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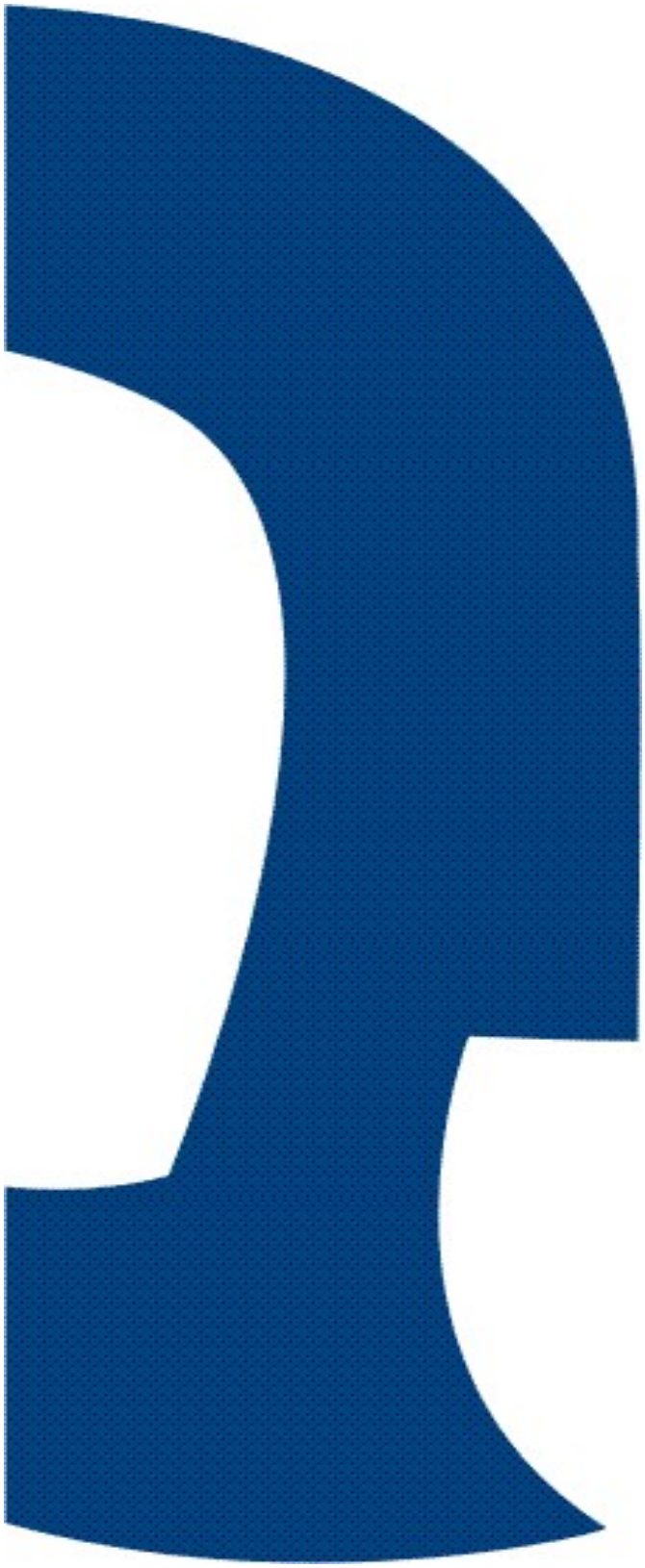

Dietary inclusion of banana flower in aquafeeds: histological effects in tilapia (*Oreochromis niloticus*) and sea bass (*Dicentrarchus labrax*)

Álvaro Rodríguez Rodríguez

Curso 2020/2021

Lidia Esther Robaina Robaina
Sara Ramírez Bolaños

Trabajo Fin de Título para la obtención del título de Grado en Ciencias del Mar

Datos personales del alumno:

- Nombre: Álvaro Rodríguez Rodríguez
- DNI:
- Fecha de nacimiento:
- Teléfono:
- Correo institucional:

Datos personales de la tutora:

- Nombre: Lidia Esther Robaina Robaina
- Departamento: Biología
- E-mail:

Datos personales de la co-tutora:

- Nombre: Sara Ramírez Bolaños
- Departamento: Grupo de Investigación en Acuicultura (GIA). IU-ECOAQUA, ULPGC
- E-mail:

Research project:

Project partially funded by to the Project “Solutions through the new use for a waste of banana crop to develop products in aquaculture and plastics sector - LIFE BAQUA”, under the program LIFE Environment and Resource Efficiency project application ((LIFE15 ENV/ES/000157; 2016-2020)”.

