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Evaluation of an antioxidant diet for tilapia *Oreochromis niloticus* with mango and roselle by-products inclusion

Evaluación de una dieta antioxidante para tilapia *Oreochromis niloticus* con inclusion de subproducto de mango y jamaica

Concepción-Brindis A[1], Jiménez-Ruíz EI[2]*, Sumaya-Martínez MT[2], Rincones-López JE[3], Sánchez-Herrera LM[2], Peraza-Gómez V[4], Enedina-Medina-Carrillo R[5], Spanopoulos-Hernández M[6]

[1] Programa de Doctorado en Ciencias Biológico Agropecuarias, Universidad Autónoma de Nayarit, Carretera Tepic-Compostela km 9, C.P. 63780, Xalisco, Nayarit, México.
[2] Unidad de Tecnología de Alimentos, Secretaría de Investigación y Posgrado, Universidad Autónoma de Nayarit, Ciudad de la Cultura s/n, C.P. 63000, Tepic, Nayarit, México. E
[3] Programa de Maestría en Ciencias Biológico Agropecuarias, Universidad Autónoma de Nayarit, Carretera Tepic-Compostela km 9, C.P. 63780, Xalisco, Nayarit, México.
[4] Unidad Académica Escuela Nacional de Ingeniería Pesquera, Universidad Autónoma de Nayarit, Carretera a San Blas km. 12, C.P. 63740, Bahía de Matanchén, San Blas, Nayarit, México.
[5] Unidad Académica de Ciencias Químico Biológicas y Farmacéuticas, Universidad Autónoma de Nayarit, Ciudad de la Cultura s/n, 63000 Tepic, Nayarit, México.
[6] Instituto Tecnológico de Mazatlán, Corsario 1 # 203 Col. Urias, C.P. 82070, Mazatlán Sinaloa, México.

ABSTRACT

The aquaculture industry experiences a growing interest in implementing antioxidants from natural origin in diets because the synthetic ones are associated to carcinogenic and teratogenic effects. Additionally, synthetic antioxidants have been included in such diets to prevent lipid oxidation during storage and distribution, without a perspective of the organisms health, since they require micronutrients, such as bioactive compounds, mainly antioxidants. Thus, mango and roselle by-products (rich in bioactive compounds) were included in an aquaculture diet, assessed on its antioxidant properties by distincts technics: antiradical activity 2, 2-diphenyl-1-pricrylhydrazyl (DPPH●), ferric reducing antioxidant power (FRAP) and phenolic compounds concentration. In addition, with this feed, tilapia *Oreochromis niloticus* fingerlings-juveniles were fed and compared with a reference and commercial diets respect their performance in a laboratory scale, according to the main parameters evaluated in aquaculture. With respect to the results obtained by the 3 techniques used, antioxidant properties of the diet are increased with the inclusion of the novel inputs. No differences were found between supplied feeds for biological parameters assessed in the tilapia, so no negative effect was considered during feeding with the novel functional diet. Therefore, the potential use of the evaluated by-products in commercial diets is inferred and an assessment of the functional feed during the next rearing stages of the species under commercial rearing conditions is suggested.
Keywords: mango; roselle; by-product; tilapia diet

