible interactions between this source of protein with the starch used.

However, it was impossible to correlate the content of moringa flour with BD of all diets, mainly due to the complexity of the different formulations tested, coinciding as reported (Ramirez-Ascheri, Ciacco, Riaz & Lusas 1995). The characteristics of the starch granules can be improved, since the dietary starch is pregelatinized, generating positive effects on the growth and physiology in the Tilapia as described (Case, Hamman & Schwartz 1992; Kanmani *et al*., 2018). Additionally, if the degree of gelatinization is increased, better integration of air in the pellet matrix is generated, which allows a greater expansion and decreases the speed of sinking (De Cruz *et al.*, 2015). The behavior of the pellet is influenced by the extrusion conditions, *i.e.* parameters like temperature, shear, and moisture content, as well as their previous management and during the process (Davis, 2015; Krueger, Thommes & Kleinebudde, 2014). Another important factor to consider is the nature of the blends, that is to say, that the components proposed for the formulation of the diets are not only rich in starch but also in proteins, whose inclusion in an extrusion process is difficult, because of its origin (Ayisi *et al.*, 2017; Davis, 2015; Liu *et al.*, 2011). Taking into account that the reports on obtaining directly expanded foods produced by extrusion, explain the expansion index and the values obtained, by BD based in the characteristics of the starches used. From this perspective, it would have to consider the components rich in proteins that can act as diluents and reducers of the expansion index, affecting the distribution of water in the formulated matrices and the macrostructure of the extruded pellet. However, proteins can contribute to the formation of extensive networks through covalent bonds and electrostatic interactions that can be propitiated during the extrusion process.

Water absorption index (WAI) and water solubility index (WSI)

The water absorption index (WAI) represents the hydrophilic aspect of the mixture formulation, while the WSI is considered to be a measure of hydrophobic behavior (Ayadi, Fallahi, Rosentrater & Muthukumarappan, 2013). Typically, the WAI is related to the starch content present in the diets, because it has the ability to absorb water when the starch granules are damaged. This means that the swelling in the excess water can be used as an index of the degree of gelatinization of it (Alcázar-Alay & Almeida Meireles, 2015; Chevanan, Muthukumarappan & Rosentrater, 2009; De Cruz *et al.*, 2015). However, the proposed formulations were not only enriched with starch but also based on proteins, with the hydrophilic balance present in the formulation. This result depends of the protein denaturation degree on the extrusion process, which induces changes on solubility profiles of proteins (Ayadi *et al.*, 2013; Ojokoh, Yimin, & Eromosele, 2014). The mixtures used in these studies were reflected in the WAI values, which range between 3.37% and 4.84%. Higher values of WAI could be associated with the use of

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high temperatures and the destruction of the crystalline structure of starch, which allows it to absorb more water (Chevanan *et al.*, 2009). Besides, it is essential to consider whether the compression is higher than the friction forces, since the granules are forced to generate more significant starch destruction. Obtained materials with low WAI values may be associated with restricted availability of water for the starch granule, due to a more compact structure   (Anderson *et al.*, 1970).

WSI is related to the macromolecular degradation of starch and the different proteins present in the formulations. This represents a measure of the number of soluble polysaccharides and polypeptides that were produced by the degradation of the starch components (Camire, Camire & Krumhar, 1990; Case *et al.*, 1992, Wang *et al.*, 1993) and the different proteins due to the high shear stresses to which they were subjected inside the extruder.

The values of the solubility index in water ranged between 10.49% and 14.53%. The diets with the inclusion of 40% of yellow corn generated the increase in the value of WSI. Similar results for WSI with increases in the level of starch were observed in previous studies. Generally, extrusion cooking denatures proteins and releases hydrophobic amino acids that reduce water solubility (Camire *et al.*, 1990; Kannadhason, Muthukumarappan & Rosentrater, 2009) and depolymerize starch as well as other macromolecules present in the mixture, which lead to the dextrinization of amylose and amylopectin (Anderson *et al.*, 1970), allowing proteins to interact with starch through the formation of cross-links (Davis, 2015; Fernández-Gutiérrez, San Martín-Martínez, Martínez-Bustos & Cruz-Orea, 2004).