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Abstract

Fish feed represent generally the most expensive production cost in aquaculture. Currently much of the research in aquaculture is focused on the search for sustainable substitutes for fish meal and oils, with interesting alternatives from new vegetable by-products. One of these is generated by the cultivation of bananas, with which the Life Baqua project is working, whose main objective is to establish a new model of circular economy for the use of this waste. The aim is to develop antioxidant additives to complement the preparation of feed for fish in aquaculture. The species chosen for the study are tilapia (*Oreochromis niloticus*) and sea bass (*Dicentrarchus labrax*), which are fed various diets composed of different levels of by-product. A histopathological study of liver was subsequently carried out to determine the effect of the different diets on the fish.

Introduction

Aquaculture is the cultivation of aquatic organisms in coastal and inland areas that involves procedures in the breeding process to increase production (FAO, 2018a). It is the fastest-growing animal food production sector worldwide, representing nowadays about 50% of the world's food fish (James S. Diana, 2009; Kumar & Engle, 2016; Ottinger *et al.,* 2016). Forecasts on food security indicate that aquaculture has great potential to produce more fish in the future and thus compensate stagnating supplies form capture fisheries (Natale *et al.,* 2012; Ottinger *et al.,* 2016).

Fish is one of the most trated food commodities worldwide (Allison, 2011; Asche *et al.,* 2015; Kumar & Engle, 2016; Ottinger *et al.,* 2016) and the main source of valuable animal protein in many regions of the world (Béné *et al.,* 2015; Ottinger *et al.,* 2016). Aquaculture plays important roles in providing food and income in many developing countries, either as a stand-alone activity or in association with crop agriculture and livestock rearing (Allison, 2011). Thus, nowadays, world per capita consumption of aquatic products has increased up from 9 kg in 1961 to 20.5 kg in 2017, thanks to the incessant increase in productions, improvements in fish conservation techniques, reducing food waste and about more efficient distribution channels, in addition to increase in disposable income (FAO, 2018b).

According to different statistic sources, the vast majority of the world aquaculture takes place in Asia, specifically 92%, as it is also the majority of continental fishing

(52%); the rest of aquaculture production is distributed in America (3.2%), Europe (2.7%), Africa (2%) and Oceania (0.2%) (APROMAR, 2019).

Aquaculture growth has been driven by rising demand from growing and urbanizing populations, stagnating supplies from capture fisheries, investment in education and technology research (Allison, 2011). In contrast to the situation with wild fisheries, the trend in aquaculture is toward increasing production (James S. Diana, 2009). To supply the growing global demand for healthy and nutritious aquatic products an adequate production from aquaculture and fisheries, two activities that should continue hand in hand in the coming decades.

World aquaculture production reached in 2017 the 11.9 million tons, 3.5% more than 2016, and surpassing fishing production at 18.3 million tons and reached a value of 199.6 billions euros. In 2017, 1,353,201 tonnes of aquaculture products were harvested in the European Union, with a value of 4,147 million euros. The main species produced in the EU are mussel, with 493,844 tonnes, followed by Atlantic salmon (209,180 tonnes) and rainbow trout (185,316 tonnes). This figure represents an increase of 4.8% compared to the market position in 2016, although it is still below the maximum (APROMAR, 2019). Spain is the member state of EU with a largest aquaculture harvest in 2017 (23% of the total), however representing only the fourth position (12.2%), when considering the value of production (APROMAR, 2019); thus, for 2017 the Spanish aquaculture harvest figures show a total of 313,538 tonnes (mussels 241,785 tonnes, sea bass 21,269 tonnes, rainbow trout 17,948 tonnes and sea bream 13,643 tonnes as the main species), and a total value at first sale of 451.5 million euros (APROMAR, 2019).