RESUMEN

En la industria acuícola existe un creciente interés por la implementación de antioxidantes de origen natural en dietas, debido a que los sintéticos están asociados a efectos cancerígenos y teratogénicos. Además, estos son incluidos para prevenir la oxidación de los lípidos en dichas dietas durante su almacenamiento y distribución, sin una perspectiva de sanidad de los organismos, que requieren micronutrientes como compuestos bioactivos, principalmente antioxidantes. Debido a lo anterior, se evaluó la inclusión de un subproducto de mango y jamaica (ricos en compuestos bioactivos) en una dieta acuícola sobre sus propiedades antioxidantes utilizando distintas técnicas: actividad antirradical 2,2-difenil-1-pricrilhidracil (DPPH●), reducción del ion férrico (FRAP, pos sus siglas en inglés) y concentración de compuestos fenólicos. Además, con esta dieta se alimentaron crías y juveniles de tilapia *Oreochromis niloticus* para evaluar su desempeño, comparada con una dieta base de referencia y una comercial, de acuerdo con los principales parámetros evaluados en la acuacultura. En los resultados obtenidos mediante las 3 técnicas utilizadas, las propiedades antioxidantes se incrementan con la inclusión de los insumos novedosos. En cuanto a los parámetros biológicos evaluados en la tilapia, no se encontraron diferencias entre los diferentes alimentos suministrados, por lo que se considera que no hubo un efecto negativo durante la alimentación con la dieta funcional novedosa. Por lo anterior, se infiere un uso potencial de los subproductos evaluados en dietas comerciales, sugiriendo la evaluación del alimento funcional en etapas de engorda de la especie y bajo condiciones de cultivo a escala comercial.
Palabras clave: mango; jamaica; subproducto; dieta tilapia

INTRODUCTION

Tilapia *Oreochromis niloticus* is valued in aquaculture because of its fast growth, disease tolerance, and other biotic and abiotic factors, which makes it a highly deman-

*Autor para correspondencia: Edgar Iván Jiménez Ruíz
Correo electrónico: jiru80@gmail.com
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ded species (Castillo-Juárez *et al.,* 2009; Torres *et al.,* 2012). Another advantage is that this species is omnivorous, whose feeding may include proteins, carbohydrates, vegetable oils, aquatic plants, animal by-products, fermented yeasts, and in some cases agroindustrial by-products in commercial feeds (González-Salas *et al.,* 2014).

The process of making aquaculture feed commonly includes butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) or tertiary butylhydroquinone (TBHQ), which are synthetic antioxidants used as additive in feed or products susceptible to lipid oxidation, however, they may show cytotoxic and carcinogenic effects (Laguerre *et al.,* 2007). Thus, the food industry shows a growing interest to substitute synthetic antioxidants with natural ones to be applied in food for human and animal consumption (Slimestad and Solhelm, 2004). Some alternatives as possible source of these bioactive compounds are using by-products as ingredients during aquaculture diet processing.

With respect to agroindustrial products, the state of Nayarit, Mexico produces more than 266,000 t of mango (*Mangifera indica*) annually, subjected to an agroindustrial process that generates large volumes of by-products (peel, kernel, and pulp adhered to both). These by-products contain an important concentration of bioactive compounds related with beneficial health effects, among which those that highlight are degenerative process prevention, such as cardiovascular diseases, antimicrobial, antioxidant, and antiviral activities (Liu, 2003; Soonga and Barlow, 2004; Maisuthisakul and Gordon, 2009). Furthermore, they are used minimally in animal feed but may turn out to be a stable ingredient by using processing technologies, such as flour production, and avoid or reduce the contamination that this activity produces since these they are considered waste and commonly deposited in open-air dumping grounds (Lorenzo-Santiago *et al.,* 2018).

Likewise, the State of Nayarit, Mexico has the agroindustrial resource of roselle (*Hibiscus sabdariffa*), which produces more than 253 t annually. Currently, this industry has been in development process and during production, harvest, and calyx dry out by-products are generated (calyxes not suitable for commercialization). These by-products contain anthocyanins, polyphenols, and ascorbic acid, among other compounds with antioxidant, antibacterial, and diuretic properties (Reanmongkol and Itharat, 2007; Castillo-Juárez *et al.,* 2009), which may enhance the concentration of bioactive compounds -mainly antioxidants- in a balanced feed.

