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Corn husk extracts as an antioxidant additive in diets for Nile tilapia (*Oreochromis niloticus*) fingerlings: Effect on growth performance, feed intake and toxicity

Extractos de hoja de mazorca de maíz como aditivo antioxidante en dietas para juveniles de tilapia del Nilo (*Oreochromis niloticus*): Efectos sobre el crecimiento, el consumo de alimento y la toxicidad

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ABSTRACT

Corn stover, a corn harvest by-product, can be used to add value to feeds for farmed fish through their extracts, which are rich in phenolic compounds (PCs) and could confer antioxidant properties and possible health benefits to fish. In this study, PCs from corn husk extracts (CHE) were identified by ultra-performance liquid chromatography-electrospray ionization-quadrupole time of fly-mass spectroscopy (UPLC-ESI-Q-ToF-MS/MS), and used to provide antioxidant potential to diets for Nile tilapia fingerlings. Three experimental diets for tilapia were formulated with 0, 100 and 200 mg PCs from CHE/kg feed and their antioxidant capacity was determined by DPPH• and ORAC assays. A total of 180 Nile tilapia fingerlings (0.35 ± 0.06 g) were fed for 14 days to determine the effect of experimental diets on feed intake and growth performance. PCs from CHE such as ferulic, *p*-coumaric and chlorogenic acids were the most abundant. The inclusion of PCs into tilapia diets increases linearly ($p < 0.05$) their antioxidant capacity (DPPH and ORAC). Growth performance and feed intake increased linearly ($p < 0.05$) as the level of CHE inclusion increased. Results suggest that the CHE can provide antioxidant potential to tilapia diets, promote growth performance and feed intake without toxic effects.

Keywords: Phenolic compounds, antioxidants, tilapia, corn by-products.

RESUMEN

La mazorca de maíz, un subproducto de la cosecha de maíz, puede usarse para agregar valor a los alimentos para peces de cultivo a través de sus extractos, que son ricos en compuestos fenólicos (PCs) y los cuales podrían conferir propiedades antioxidantes y posibles beneficios a la salud de los peces. En este estudio, las PCs de los extractos de cáscara de maíz (CHE) se identificaron mediante cromatografía líquida de ultra resolución-tiempo de vuelo con cuadrupolo acoplado a espectrometría de masas con ionización por electrospray (UPLC-ESI-Q-ToF-MS/MS), y se utilizaron para proporcionar potencial antioxidante a las dietas para alevines de tilapia del Nilo. Se formularon tres dietas experimentales para tilapia con 0, 100 y 200 mg de PCs de CHE/kg de alimento y su capacidad antioxidante se determinó mediante ensayos DPPH• y ORAC. Se alimentaron un total de 180 alevines de tilapia del Nilo (0.35 ± 0.06 g) durante 14 días para determinar el efecto de las dietas experimentales, sobre el consumo de alimento y el crecimiento. Los PCs de CHE como los ácidos ferúlico, *p*-cumárico y clorogénico fueron las más abundantes. La inclusión de PCs en las dietas para tilapia, aumenta linealmente ($p <0.05$) su capacidad antioxidante (DPPH• y ORAC). El crecimiento y el consumo de alimento aumentaron linealmente ($p <0.05$) a medida que aumentó el nivel de inclusión de CHE. Los resultados sugieren que el CHE puede proporcionar potencial antioxidante a las dietas para tilapia y promover el crecimiento y el consumo de alimento de esta especie sin efectos tóxicos.

Palabras clave: Compuestos fenólicos, antioxidantes, tilapia, residuos de maíz.

INTRODUCTION

In Mexico, corn is the most cultivated crop with a production of more than 27.9 million tons in 2019 (SIAP, 2019). The grain is a rich source of carbohydrate and protein and is mainly used for human and farm animal consumption. The corn stover (leaves, stems, panicles, husk and cobs), which has a high fiber and low protein content, is commonly used to feed livestock during the cold season and as compost (Reyes *et al*., 2013). However, such by-products can be used to add value to diets of some animals, for instance in the aquaculture field.