Sea bass, *Dicentrarchus labrax* (Linnaeus, 1758), is widespread cultured and a very important commercial marine fish species in the Mediterranean Sea (Alasalvar *et al.,* 2002; Arechavala-Lopez *et al.,* 2012; Di Trapani *et al.,* 2014). Globally in 2016, a total of 191,033 tonnes were produced compared to 5,752 tonnes form fisheries (FAO, 2017a), being Greece, Turkey, Italy, Spain, Croatia and Egypt the largest producers (FAO, 2019a). In 2017 the production of sea bass in Spain was 17,655 tonnes with the Canary Island being the main producer with 5,804 tonnes (Ministerio de Agricultura, Pesca y alimentación, 2018). Sea bass is distributed in the eastern Atlantic from Morocco to Norway but can also be found in the Mediterranean Sea and the Black Sea (Haffray *et al*., 2006).

Sea bass are eurythermic (5-28 °C) and euryhaline (3‰ to full strength sea water); thus, they are able to frequent coastal inshore waters, and occur in estuaries and brackish water lagoons. Sometimes they venture upstream into freshwater. There is only one breeding season per year, which takes place in winter in the Mediterranean population (December to March), and up to June in Atlantic populations. Seabass spawn small (1.02-

1.39 mm) pelagic eggs in water with salinities lower than 35‰, near to river mouths and estuaries or in littoral areas where the salinity is high (≥30‰). Being not particularly sensitive to low temperature some fish may over-winter in coastal lagoons instead of returning to the open sea. Seabass are predators and their feeding range includes small fish, prawns, crabs and cuttlefish (FAO, 2005a). *Dicentrarchus labra*x has mouth moderately protractile with teeth in a crescentic band; a body rather elongate, opercle with 2 flat spines and preopercle with large, forward-directed spines on its lower margin. Two separate dorsal fins; the first with 8 to 10 spines; the second with 1 spine and 12 to 13 soft rays. Anal fin with 3 spines and 10 to 12 soft rays. Characteristics small scales and a colourfull lateral line. Caudal fin moderately forked. Colour silvery grey to bluish on the back, silvery on the sides, belly sometimes tinged with yellow. Young with some dark spots on upper parto f body but adults never spotted and a didduse spot on the edge of opercle. (FAO, 2005a).

On the other side, tilapia species are on the top of the world fish species production being its culture increasing up in different production systems. Among Tilapia species, *Oreochromis niloticus* (Linnaeus, 1758), has a great adaptability to different ambient conditions, being found in a variety of freshwater habitats such as rivers, lakes and channels. They live mainly in tropical regions and can be found as an exotic species in Central America, the southern Caribbean, southern North America and Southeast Asia (FAO, 2017b).

Tilapia is a specie that prefers to live in shallow water. It is an omnivorous feeder that feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, debris and bacterial layers associated with debris. In ponds, sexual maturity is reached at the age of 5 or 6 months. Spawning starts when the temperature reaches 24 ºC. Incubation and aging is completed in a period of 1 to 2 weeks, depending on the temperature. The number of eggs is proportional to the weight of the female's body. They can live for more than 10 years and reach a weight of 5 kg (FAO, 2005b). It has ease of handling, adapts to different conditions, easy reproduction, high resistance to disease and high productivity (Prieto Ortega, 2008). This specie has a compressed body; the depth of the caudal peduncle is equal to its length. Cycloid scales. Absent bulge on the dorsal Surface of the snout. The length of the upper jaw shows no sexual dimorphism. The first branchial arch has between 27 and 33 branchial filaments. The lateral line is interrupted. Continuous stiff and soft spines on back fin. Dorsal fin with 16 or 17 spines and between 11 and 15 rays. The anal fin has 3 spines and 10 or 11 rays. Truncated tail fin. The pectoral, dorsal and caudal fins acquire a reddish coloration in the spawning season; dorsal fin with numerous black lines (FAO, 2005b).