Additionally, rearing tilapia -same as other species- may show pathological events due to stress generated by management or so by adverse conditions in the enviroment, such as high ammonium concentration, low temperatures, and dissolved oxygen levels. At the same time, this situation may unleash mortalities that impact productivity and feasibility of the activity (Gallage *et al.,* 2016; Zeitoun *et al.,* 2016). In accordance with the above, natural antioxidants contained in aquaculture diets may impact beneficially on the organisms health to counteract events such as oxidative stress. Therefore, the objective of this research was to assess antioxidant properties of a diet including mango and roselle by-products for tilapia (*Oreochromis niloticus*) during fingerling up to juvenile stages and their effects on biological production parameters.

MATERIALS AND METHODS

Preparation of ingredients with antioxidant properties

By-products flour (peel, kernel, and pulp adhered to both) was obtained from mango "Ataulfo" (*Mangifera indica*) with fruits classified in the agroindustry as second class or waste because it does not comply with quality indexes for national or international marketing. Pulping was performed mechanically, and the by-product was dried at 45 °C for 12 h. Subsequently, it was passed through a hammer mill to then be sieved to a 250 µm particle size. On the other hand, an aqueous roselle (*Hibiscus sabdariffa*) extract was prepared with 1 g of ground roselle calyxes in 100 mL of distilled water (100 °C) for 3 min and then filtered through Whatman # 4 filter (Little Chalfont, Buckinghamshire, UK) (Prenesti *et al.,* 2005). This extract was used for dough hydration during feed production in one of the treatments described as follows.

Proximal composition of ingredients

For the experimental diets formulation, it was necessary to first perform proximate composition of the ingredients, according to the methods described by the Association of Official Analytical Chemists International (AOAC, 2012) to determine moisture (drying in an oven at 95-100 °C to constant weight), protein (crude protein: total nitrogen by micro-Kjeldahl method), lipids (crude lipid: petroleum ether extraction by Soxhlet method), and ash (incineration in a muffle furnace at 550 °C for 12 h).

Experimental feeds

Two feed diets were obtained, one as reference (RD) and the other one with mango by-product and roselle (MRD) extract inclusion; both diets contained 35 % protein and 8-12 % lipids, based on the proposal by Bureau and Cho (1994) and requirements issued by Mjoun *et al.* (2010) and FAO (2021).

The following ingredients were used: fish meal, soy, and wheat flour, fish oil, gluten, choline chloride, vitamins, and minerals; RD contained only the ingredients previously mentioned while MRD included 10 % mango by-product flour and 65 mL aqueous roselle extract for each 100 g of dry ingredient mixture (Table 1). The dry ingredients were mixed, subsequently, fish oil, water (RD) or roselle extract (MRD) were added, pelletized in a meat grinder with a 3 mm die, and finally dried at 45 °C for 12 h in a horizontal flux oven at air speed of 2 m/s. A commercial feed (CD) widely used in the region -reporting a minimum of 35 % and 8 % protein and lipids, respectively- was used in case of a third group. Diets were also analyzed for their proximal composition according to the techniques previously mentioned (AOAC, 2012).

Table 1. Formulation of experimental diets.
Tabla 1. Formulación de dietas experimentales.

Component (g/100g)	Diets	
	(RD)	(MRD)
Wheat flour	38.00	28.00
Soy flour	22.00	22.00
Fish meal	29.00	29.00
Fish oil[d]	4.30	4.30
Gluten	5.00	5.00
Vitamin Pre-mixture[a]	0.50	0.50
Vitamin C[c]	0.20	0.20
Mineral Pre-mixture[b]	0.50	0.50
Choline chloride[f]	0.50	0.50
Mango by-product flour	0.00	10.00
Total	100	100
Water	65 mL/100g	0.00
Roselle extract	0	65 mL/100g