Corn stover can be used as a source of PCs with antioxidant properties (Vazquez-Olivo, 2017). Among these PCs, hydroxycinnamic acids such as ferulic, ρ-coumaric, caffeic and sinapic acids are the most abundant. These types of plant metabolites participate as intermediates in the biosynthesis of lignin bound to cell wall components (Adom

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and Liu, 2002). In corn, ferulic acid is the major phenolic compound and it is bound in grain cell walls. Nevertheless, corn husk has the highest concentration of PCs with ferulic and ρ-coumaric acids being the most abundant (Žilić *et al*., 2012; Vazquez-Olivo, 2017).

Hydroxycinnamic acids exert antioxidant effects such as free radical scavenging, metal chelation and modulation of enzymatic activity, which prevent metabolic and chronic diseases in mammals and other species (Ou and Kwok, 2004; Itagaki *et al*., 2009; Paiva *et al*., 2013; Pei *et al*., 2016; Zhu *et al*., 2018). Despite the potential effect of phenolic acids on the health status of organisms including humans, they have been associated with astringent and bitter flavors (Huang and Zayas, 1991), and they are highly susceptible to environmental factors such as intense lighting and high temperatures (Sohn and Oh, 2003; Arceusz *et al*., 2013; Makila *et al*., 2016).

In aquaculture, the use of PCs as feed additives has been implemented in order to favor the primary defense antioxidant system, resistance to oxidative stress and post-mortem muscle preservation, as well as to modulate beneficial microbial communities in the gut of farmed organisms (Gómez-Guillén and Montero, 2007; Giannenas *et al*., 2012; Maqsood *et al*., 2013; Magrone *et al*., 2016; Papuc *et al*., 2017; Sun *et al*., 2017; Oniszczuk *et al*., 2019; Lizarraga-Velázquez *et al*., 2019). Although PCs exert extensive antioxidant effects which have consequent health benefits, their inclusion in fish diets can modify feed intake, and have an impact on survival and growth performance of fish (El-Mesallamy *et al*., 2016; Omnes *et al*., 2017). Thus, given that the Nile tilapia (*Oreochromis niloticus*) is one of the most cultivated freshwater fish which can tolerate a multitude of environmental factors and exhibits a moderate protein requirement that can be derived from plant origin (Montoya-Camacho *et al*., 2019), it is a good model for the current study.

Therefore, the aim of this study was to characterize the PCs profile of CHE and determine their antioxidant capacity *in vitro* when added to fish diets, as well as to evaluate their effect on growth performance, feed intake and **toxicity** in Nile tilapia fingerlings.

MATERIALS AND METHODS

Extraction of PCs from corn husk

Fresh corn husk was dried with forced air at 40°C during 24 h and ground to a particle size of 250 µm. PCs were extracted according to Adom and Liu (2002) with minor modifications. Fresh corn husk powder (0.5 g) was homogenized in 10 mL of 80 % ethanol, and shaken in an Orbit Environ Shaker (Lab-Line, USA) at 200 rpm for 12 h at room temperature. The mixture was centrifuged (3000 *g*, 15 min, 4 °C) in an Allegra X-30R model A99470 centrifuge (Beckman Coulter, Germany), and the supernatant with the free PCs was collected and stored at -20 °C until use. The pellet was digested with 10 mL of 2 M sodium hydroxide at 95 °C for 30 min in order to extract bound PCs. Subsequently, samples were incubated at room temperature with shaking at 200 rpm during 1 h. The mixture was neutralized with 2 mL of 37 % HCl and 10 mL of hexane was used to remove lipids from the extract through centrifugation. The supernatant was removed, and the pellet was washed five times with 10 mL of ethyl acetate to drag PCs. Ethyl acetate was evaporated at 35 °C under vacuum conditions and PCs were reconstituted with 2 mL of 80 % ethanol and stored at – 20 °C until use. Supernatants stored were mixed to quantify the total PCs content.

Quantification of total PCs

Total PCs content was determined using the methodology described by Singleton *et al*. (1999); 10 µL of each extract were diluted with 230 µL of distilled water. Afterwards, 10 µL of Folin-Ciocalteu reagent were added and the mix was incubated for 3 min in darkness, and then it neutralized with 25 µL of sodium carbonate 4 N. The samples were incubated for 120 min and measured at 725 nm in a Synergy™ HT microplate reader (Biotek, USA). Total PCs content was determined using gallic acid as standard. Results were reported as mg of gallic acid equivalent (mg GAE)/100 g of dry weight (DW). From the known concentration of total PCs, dilutions in concentrations of 100 and 200 mg of PCs from CHE were prepared.