Sinking velocity (SV)

The pellet must float for a limited period of time before it sinks slowly to provide the fish a more reasonable chance to consume the pellet before it reaches the bottom (Himadri *et al*., 1993). Therefore, sinking rate represents a critical characteristic for the preparation of food in aquaculture. It tends to sink quickly, and the species does not eat at the bottom of the tank; it will be lost food. Therefore, the stability of the food may be related to the absorption of water during the flotation of the food on the surface (Chevanan *et al*., 2009).

In five of the eight diets formulated, SV values were obtained below the reported, range of 2 cm/s by other authors (Chevanan *et al*., 2009, Davis, 2015, Kannadhason *et al*., 2009). Typically, the SV depends on the magnitude of the expansion and the physicochemical changes that occur within the barrel of the extruder, and so this has an effect. The expansion affects the density of the extrudates. In this sense, the magnitude of the physicochemical changes impacts the water absorption capacity and the structural integrity of the extrudates, which equally affect the SV (Chevanan *et al*., 2009, Davis, 2015).

Hardness (H)

Cut resistance is related to the degree of gelatinization and degradation of starch, as well as to changes in the conformation of proteins (Case *et al.*, 1992). A very compact pellet causes the rise to in shear strength, perhaps by the amylose released from gelatinization, and the crystallinity of the extrudates; reaffirming the texture of the product (Ojokoh *et al*., 2014). A relationship between the expansion rate and the shear force applied in extruded corn products has been reported; this indicates that the products with the highest expansion rates also had the lowest cut resistance values (Chang & El-Dash, 2003). The hardness of products manufactured by extrusion represents a crucial factor for the industry. It is an important trait for nutrition and commercialization; if the product obtain a low hardness, it will imply that it could break easily on packing and distribution operations.

Fourier transform infrared spectroscopy (FTIR) analysis

The structures found in the FTIR show the heterogeneity of the proposed mixtures; having a variability of intensity between the blends and the pellets because of the thermal process used; being visible in the characteristic spectral peaks of the raw ingredients, like fishmeal and yellow corn which have been reported. In the case of Moringa, the literature presents spectra of seeds and flowers with few differences between them, which corroborates the fact that the composition between the

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different parts of the tree is similar and is some what variable (Anand *et al.*, 2016; Arias *et al.*, 2016; Pineda-Gómez *et al.*, 2012; Tavengwa, Cukrowska & Chimuka, 2016).

Structural characterization

Viscosity analysis

The extrusion process decreases the viscosity of the different formulations. This decrease can be directly related to the gelatinization process of the starches present in yellow corn flour; however, the remaining viscosity can be directly related to the amount and type of protein present in each formulation, since it depends on whether the protein is from fishmeal or moringa leaf (Becker, Hill & Mitchell, 2001).

Scanning electron microscopy (SEM)

In the micrographs can be see the interactions of the amorphous moringa structures with the rest of the ingredients, which it is observed in the cross-sectional and longitudinal sections. A surface with little porosity are ideal for feeding Tilapia, sometimes the use of the extruder it generates the integration of air in the walls of the food, compromising its physicochemical characteristics according to the reported (Davis, 2015; Kanmani *et al.*, 2018).

Conclusions

The addition of Moringa leaf flour modified the proximal chemical composition, as well as the physical properties of the extrudate, being the D3 diet the one that presented an ideal protein content for the adult stage, apart from having physicochemical characteristics that shape it as the best formulation, capable of efficient feeding in *Oreochromis niloticus*. The low sink rate of the proposed foods will allow the food to float so that the fish can eat and reduce over-breeding. Adequate changes in the particular content of ingredients had in the extrusion process, significant effects on certain processing conditions and physical properties of the extrudate (Adequate nutrimental balance, palatability, buoyancy, density, and hardness), so they can be modified using various combinations of the same. The heterogeneity of the mixtures only allowed to observe some of the effects.