RD: Reference diet.
MRD: Diet with mango and roselle by-product inclusion.
[a] Vitamin pre-mix: vitamin A, 2400 IU o mg/g; vitamin D3, 2250 IU; vitamin E, 160 g; vitamin K3, 8 g; vitamin B1, 20 g; vitamin B2, 40 g; pantothenic acid, 60 g; nicotinic acid, 160 g; vitamin B6, 16 g; folic acid, 4 g; vitamin B12, 80 mg; biotin, 500 mg; vitamin C, 0.2 g; choline (as chloride) (Courtesy of Trouw Nutrition México S.A. de C.V.).
[b] Mineral pre-mix: manganese, 100 g; zinc, 160 g; iron, 200 g; copper, 20 g; iodine, 5 g; selenium, 0.40 mg; cobalt 0.60 mg (Courtesy of Trouw Nutrition México S.A. de C.V.).
[c] Courtesy of DSM Nutritional Products México S.A. de C.V.
[d] Courtesy of Dr. Milton Spanopoulos Hernández, Instituto Tecnológico de Mazatlán, Sinaloa.
[e] Courtesy of Maz Industrial S.A de C.V., Mazatlán, Sinaloa.
[f] Courtesy of M. Sc. Yessica Silva Carrillo.

Evaluation of antioxidant properties

Antiradical DPPH● activities. To measure this parameter, feed was subjected to ethanolic extraction by reflux for 3 h. The extracts were filtered through Whatman # 4 filter (Little Chalfont, Buckinghamshire, UK), and then stored at 4 °C until their analyses. The antiradical activity determination was performed using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH●) as a free radical test, following the methodology reported by Morales and Jiménez-Pérez (2001). The antioxidant activity was expressed as micromoles Trolox equivalent/gram of sample (µmol TE/g). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic) is a reference molecule that shows a strong antiradical activity.

Ferric reducing antioxidant power (FRAP). In this analysis, the ethanolic extracts previously described were used for the antiradical activity. The ferric reducing antioxidant power assay was performed following the technique described by Hinneburg *et al.* (2006). Quantification was made at an absorbance of 700 nm, and Fe (III) reduction was determined as micromoles Trolox equivalent/gram of sample (µmol TE/g).

Total phenolic compounds concentration. For this analysis, the ethanolic extracts obtained were used as previously described. The technique was performed according to that reported by Stintzing *et al.* (2005), in which Folin-Ciocalteu reagent was used. The concentration of these compounds was obtained from a standard curve of gallic acid (0-400 mg/L) and expressed as miligrams gallic acid equivalent/g of sample (mg EGA/g).

Experimental design and bioassay conditions

For this study, a completely randomized experimental design was applied with homogeneous organisms in weight, age, and same batch. Three treatments were established, corresponding to the three diets mentioned (RD, MRD and CD), each one with three replicates.

Specimens of tilapia *Oreochromis niloticus* variety *Chitralada* -acquired commercially with a supplier in Jamay, Jalisco, Mexico- were used in the experimental rearing system (Laboratory of Bioassays of the Food Technology Unit at the Universidad Autónoma de Nayarit, Mexico). Sixty-two organisms with a weight of 1.08 ± 0.04 g were sown in each one of the nine experimental 500 L tanks (three replicates per treatment). Feeding was performed manually three times a day (08:00, 12:00, 16:00 h) maintaining the conditions within the optimum intervals for the species, using heaters with thermostat and air pumps (temperature 29.56 ± 0.31 °C; dissolved oxygen 4.60 ± 0.02 mg/L; pH 7.06 ± 0.01). Morphometric analyses were performed every 15 days to adjust diet supply per feed percentage, starting with 15 % of average organism weight and decreasing according to fish development until 4 % after 10 weeks of bioassay length.

Growth performance and feed utilization indices

Biological assessment was developed through morphometric data and quantity of food ingested during the development of the experimental organisms according to the following methodologies: weight gain: WG (Utne, 1979); specific growth ratio: SGR (Ricker, 1979); feed conversion ratio: FCR (Kilambi and Robinson, 1979); protein efficiency ratio: PER (Hepher, 1988); survival ratio: SR (Halver and Hardy, 2002).