PCs profile determination by UPLC-ESI-Q-ToF-MS/MS

The PCs content of reconstituted free and bound solutions of CHE was determined through Ultra-Performance Liquid Chromatography (UPLC) using an ACQUITY UPLC; H-Class system (Waters, Massachusetts, USA) coupled to a G2 XS Quadrupole-Time-of-Flight (Q-Tof) mass spectrometer (Agilent, California, USA) equipped with electrospray ionization (ESI). PCs were separated by UPLC at 40 °C with an ACQUITY BEH C18 (1.7 µm, 2.1 x 50 mm) column using a mobile phase composed of 0.1 % formic acid (A) and acetonitrile (B) at a flow rate of 0.3 mL/min. The gradient procedure was as follows: 0 min, 95% (A); 5 min, 70% (A); 9 min, 30% (A); 14 min, 0% (A); 14.5 min, 0% (A); 15 min, 95% (A); 16 min, 95% (A). An electrospray source in negative mode was used to collect mass spectra under the following conditions: nitrogen gas; desolvation temperature, 350 °C; desolvation gas, 13.3 L/min; capillary voltage, 1500 V; and fragmentor voltage, 30 V. The standard solutions were injected in different concentrations to make the calibration curve. Linear regression was used to evaluate relationships of concentration vs area sum, under the same conditions for each analyzed sample. Compounds were quantified using caffeic, chlorogenic, *p*-coumaric, ferulic, p-hidroxybenzoic and sinapic acids, as well as rutin as standards.

Experimental diets

PCs from CHE were incorporated into Nile tilapia diets which were formulated with approximately equal amounts of crude proteins (350 g/kg) and lipids (100 g/kg) (Jauncey, 2000). A basal diet without PCs was formulated as

previously described by Hernández *et al*. (2010) and denoted as Ex-0. Two basal diets were supplemented with 100 and 200 mg PCs/kg feed, which were denoted as Ex-100 and Ex-200, respectively. The dry ingredients (Table 1) were ground in a hammer mill to a particle size of 250 µm. Macronutrients (fish meal, soybean meal, wheat flour) were mixed in a Hobart mixer (model A-200, Ohio, USA); and the micronutrients (carboxymethyl cellulose, vitamin premix, mineral premix, and vitamin C) were then added. Fish oil and soy lecithin were added to the mixture of macro and micronutrients. Finally, PCs from CHE were previously homogenized in warm water and added until a homogeneous mixture was obtained.

Diets were extruded by using the twin-screw extruder TSE 20/40 (Brabender, Germany) under the following conditions: 30, 50, 70, 100, 100, 100 °C for each stage and 3.3 to 5.0 bar of pressure. Pellets were dried with forced air at 37 °C for 12 h. Sieves were used to remove fine particles, and the pellets were manually reduced to a size of approximately 0.5 mm. Pellets were kept in sealed labelled containers and maintained at -20 °C until use.

Table 1. Ingredients, proximate composition and phenolic content of experimental diets.
Tabla 1. Ingredientes, composición proximal y contenido fenólico de las dietas experimentales.

Ingredients (g/kg)	Diet		
	Ex-0	Ex-100	Ex-200
Fish meal (sardine)[a]	453.4	453.4	453.4
Fish oil[b]	65	65	65
Soybean meal[c]	120	120	120
Wheat flour[b]	350	349.9	349.8
Carboxymethyl cellulose[b]	1	1	1
Vitamin premix[d]	5	5	5
*Mineral premix[d]	5	5	5
**Vitamin C[e]	0.6	0.6	0.6
CHE[f]	0	0.1	0.2
Composition (g/kg)			
Dry matter	937.28	916.64	918.97
Crude protein	358.60	356.50	355.60
Crude lipid	95.00	94.00	109.60
Ash	85.40	83.50	83.50
NFE[g]	398.28	382.64	370.19
Total phenolic compounds	0.00	0.016	0.081