Acknowledgments

References

Alcázar-Alay, S. C., & Almeida Meireles, M. A. (2015). Physicochemical properties, modifications and applications of starches from different botanical sources. *Food Science and Technology* (*Campinas*), *35*(2), 215-236. doi: http://dx.doi.org/10.1590/1678-457X.6749

Anand, K., Tiloke, C., Phulukdaree, A., Ranjan, B., Chuturgoon, A., Singh, S., & Gengan, R. M. (2016). Biosynthesis of palladium nanoparticles by using *Moringa oleifera* flower extract and their catalytic and biological properties. *Journal of Photochemistry and Photobiology B: Biology*, *165*, 87-95. doi: https://doi.org/10.1016/j.jphotobiol.2016.09.039

Anderson, R. A., Conway, H. F., & Peplinski, A. J. (1970). Gelatinization of corn grits by roll cooking, extrusion cooking and steaming. *Starch, Biosynthesis Nutrition Biomedical*, *22*(4), 130-135. doi: https://doi.org/10.1002/star.19700220408

Pérez-Viveros, K. J., Gutiérrez-Dorado, R., Téllez-Jurado, A., Gómez-Aldapa, C. A., Reyes-Moreno, C.,
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diet of the adult stage of *Oreochromis niloticus*. | 1-17

Association of Official Analytical Chemists (AOAC). (1990). *Official methods of analysis*. Virginia, USA: Association of Official Analytical Chemists (AOAC)

Arias-Moscoso, J. L., Cuevas-Acuña, D. A., Rivas-Vega, M. E., Martínez-Córdova, L. R., Osuna-Amarilas, P., & Miranda-Baeza, A. (2016). Physical and chemical characteristics of lyophilized biofloc produced in whiteleg shrimp cultures with different fishmeal inclusion into the diets. *Latin American Journal of Aquatic Research, 44*(4), 769-778.

Ayadi, F. Y., Fallahi, P., Rosentrater, K. A., & Muthukumarappan, K. (2013). Modeling Single-Screw Extrusion Processing Parameters and Resulting Extrudate Properties of DDGS-Based Nile Tilapia (*Oreochromis niloticus*) Feeds. *Journal of Food Research*, *2*(2), 11-28. doi: http://dx.doi.org/10.5539/jfr.v2n2p11

Ayisi, C. L., Zhao, J., & Rupia, E. J. (2017). Growth performance, feed utilization, body and fatty acid composition of Nile tilapia (*Oreochromis niloticus*) fed diets containing elevated levels of palm oil. *Aquaculture and Fisheries*, *2*(2), 67-77. doi: https://doi.org/10.1016/j.aaf.2017.02.001

Azaza, M. S., Wassim, K., Mensi, F., Abdelmouleh, A., Brini, B., & Kraïem, M. M. (2009). Evaluation of faba beans (*Vicia faba* L. *var. minuta*) as a replacement for soybean meal in practical diets of juvenile Nile tilapia *Oreochromis niloticus*. *Aquaculture*, *287*(1-2), 174-179. doi: https://doi.org/10.1016/j.aquaculture.2008.10.007

Becker, A., Hill, S. E., & Mitchell, J. R., (2001). Milling—A further parameter affecting the rapid visco analyser (RVA) profile. *Cereal Chemistry Journal*, *78*(2), 166-172. doi: https://doi.org/10.1094/CCHEM.2001.78.2.166

Camire, M. E., Camire, A., & Krumhar, K. (1990). Chemical and nutritional changes in foods during extrusion. *Critical Reviews in Food Science and Nutrition, 29*(1), 35-57. doi: https://doi.org/10.1080/10408399009527513

Carter, C. G., & Hauler, R. C. (2000). Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture*, *185*(3-4), 299-311. doi: https://doi.org/10.1016/S0044-8486(99)00353-1

Case, S. E., Hamman, D. D., & Schwartz, S. J. (1992). Effect of starch gelatinization on physical properties of extrudate wheat- and corn- based products. *Cereal Chemistry*, *64*(4), 401-404.