Statistical analyses

Data were subjected to a one-way analysis of variance (ANOVA), taking as main factor the three different feeds used during the experimental stage, and when significant differences were found, a multiple Tukey's test was performed considering a level of significance of 5 %. The analyses were performed with IBM SPSS Statistics 26 program (New York, U.S.A).

RESULTS AND DISCUSSION

Proximal composition of ingredients and experimental diets

Table 2 shows the proximal composition of the ingredients used to produce the experimental diets. Gluten was

Table 2. Proximate composition of ingredients and experimental diets.
Tabla 2. Composición química de insumos y dietas experimentales.

Ingredient/ Diet	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)
FM	5.52 ± 0.85	60.61 ± 4.02	13.86 ± 0.34	19.32 ± 0.58
FO	0.92 ± 0.48	-	97.57 ± 0.37	-
WF	11.62 ± 0.03	13.55 ± 0.41	1.93 ± 0.08	0.79 ± 0.01
SF	10.08 ± 0.11	52.71 ± 1.60	1.24 ± 0.02	7.62 ± 0.13
GL	7.11 ± 0.09	72.42 ± 2.84	1.10 ± 0.34	0.92± 0.03
MBF	15.22 ± 0.85	6.19 ± 0.98	2.15 ± 0.11	3.59 ± 0.40
CD	7.94 ± 0.07^{b}	35.34 ± 0.41^{a}	9.40 ± 0.24^{b}	9.75 ± 0.06^{b}
RD	7.40 ± 0.16^{a}	35.42 ± 0.71^{a}	8.06 ± 0.03^{a}	9.25 ± 0.05^{a}
MRD	7.55 ± 0.40^{ab}	35.57 ± 0.49^{a}	8.10 ± 0.03^{a}	9.70 ± 0.05^{b}

FM: fish meal; FO: fish oil; WF: wheat flour; SF: soy flour; GL: gluten; MBF: mango by-product flour; CD: Commercial diet; RD: Reference diet; MRD: Diet including mango and roselle by-products.
Means in the same column with different superscript letters are statistically different (P < 0.05).

the ingredient with the greatest protein content: 72.42 ± 2.84 %, followed by fish meal: 60.61 ± 4.02 % and soy flour: 52.71 ± 1.60 %. Protein level for the ingredients commonly used in aquaculture feed have been previously reported. Tacon (1989) reported protein content of 45.8% in gluten, Álvarez *et al.* (2007) 62 % in fish meal and 44.2 % in soy flour, and recently, Jiménez-Ruíz *et al.* (2019) reported protein levels of 65, 55.92 and 43.05 % in gluten, fish meal, and soy flour, respectively. The differences found may be attributed to wheat and soy flour type, harvest season, and extraction temperature, whereas variation in protein percentage in fish meal may be due to manufacturing methods, organs or species used as raw matter, and season of the year. Corn and wheat gluten are some of the accepted inputs to be used in aquaculture diets where the maximum inclusion percentage recommended oscillates from 9 to 12.5 % conforming total feed (Koops *et al.,* 1982). This research included 9 % of wheat gluten and was used mainly as natural ligant (Devresse, 2000).

As to lipids, the greatest concentration was found in fish oil with 97.0 ± 0.06 %, followed by fish meal with 13.86 ± 0.34 % and mango by-product flour with 2.15 ± 0.11 %. Lipid content of this last one is attributed to the seed. Some studies have reported concentration from 12.8 % to 16.79 % in this part of the fruit (Pascual-Bustamante and Vilchis-Martínez, 2008; Rojas and Burbano, 2011).

Table 2 also shows the percentage composition of experimental diets that were initially projected and formulated with a protein content of 35 % and 8-12 % lipids, according to the nutritional requirements previously mentioned for the species (RD and MRD). The percentage content was isoproteic and isolipidic, since it did not show significant differences in the principal components (proteins and lipids) of these feeds ($P > 0.05$).