[a] Selecta de Guaymas, S.A de C.V, Guaymas, Sonora México.
[b] Droguería cosmopolita, S.A. de C.V. México, D.F., México.
[c] Proteínas marinas y agropecuarias S.A. de C.V., Guadalajara, Jalisco, México.
[d] Trout Nutrition México S.A. de C.V. (by cortesy). *Mineral premix composition: Manganese, 100.00 g; Magnesium, 45.00 g; Zinc, 160.00 g; Iron, 200.00 g; Copper, 20.00 g; Iodine, 5.00 g; Selenium, 0.40 g; Cobalt 0.60 g. **Vitamin premix composition: Vitamin A, 2400 IU/g; Vitamin D3, 2250 UI/g; Vitamin E, 160.00 g; Vitamin K3, 8.00 g; Thiamine B1, 20.00 g; Riboflavin B2, 40 g; Pyridoxine B6, 16.00 g; Vitamin B12, 80.00 mg; Pantothenic acid, 60.00 g; Nicotinic Acid, 160.00 g; Folic Acid, 4.00 g; Biotin, 0.50 g; Vitamin C, 100.00 g; Choline 300.00 g, Excipient 1046.85 g.
[e] DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, Mexico.
[f] Phenolic compounds from Corn hursk extract
[g] NFE (Nitrogen free extract) calculated by subtraction, 100-(crude protein + crude fat + moisture + ash + PCs).

Analysis of ingredients and diets

The proximal chemical analysis of ingredients and diets was conducted by triplicate. Moisture (method 934.01), lipid (method 920.39), and ash (method 942.05) contents were determined using standard methods described in AOAC (2005). Moisture was determined using a Craft stove (Felisa, Jalisco, Mexico). Lipid content was analyzed using a micro Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec, Hoganäs, Sweden) using petroleum ether as the extractor solvent. Ash content was determined gravimetrically by combustion in a muffle furnace (Fisher Scientific International, Inc. Pennsylvania, USA) at 550°C for 6 h.

The crude protein level was determined by Dumas combustion method (Ebling, 1968) using a Flash 2000 CHN/O Elemental analyzer (Thermo Scientific, Milan, Italy).

The final concentration of PCs in the experimental diets was analyzed following the same methodology used for the extraction of free PCs from the corn husk (Adom and Liu, 2002). PCs content was determined with the Folin–Ciocalteu reagent as mentioned above.

Antioxidant capacity from diets

The antioxidant capacity of extruded diets was determined by the oxygen-radical absorbance capacity (ORAC) assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) antioxidant assay. The ORAC assay was performed according to Huang *et al*. (2002). Briefly, 25 µL of extract were added to each plate, then 150 µL of flourescein 0.96 µM dispensed for reduction and induced by 50 µL of peroxyl radical generator (2,2'-Azobis (2-amidinopropane) dihydrochloride, AAPH 95.8 µM). Measurements were performed at 485 nm by excitation and 580 nm by emission for 70 min every 70 seconds at 37 °C, using a Synergy™ HT microplate reader (BioTek, USA).

The DPPH• radical scavenging capacity was determined according to Brand-Williams *et al*. (1995). In a 96-well microplate, 280 µL of DPPH• reagent (200 µM in ethanol) were added to 20 µL of the extract. The absorbance was recorded at 540 nm. Results were expressed as mmol trolox equivalents (TE)/g of diet. Data are reported as mean ±SD for at least three replications.

Fish culture conditions and feeding

All male tilapia fingerlings were produced in a commercial farm facility in the south of Sinaloa. The fish were acclimatized at the Finfish Hatchery, from the Research Center for Food and Development CIAD, Mazatlán Unit.

A total of 180 fish with a mean initial weight of 0.35 ± 0.06 g were evenly distributed into 12 tanks of 70 L capacity, whereby each tank consisted of 15 fish. A completely randomized experimental design with four replicates per treatment was used. Nile tilapias were fed to apparent sati-

ety three times a day (9:00, 12:00 and 16:00 h) for 14 days to evaluate the toxicity of PCs from CHE and their possible effect on feed intake and growth performance (Ahmed *et al*., 2017). Daily feed intake was measured throughout the experimental period. Nile tilapias were maintained in a recirculation system which included a settling tank with dissolved oxygen 5.0 ± 0.3 mg/L and a temperature of 27 ± 0.5 °C that was monitored with a dissolved oxygen meter (YSI, Ohio, USA); ammonia, nitrite and nitrate were evaluated daily with Insta-Test® strips (LaMotte Company, Maryland, USA). 20% of the water volume was replaced with fresh water every two days and maintained with natural photoperiod.