Chang, Y. K., & El-Dash, A. A. (2003). Effects of acid concentration and extrusion variables on some physical characteristics and energy requirements of cassava starch. *Brazilian Journal of Chemical Engineering*, *20*(2), 129-137. doi: http://dx.doi.org/10.1590/S0104-66322003000200006

Chevanan, N., Muthukumarappan, K., & Rosentrater, K. A. (2009). Extrusion studies of aquaculture feed using distillers dried grains with solubles and whey. *Food and Bioprocess Technology*, *2*(2), 177-185. doi: https://doi.org/10.1007/s11947-007-0036-8

Davis, D. A. (2015). *Feed and Feeding Practices in Aquaculture*. Cambridge: Woodhead Publishing

De Cruz, C. R., Kamarudin, M. S., Saad, C. R., & Ramezani-Fard, E. (2015). Effects of extruder die temperature on the physical properties of extruded fish pellets containing taro and broken rice starch. *Animal Feed Science and Technology*, *199*, 137-145. doi: https://doi.org/10.1016/j.anifeedsci.2014.11.010

Draganovic, V., Van der Goot, A. J., Boom, R., & Jonkers, J. (2011). Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. *Animal Feed Science and Technology*, *165*(3-4), 238-250. doi: https://doi.org/10.1016/j.anifeedsci.2011.03.004

El-Sayed, A. F. M. (2006). *Tilapia culture*. UK: CABI Publishing

Food and Agriculture Organization of the United Nations (FAO). (2016). *The state of world fisheries and aquaculture*. Rome: Food and Agriculture Organization of the United Nations (FAO)

Food and Agriculture Organization of the United Nations (FAO). (2017). Aquaculture feed and fertilizer resources information system. Retrived October 7th, 2017 from http://www.fao.org/fishery/affris/species-profiles/nile-tilapia/en/.

Fernández-Gutiérrez, J. A., San Martín-Martínez, E., Martínez-Bustos, F., & Cruz-Orea, A. (2004). Physicochemical Properties of Casein-Starch Interaction Obtained by Extrusion Process. *Starch Biosynthesis Notrition Biomedical*, *56*(5), 190-198. doi: https://doi.org/10.1002/star.200300211

Gujska, E., & Khan, K. (1990). Effect of temperature on properties of extrudates from high starch fractions of navy, pinto and garbanzo beans. *Journal of Food Science*, *55*(2), 466-469. doi: https://doi.org/10.1111/j.1365-2621.1990.tb06788.x

Hassaan, M. S., Soltan, M. A., & Abdel-Moez, A. M. (2015). Nutritive value of soybean meal after solid state fermentation with *Saccharomyces cerevisiae* for nile tilapia, *Oreochromis niloticus*. *Animal Feed Science and Technology*, *201*, 89-98. doi: https://doi.org/10.1016/j.anifeedsci.2015.01.007

Herath, S. S., Haga, Y., & Satoh, S. (2016). Effects of long-term feeding of corn co-product-based diets on growth, fillet color, and fatty acid and amino acid composition of nile tilapia, *Oreochromis niloticus*. *Aquaculture*, *464*, 205-212. doi: https://doi.org/10.1016/j.aquaculture.2016.06.032

Himadri, K. D., Hattula, M. T., Myllymäki, O. M., & Mälkki, Y. (1993). Effects of formulation and processing variables on dry fish feed pellets containing fish waste. *Journal of the Science of Food and Agriculture*, *61*(2), 181-187. doi: https://doi.org/10.1002/jsfa.2740610208

Kanmani, N., Romano, N., Ebrahimi, M., Nurul Amin, S. M., Kamarudin, M. S., Karami, A., & Kumar, V. (2018). Improvement of feed pellet characteristics by dietary pre-gelatinized starch and their subsequent effects on growth and physiology in tilapia. *Food Chemistry*, *239*, 1037-1046. doi: https://doi.org/10.1016/j.foodchem.2017.07.061

Kannadhason, S., Muthukumarappan, K., & Rosentrater, K. A. (2009). Effects of Ingredients and extrusion parameters on quafeeds containing DDGS and tapioca starch. *Journal of Aquaculture Feed Science and Nutrition*, *1*(1), 6-21.