One of the aims of incorporating mango and roselle by-products in feed for tilapia is substituting synthetic antioxidants (mainly BHA and BHT) currently used to reduce deterioration, rancidity, and oxidative discoloration of lipids in feed (Abdalla *et al.,* 2007). Additionally, it is important to consider the possible beneficial effects in organism health and their capacity to deal with events such as oxidative stress and pathologies that may occur. Nevertheless, more studies such as this one, where it is considered antioxidant properties of the proposed diets, growth performance and feed utilization indices are necessary.

Antiradical activity against DPPH●

Table 3 shows the results of antioxidant activity with the radical DPPH●, observing significant differences between diets ($P < 0.05$), where MRD showed 2.5 times greater antiradical activity than RD and three times more than CD. These results could be the effect of including mango and roselle by-products, which have an important antioxidant activity mainly related with anthocyanines and total phenolic compounds.

It should be mentioned that the antioxidant activity in CD may be due to the addition of synthetic antioxidants during its formulation, which are usually not declared in their labelling (Lundebye *et al.,* 2010). However, diets as MRD showing a high antioxidant activity without the incorporation of these synthetic compounds represent a great opportunity for utilizing agriculture products high in antioxidant compounds, which do not pose a health risk, differing from those synthetic of commercial use (Laguerre *et al*., 2007). On the other hand, the antiradical activity in RD may be attributed to the bioactive compounds found in the basic ingredients such as wheat and soy flour and pre-mixture of vitamins (Devi *et al.,* 2009; Menga *et al.,* 2010; Shewry *et al.,* 2010; Lv *et al.,* 2012).

Diets for tilapia have been previously formulated with the inclusion of novel ingredients such as agroindustrial by-products with antioxidant properties, some have even assessed antiradical DPPH● capacity. Among the main studies are those by Jiménez-Ruiz *et al.* (2019) with avocado by-product, Ahmed *et al.* (2015) with edible mushroom, and Carbonera *et al.* (2014) with acerola extract by-product. In general, these studies attribute the antioxidant capacity against DPPH● radical to the bioactive compounds, among which those that stand out are phenolic type in each one of the ingredients used in food. As mentioned previously, in the case of this study, a greater MRD antiradical activity than in RD may be attributed to phenolic bioactive compounds found in mango and roselle by-products used (Soong and Barlow, 2004; Maisuthisakul and Gordon, 2009; Medina-Carrillo *et al.,* 2013).

Ferric reducing antioxidant power (FRAP)

Table 3 shows the results of the diets with respect to antioxidant capacity by the FRAP method. In this case, MRD again showed the highest values compared to the other diets ($P < 0.05$). Although CD showed an acceptable acting by the analysis of this technique, it was likely related to the inclusion of synthetic compounds in its formula (Lundebye *et al.,* 2010). On the other hand, when RD was compared to MRD, the difference in ion Fe (III) reduction capacity was at-

Table 3. Antioxidant properties of the experimental diets.
Table 3. Propiedades antioxidantes de dietas experimentales.

Diet	DPPH● (µmol TE/g)	FRAP (µmol TE/g)	TPC (mg GAE/g)
CD	4.23 ± 0.24[a]	11.04 ± 0.14[b]	1.54 ± 0.10[b]
RD	5.23 ± 0.48[b]	9.91 ± 0.02[a]	0.96 ± 0.24[a]
MRD	13.25 ± 0.19[c]	11.99 ± 0.05[c]	2.51 ± 0.07[c]

CD: Commercial diet; RD: Reference diet; MRD: Diet including mango and roselle by-products.
DPPH●: Antiradical activity DPPH●.
FRAP: Capacity for iron Fe III ion reduction.
CFT: Concentration of total phenolic compounds.
Means in the same column with different superscript letters are statistically different (P < 0.05).

tributed to the inclusion of the non-conventional ingredients assessed. This result can be explained because they were the only difference with respect to the elements incorporated during diets formulation.