Growth parameters and feed efficiency

Once per week, fish were anaesthetized using a solution of clove oil (0.5 mL/L) and weighed to calculate their mean body weight. The feed efficiency and growth of tilapias were monitored in terms of weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and survival (S), according to the following formulas:

WG (%) = 100 x [(final body weight - initial body weight)/initial body weight;

SGR (%/day) = [100 × (ln (final mean body weight) - ln (initial mean body weight))/number of days];

FI (mg/fish) = $\sum_i$ 14 [(total feed consumption)/(number of fish)]/number of days;

FCR = feed intake/weight gain;

S (%) = (final number of fish/initial number of fish) x 100.

Statistical analysis

Data were subject to a test of normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test) before statistical analysis. The results were analyzed using one-way analysis of variance (ANOVA). Linear and quadratic effects due to PCs addition in diets were determined. All statistical procedures were conducted with IBM SPSS Statistics 25 program.

RESULTS AND DISCUSSION

PCs content in CHE

The free PCs content from corn husk was 171.33 ± 7.33 mg GAE/100 g, while that the bound PCs content was 1837.37 ± 136.64 mg GAE/100 g. Vazquez-Olivo *et al*. (2017) reported a similar content of free PCs (144.45 mg GAE/100 g), but a lower bound PCs content (1276.49 mg GAE/100 g) than the value obtained in this study. The differences among results could be attributed to the pre and postharvest conditions, drying temperature used in corn husk, as well as the differences among extraction methods (Kalt, 2005; Tomsone *et al*., 2012).

PCs profile of CHE by UPLC-ESI-Q-ToF-MS/MS

Free and bound PCs of CHE are shown in Table 2. Chlorogenic acid was the most abundant component in the free PCs fraction constituting 101.54 µg/g of corn husk, while ferulic and *p*-coumaric acids were the main components of the bound PCs fraction (12934.05 and 5731.14 µg/g from corn husk, respectively). Vázquez-Olivo *et al*. (2017) also have indicated that ferulic and *p*-coumaric acids are the major bound

Table 2. Phenolic compounds of corn husk extract identified by UPLC-ESI-Q-ToF-MS/MS.
Tabla 2. Compuestos fenólicos en extracto de hoja de mazorca identificados por UPLC-ESI-Q-ToF-MS/MS.

Compound	Retention time (min)	m/z $[M-H]^-$	Fragment ions MS/MS (m/z) $[M-H]^-$	Molecular formula	Concentration (µg/g)[a]
Free PC					
Chlorogenic acid	3.73	353.095	191.05, 353.08	$C_{16}H_{18}O_9$	101.54
p-Coumaric acid	5.06	163.047	119.04, 163.03	$C_9H_8O_3$	7.60
p-Hydroxybenzoic acid	3.79	137.032	122.88, 137.01	$C_7H_6O_3$	2.16
Sinapic acid	5.46	223.068	164.04, 193.01, 208.03	$C_{11}H_{12}O_5$	**
Caffeic acid	4.17	179.042	135.04, 179.03	$C_9H_8O_4$	**
Ferulic acid	5.45	193.058	134.03, 160.83, 195.80	$C_{10}H_{10}O_4$	**
Rutin	5.14	609.153	609.14, 611.20	$C_{27}H_{30}O_{16}$	**
Bound PC					
Ferulic acid	5.45	193.058	134.03, 178.02, 193.04	$C_{10}H_{10}O_4$	12934.05
p-Coumaric acid	5.06	163.047	119.04, 163.03	$C_9H_8O_3$	5731.14
p-Hydroxybenzoic acid	3.79	137.032	137.02	$C_7H_6O_3$	151.65
Caffeic acid	4.17	179.042	135.04, 179.04	$C_9H_8O_4$	**

[a] Milligrams of phenolic compound per gram of corn husk dry matter. **Traces.