Krueger, C., Thommes, M., & Kleinebudde, P. (2014). Influence of storage condition on properties of MCC II-based pellets with theophylline-monohydrate. *European Journal of Pharmaceutics and Biopharmaceutics*, *88*(2), 483-491. doi: https://doi.org/10.1016/j.ejpb.2014.06.006

Labib, E. M. H., Zaki, H. A. M. A., & Mabrouk. (2012). Nutritional studies on partial and total replacement of fishmeal by Jojoba Meal (*Simmondsia chinensis*) in nile tilapia (*Oreochromis niloticus*) fingerlings diets. *APCBEE Procedia*, *4*, 196-203. doi: https://doi.org/10.1016/j.apcbee.2012.11.033

Liu, S., Alavi, S., & Abughoush, M. (2011). Extruded moringa leaf–oat flour snacks: Physical, nutritional, and sensory properties. *International Journal of Food Properties*, *14*(4), 854-869. doi: https://doi.org/10.1080/10942910903456358

Ojokoh, A. O., Yimin, W., & Eromosele, O. S. (2014). Effect of some extrusion variables on fermented maize-soybean blend. *Journal of Food Science and Technology, 52*(9), 5763-5771. doi: https://doi.org/10.1007/s13197-014-1689-8

Pereira, R., Valente, L. M. P., Sousa-Pinto, I., & Rema, P. (2012). Apparent nutrient digestibility of seaweeds by rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). *Algal Research*, *1*(1), 77-82. doi: https://doi.org/10.1016/j.algal.2012.04.002

Pineda-Gómez, P., Acosta-Osorio, A. A., Coral, D. F., Rosales-Rivera, A., Sanchez-Echeverri, L. A., Rojas-Molina, I., & Rodríguez-García, M. E. (2012). Physicochemical characterization of traditional and commercial instant corn flours prepared with threshed white corn. (*Ciencia y Tecnología Alimentaria*) *CyTA - Journal of Food*, *10*(4), 287-295. doi: https://doi.org/10.1080/19476337.2011.653694

Ramirez-Ascheri, J. L., Ciacco, C. F., Riaz, M. N., & Lusas, E. W. (1995). Efecto de la formulación sobre la expansión y viscosidad de snacks (pellets) producidos por extrusión termoplástica. *Alimentaria*, *33*(268), 111-118.

Richter, N., Siddhuraju, P., & Becker, K. (2003). Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture*, *217*(1-4), 599-611. doi: https://doi.org/10.1016/S0044-8486(02)00497-0

Sá, M. V. C., Sabry-Neto, H., Cordeiro-Júnior, E., & Nunes, A. J .P. (2013). Dietary concentration of marine oil affects replacement of fish meal by soy protein concentrate in practical diets for the white shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition*, *19*(2), 199-210. doi: https://doi.org/10.1111/j.1365-2095.2012.00954.x

Schwitzguébel, J. P., & Wang, H. (2007). Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Environmental Science and Pollution Research-International, 14*(7), 452-462. doi: https://doi.org/10.1065/espr2007.05.426

Silva-Soares, N. F., Nogueira-Alves, A., Beldade, P., & Mirth, C. K. (2017). Adaptation to new nutritional environments: larval performance, foraging decisions, and adult oviposition choices in *Drosophila suzukii*. *BMC Ecology*, *17*(21), 21. doi: https://doi.org/10.1186/s12898-017-0131-2

Tavengwa, N. T., Cukrowska, E., & Chimuka, L. (2016). Application of raw and biochared *Moringa oleifera* seed powder for the removal of nitrobenzene from aqueous solutions. *Desalination and Water Treatment*, *57*(53), 25551-25560. doi: https://doi.org/10.1080/19443994.2016.1151381

Thongrod, S., (2007). Analysis of feeds and fertilizers for sustainable aquaculture development in Thailand. In: M. R. Hasan, T. Hecht, S. S. De Silva, & A. G. J. Tacon (Eds.). *Study and analysis of feeds and fertilizers for sustainable aquaculture development 497*(pp. 309-330). Roma, Italia: Fisheries Technical Paper (FAO)

Wang, W. M., Klopfenstein, C. F., & Ponte, J. G. J. (1993). Effects of twin-screw extrusion on the physical properties of dietary fiber and other components of whole wheat and wheat bran and on the baking quality of the wheat bran. *Cereal Chemistry, 70*(6), 707-711.