The DPPH● method has been reported to be directly related with the evaluation of antioxidant capacity, mainly due to hydrophobic compounds, while FRAP relates to the hydrophilic type. Thus, the fact the MRD showed similar values for both techniques may be indicating that the non-conventional ingredients are contributing to a mechanism of action related to both types of systems. The mango and roselle by-products have been previously reported to show the capacity for ion Fe (III) reduction (Medina-Carrillo *et al.,* 2013; Sumaya-Martínez *et al.,* 2019).

Currently, not many reports exist of tilapia diet that have been alnalyzed by this method. Some works related with feed for the same species are those Jiménez-Ruiz *et al.* (2019), using avocado by-product, Carbonera *et al.* (2014) with acerola waste, and Rahman *et al.* (2017) in feed for shrimp using *Tetraselmis chuii* microalgae biomass. These works agree in that antioxidant properties of the experimental diets by this technique are attributed to bioactive compounds added when the assessed non-convenitonal ingredients were incorporated.

Total phenolic compounds concentration

The diets evaluated showed significant ($P < 0.05$) differences in regard to these bioactive compounds, where greater concentration was found in MRD with 2.50 µmol GAE/g with respect to RD and CD, which showed 0.95 and 1.53 µmol GAE/g, respectively (Table 3). The increase in more than double the value of MRD with respect to RD is attributed to the contribution of the phenolic compounds from mango by-product and roselle extract. These results agree with previous works where both non-conventional ingredients included in this study have demonstrated to be rich in such compounds (Medina-Carrillo *et al.,* 2013; Sumaya-Martínez *et al.,* 2019). In the case of mango, the main phenolic compounds found are mangiferin, catechin, epicatechin, chercetin, isochercetin and anthocyanins (Masibo and He, 2008). With respect to roselle, proanthocyanins, catechins, anthocyanins, chlorogenic acid and galic acid have been mainly mentioned (Ramírez-Rodríguez *et al.,* 2011; Reyes-Luengas *et al.,* 2015). Ajila *et al.* (2012) mentioned to the phenolic compounds as main components to consider when studying the potential use of agroindustrial waste in animal feed.

On the other hand, the total phenolic compound concentration in CD and RD may also be explained by the diet formulation since for example in RD ingredients such as wheat and soy flour, and gluten were used, which have demonstrated to contain such compounds (Pimiä *et al.,* 2001; Milner, 2004; Menga *et al.,* 2010; Shewry *et al.,* 2010). In the case of commercial feed, the concentration found may be attributed to the addition of antioxidants such as BHA, BHT, and TBHQ that are synthetic phenolic compounds (Lundebye *et al*., 2010). With respect to this, although CD showed considerable values in the test, MRD continued having an advantage in providing such compounds in feed from a natural source.

Few works have been found related directly with this study, where total phenolic compounds have been determined in diets for tilapia, including non-conventional ingredients from agroindustrial by-products. Previously, Galeana-López *et al.* (2020) worked with a corn husk extract (CHE), and their results suggest that its inclusion improve antioxidants properties in the feed and promote growth performance; Jiménez-Ruiz *et al.* (2019) with avocado by-product, who propose the use of this paste due to its antioxidant properties and the value added that would be granted; Ahmed *et al.* (2015) utilizing an edible mushroom reported a beneficial effects in organisms respect to oxidative stress, Obasa *et al*. (2013) with fermented mango seed replacing at 50% of yellow maize without affect growth and nutrient utlization indices, and Lim and Lee (2011) with fermented soy flour and cotton seed incorporated in feed reported a enhancement in antioxidant activity of both diet and fish. Most of these works have reported a greater total phenolic compounds concentration than MRD, which could be attributed to the different raw matter used. However, in this study, to obtain the ingredients such as mango by-product and roselle extract, required minimal processes to incorporate them to the diet, without the need for laborious extractions or fermentations to improve their functional properties.