PCs found in corn husk. However, these authors reported values of ferulic and *p*-coumaric acids (4555.00 and 3515.50 µg/g, respectively), which are lower than values obtained in our study. The results in both studies indicate that main PCs from corn husk are found bound to polysaccharides of the wall cell and lignin (Mounguengui *et al*., 2015).

PCs content in experimental diets

The concentration of PCs from experimental diets (Table 1) showed a reduction compared to the initial concentrations (100 and 200 mg PCs/kg feed). The basal diet Ex-0 was adjusted to 0 mg PCs/kg, considering that it does not contain CHE. Therefore, the final concentrations of experimental diets were of 16.11 mg PCs/kg for Ex-100 and 80.80 mg PCs/kg for Ex-200. The thermolability of PCs is one of the main drawbacks if they are outside of their natural matrix (for example bound to lignin), so despite the benefits of PCs, caution should be taken when integrating these compounds into diets (Ribas-Agustí *et al*., 2018). Our results suggest that the reduction of the PCs content in tilapia diets is due to the high temperatures used during the extrusion. Therefore, we consider that the use of PCs as fish feed additives with antioxidant properties, involves the optimization of the fish feed manufacturing process.

Antioxidant capacity of experimental diets

The use of extracts or meal from plants as feed additives for fish has intensified, due to their content of bioactive compounds such as PCs, which exhibit antioxidant properties (Catap *et al*., 2015; Villasante *et al*., 2015; Lizárraga-Velázquez *et al*., 2018; Lizárraga-Velázquez *et al*., 2019). Consequently, they could confer antioxidant capacity to the feed. In the present study, the antioxidant capacity measured by DPPH• and ORAC assays showed a linear increase ($p < 0.05$) according to the augmentation of PCs inclusion (Table 3). Previous studies have reported similar results in tilapia diets supplemented with plants as sources of natural antioxidants. Jiménez-Ruiz *et al*. (2019) indicated that dietary inclusion of avocado by-product paste increase the antioxidant capacity (DPPH•) of tilapia diets. In addition, the use of soybean meal and cottonseed meal as feed additives increase the DPPH• scavenging ability of tilapia diets (Lim and Lee, 2011). The increase in the antioxidant activity reported in these studies might be attributed to the bioactive compounds mix found in the plant matrix used. However, our results are mainly attributed to the presence of PCs such as ferulic and *p*-coumaric acids, since these are the major components in the CHE.

On the other hand, Table 3 shows that DPPH• scavenging ability showed values lower than antioxidant capacity measured by ORAC assay. The differences between the results of the two assays analyzed may be due to the different mechanisms of antioxidant action measured in each method. For example, DPPH• measures the ability of PCs to transfer electrons to free radicals, while ORAC assay is based on their hydrogen donating ability (Schaich *et al*., 2015). This indicate that the experimental diets have capacity to scavenge free radicals through hydrogen atom donation. However, further investigation in fish in order to validate their antioxidant capacity *in vivo* is needed.

Table 3. Antioxidant capacity of experimental diets.
Tabla 3. Capacidad antioxidante de dietas experimentales.

Assay	Diet			SEM	*P*	
	Ex-0	Ex-100	Ex-200		Linear	Quad
DPPH• (mmol TE/g) [a]	2.31 ± 0.03	2.45 ± 0.05	2.66 ± 0.03	0.035	<0.001	0.103
ORAC (mmol TE/g) [a]	4.24 ± 0.02	4.31 ± 0.01	4.36 ± 0.02	0.015	0.001	0.021

Values are mean ± SD, n = 3. [a] TE= Trolox equivalents.

Growth performance, feed intake, feed efficiency and survival

Experimental diets were well accepted by tilapias throughout the entire experiment. Table 4 indicates that FI and growth performance parameters as FW, WG and SGR showed a linear increase ($p < 0.05$) as the level of PCs inclusion increased. Even though the FCR mean decreased as PCs incremented in the diets, this was not statistically significant. The survival percentage was not affected by increasing of PCs in diets.