In aquaculture fish feed represent generally the most expensive production cost (Kaur and Shah, 2017), being fish meals and fish oils continuously lowering in commercial feeds formulations to compliment with the increased sustainability expected from this sector. Thus, much of the aquaculture research focuses on finding sustainable substitutes for fishmeal and fish oils (González *et al.,* 2014), by the way with interesting alternatives from novel plant by-products to reduce costs of the feeds (Omoregie and Ogbemudia 1993; Kaur and Shah, 2017). These plant by-products can provide necessary nutrients for fish, while having a minimal impact on environment (Gatlin *et al.,* 2007).

Some unprocessed herbs, fruits, and vegetables can be moreover a source of many bioactive compounds, such as polyphenols. For example, the flower purple coneflower (*Echinacea purpurea*) has antioxidant properties, so feed for fish with its addition can be used as a supplement in the prevention of fish diseases due to oxidative stress (Oniszczuk *et al.,* 2016). All living beings that use oxygen for energy generation release free radicals, something that is incompatible with life unless there are defence mechanisms against these species (García Bacallao *et al.,* 2001). The ones in charge of this defence are antioxidants, which can be defined as all substances that, being present at low concentrations with respect to those of an oxidizable molecule (biomolecule), delay or prevent the oxidation of this substrate (García Bacallao *et al.,* 2001; Avello and Suwalsky, 2006; Carocho *et al.,* 2017).

Among fish tissues, the liver is of great physiological important as it is the chief site for storage of high-energy foods like glycogen and lipids, which utilizes fish at the time of emergency, and it also plays a vital role in detoxification, digestion and other physiological processes (Gaber *et al.,* 2015). In fish liver performs normally both, hepatic and pancreatic functions, and is the metabolizing organ by excellence of all substances that reach via blood (Torres *et al.,* 2010). In fish liver represent a dense organ ventrally located in the cranial region of the general cavity and its size and shape are adapted to the space available between other visceral organs. It is divided into three lobes in many Teleostei species. The fish liver is generally reddish-brown because of its rich vascularization, tending towards yellow when fat storage is high (Datta-Mushi and Dutta, 1996). In *Dicentrarchus labrax* has been seen livers of a yellowish colour due to artificial feeds responsible for lipid accumulation (Bac *et al.,* 1983; Datta-Mushi and Dutta, 1996).

Present study is integrated into the Life Baqua project (LIFE15 ENV/ES/000157; 2016-2020), which has as its main objective to establish a new circular economy model for the use of residues generated by the cultivation of bananas, specifically the pseudostem of the plant or "talo". Starting from this residue and through a mechanical process, two different raw materials can be obtained: high quality natural fiber and residual pulp. With the residual pulp of the fiber extraction process, it is intended as a

great technological novelty, the development of antioxidant additives that serve as a complement in the preparation of feed for fish feeding in aquaculture. Also, the project aims to develop the use of other waste from the banana culture, such the flowers that are removed to let the fruit grow (Life Baqua Project).

Spain is the main banana producer in the EU with 60% of total production. The Canary Islands, in turn, is an important producer within the EU, with a production of 406,225 tonnes in 2019 (ASPROCAN, 2019). The residues generated in the banana production are underused globally. Therefore, in an important region in its production such as The Canary Islands, it can be an interesting objective to develop.

The Life Baqua project was structured in three consecutive phases of dietary development, a first generation with tilapia, where the talo and flower were tested; the second generation with sea bass with the same raw materials and finally a third generation was designed from previous results where banana flower extract was tested against its residue in 2 consecutive trials with tilapia and sea bass.

As a summary, design and growth results for these generation trials and the banana flower for sea bass and tilapia were:

Tilapia. – fish were fed with control diet and four diets with banana flower flour inclusion for 76 days. After that, an osmotic challenge was performed; the water salinity was increased from 7 ppm to 21 ppm. Samples were taken at 0 hour, 3h, 24h and 48h for blood analysis, biochemical composition, histology of the liver, gill and gut and oxidation enzymes in the liver, from 2 fishes per tank and each sample moment. For tilapia, there were no significant differences between C, F3 and F0.5 in growth, so the objective of this work is justified, unless dependent of the rest of the physiological results.