Growth performance and feed utilization indices

The results for the zootechnical parameters in weight gain (WG), specific growth ratio (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival ratio (SR) are shown in Table 4. No significant differences ($P > 0.05$) between diets are observed, which may be considered as indicator that including non-conventional ingredients in MRD did not hinder development of the organisms at this growth stage. The added value given to mango and roselle by-products represents an advantage that should be considered. Furthermore, the potential benefits of the antioxidant properties that may be granted to the diets with bioactive compounds from a natural source for organisms health.

Some related studies have experimented implementing similar by-products in tilapia nilótica. Souza *et al.* (2013) worked with mango waste flour (pulp and peel) reporting

Table 4. Growth performance and feed utilization indices of tilapia *Oreochromis niloticus* fed with three experimental diets.
Tabla 4. Desempeño del crecimiento e índices de utilización del alimento de tilapia *Oreochromis niloticus* alimentada con tres dietas experimentales.

	CD	RD	MRD
Weight gain (WG, g)	42.85 ± 4.46[a]	48.72 ± 2.57[a]	44.99 ± 2.21[a]
Specific growth ratio (SGR, % day^{-1})	3.61 ± 0.13[a]	3.74 ± 0.15[a]	3.66 ± 0.08[a]
Feed conversion ratio (FCR)	1.4 ± 0.2[a]	1.3 ± 0.01[a]	1.5 ± 0.02[a]
Protein efficiency ratio (PER)	1.07 ± 0.11[a]	1.22 ± 0.06[a]	1.12 ± 0.06[a]
Survival ratio (%)	89.4 ± 2.04[a]	95.8 ± 2.42[a]	90.3 ± 3.33[a]

CD: Commercial diet; RD: Reference diet; MRD: Diet including mango and roselle by-products.
Means in the same row with different superscript letters are statistically different (P < 0.05).

the possibility of including up to 10 % in diet formulation without adversely affecting development of the organisms with respect to feeding and growth. Different from their work, this study took advantage of all the fruit by-product (peel, kernel, and pulp adhered to both). Omojowo *et al.* (2012) worked with mango peel flour with different levels of inclusion in the experimental diets (0, 12, 24, 36 and 48 %), concluding that the zootechnical parameters evaluated were more affected as such ingredient increased. In fact, although the level of inclusion of 12 % promoted better performance in the organisms, it was not better when compared to the control diet (0 %).

Omoregie (2001) evidenced better growth performance in the organisms when 10 % of mango kernel flour was included than in diets containing 20 and 30 %. Nevertheless, different from this study, the results were better when they used a commercial diet as reference. With respect to roselle, Amani *et al.* (2016) evaluated diets with inclusion of 0.5, 1, and 1.5 % of roselle calyxes and found that 1 % of this ingredient promoted better growth performance of the organisms. In this study, the final inclusion of roselle calix powder was 0.6 %, which did not affect the zootechnical parameters evaluated.

Finally, one of the challenges when non-conventional ingredients are included in animal feed may be taste and acceptance by the organisms fed. In this case, no problems were observed in feed consumption at the start of the experiment, because the organisms were in their fingerling stage, so they did not show difficulties to adapt to the experimental diet. However, when working with organisms of greater sizes that have already previously fed with a commercial feed for some time, a period of adaptation to the new diet is necessary. Previously, Omoregie (2001) reported one week as a period of adaptation to a diet for *Labeo senegalensis* that included mango and palm kernel seeds.

CONCLUSIONS

The inclusion of mango and roselle by-products promoted an increase in antioxidant properties of the diet for tilapia. Additionally, according to the biological parameters evaluated, the organisms were not affected by the inclusion of these non-conventional ingredients. Thus, the potential use of this diet for fingerling and juvenile stages is considered feasible in the near future. However, this diet should be evaluated in the next rearing stages of the organisms as well as assessment under farming conditions where the organisms are exposed to real rearing conditions.

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