The CHE contains PCs that can provide additional functional value to the tilapia diets. In this study, the addition of 200 mg/kg of CHE (Ex-200) resulted in the greatest increase in feed intake and growth performance (Table 4). Furthermore, a greater anticipatory activity to food was observed in fish fed with Ex-200 than in those fed with Ex-0 and Ex-100 diets. The diets were well tolerated, as demonstrated by the high feed intake, even when fish were apparently satiated, which might reflect a possible effect of the CHE on palatability.

Table 4. Growth parameters and feed efficiency of Nile tilapia fed experimental diets.
Tabla 4. Parámetros de crecimiento y eficiencia alimenticia de las tilapias del Nilo alimentadas con las dietas experimentales.

Variable	Diet			SEM	*P*	
	Ex-0	Ex-100	Ex-200		Linear	Quad
IW (g)	0.35 ± 0.06	0.35 ± 0.06	0.35 ± 0.06	0.00	0.177	0.380
FW (g)	0.94 ± 0.04	0.99 ± 0.04	1.03 ± 0.06	0.02	0.024	0.354
FI (mg/ fish · day)	43.07 ± 0.24	46.01 ± 0.93	47.51 ± 0.83	0.55	0.004	0.043
WG (%)	165.60 ± 5.62	178.82 ± 5.01	192.02 ± 8.25	6.29	0.023	0.355
SGR (%/d)	6.97 ± 0.15	7.32 ± 0.13	7.65 ± 0.20	0.16	0.022	0.340
FCR	1.00 ± 0.06	1.01 ± 0.10	0.97 ± 0.04	0.05	0.615	0.840
S (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	0.00	-	-

Values are mean ± SD, n = 4. IW=Initial weight; FW=Final weight; FI=Feed intake; WG=Weight gain; SGR=Specific growth rate; FCR=Feed conversion ratio; S=Survival.

During the first month after hatching (part of the fingerling stage), Nile tilapias are highly susceptible to environmental factors that significantly affect survival, and feed intake is higher in relation to body weight (Gullian-Klanian and Arámburu-Adame, 2013). Therefore, the fingerling stage was selected to evaluate the difference in feed intake, changes in palatability and possible toxic effects of CHE in diets. Individually, the PCs of CHE have been evaluated in mammals and showed low toxicity. The OECD Guidelines for the Testing of Chemicals stablished that the maximum doses for oral administration of *p*-coumaric acid in experiments is 2000 mg/kg body weight and with a known LD_{50}=2850 mg/kg for mice (Pei *et al*., 2016; Kim *et al*., 2017). In addition, dosage of 29.4 mg/kg of ferulic acid in mice did not have negative effects (Hirabayashi *et al*., 1995).

In aquaculture, Magrone *et al*. (2016) reported that diets supplemented with 100-200 mg/kg of red grape seed extract (proanthocyanidins and catechins) had no toxic effect on sea bass (*Dicentrarchus labrax* L.). On the other hand, the dietary inclusion of 10 to 15 g/kg of Roselle calyx extract, containing cyanidin, delphinidin, synergenic acid and ellagic acid as the most abundant PCs, enhanced growth performance and FI in Nile tilapia (El-Mesallamy *et al*., 2016). These results are consistent with a study by Yu *et al*. (2017) that also reported an increase in growth performance of tilapia juveniles fed with supplemented diets with 100 mg/kg of ferulic acid. These data indicate that the effect of PCs on growth performance and FI might depend on their inclusion level and the difference in their phenolic profiles, as well as, the fish species and the species' feed preferences.

CONCLUSIONS

The inclusion of PCs from CHE increased the antioxidant capacity (DPPH and ORAC) of diets for Nile tilapia. Furthermore, the supplementation of 100 and 200 mg PCs/kg of CHE improved growth performance and feed intake without toxic effects in Nile tilapia fingerlings fed during 14 days. However, further studies are necessary to both support these findings and to probe the use of PCs from CHE as fish feed additives and their antioxidant effects *in vivo* on a large scale.

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Author contributions:

Conceived and designed the experiments: JAGL. The project was led by: CH. Conducted bioassays: JAGL. Chemical assays collaboration by: JBH. The main manuscript was written by JAGL with input from CH, JBH, NLL, CELV and EYSG. All authors reviewed and approved of the final manuscript.

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