Sea bass. – fish were fed for one month with control diet, and three diets with banana flower flour inclusion. Growth and performance parameters were taken at the end of the trial apart from biochemical composition, fatty acid and digestive and liver histology, and macrophages extraction from the head kidney to evaluate the reaction to an in vitro viral infection trough the Mx gene expression, also samples for muscle and digestive enzymatic analysis were taken. From the growth side parameters and the sea bass, BF6 presented the best growth.

Objectives

The aim of present study was to evaluate possible liver histological effects of the inclusion of banana flower in feeds for tilapia (*Oreochromis niloticus*) and sea bass (*Dicentrarchus labrax*), to contribute to better understand their fish uses and commercial ingredient applications. To do this, sample recovery and histopathological analysis were performed in initial and final fish tissues, in order to determine if there was any effect or damage on the key liver tissue, and to be able to differentiate the best dietary levels from the histological point of view.

Material and methods

Integrated in the Life Baqua Project, experimental design was done in the Universidad de Las Palmas de Gran Canaria (ULPGC), concretely in the facilities of Parque Científico Tecnológico Marino de Taliarte (PCTM). For tilapia experiment twelve specimens were selected, which were fed with five diets: control diet, F0.2, F0.5, F1 and F3 (being 0, 0.2, 0.5, 1 and 3 the percentage of banana flower dietary inclusion); fish were stocked in 60 l aquarium and daily feeding 3 times (09:00, 12:00, 15:00) to apparent satiation until duplication of the initial fish weight. For the sea bass trial, twelve cylindroconical 500 L tanks (Fig. 1) with thirty specimens each were selected; fish were fed with four different diets: Control diet (C, no banana flower), BF1, BF3 and BF6 (being 1, 3 and 6 the percentage of banana flower dietary inclusion).

Fig 1. A view of the sea bass culture system used in present trial

Tanks systems for both species were controlled under GIA protocol for Recirculating Aquaculture System (RAS), with reused water continuously. The produced waste product like solid waste, ammonium and CO2, are either removed or converted into non-toxic products by the system components. The purified water is subsequently saturated with oxygen and returned to the fish tanks (Parisi *et al.,* 2014; Edwards, 2015). Specimens were fed during one month, accounting the daily amount of food supplied. Data of total weight and total length was collected during samplings. In the samplings, two fishes from each tank were sacrificed with an overdose of clove oil, an anaesthetic. Intestine and liver samples were collected and fixed in 4% formaldehyde.

Then in the laboratory, intestine and liver were cut a stored in cassette, being fixed in formaldehyde due its compatibility with majority stains used in histology, penetration speed, disinfecting agent, to be good fixing and cheap price how explain García del Moral (1993). The cassettes went to an automatic tissue processor for de hydration (Jung Histokinette, 2000) (Fig.2).

Fig 2. automatic tissue processor (Jung Histokinette, 2000).

In this processor the cassettes are submerged in a sequence of ethanol upward alcohol content 70%, 96% and 100%. The process let for 18 hours, so it´s took several weeks to get all the samples ready. Finally, the cassettes were submerged in paraffin to make blocks, making a total of 20-30 per day in this case. The samples were placed in mold with melted paraffin and fixed on a cold plate. Then blocks were cut by microtome (Leica Autocut 2055). The cutting band was placed in a ghot bath and glued on a slide. When the slides were dry they went into the stove thirty minutes to proceed with the stains.

Two types of stains were performed: haematoxylin eosin and alcian blue (Martoja and Martoja-Pierson, 1970), therefore each slide will be in duplicate. Both stains are similar but present some differences in the procedure. Below are the steps (Table 1 and 2) that were followed:

Table 1. Alcian blue stain's steps:

Xylol	2 min
Xylol	2 min
Ethanol 100%	2 min
Ethanol 100%	2 min
Ethanol 70%	2 min
Distilled water	2 min
Distilled water	2 min
Distilled water	2 min
Alcian blue	30 min
Running water	2 min
Running water	10 min
Periodic acid 0.5%	5 min
Distilled water	10 min
Schiff reactive	15 min
Running water	10 min
Harris' hematoxylin	2 min
Running water	10 min
Ethanol 96%	2 min
Ethanol 96%	2 min
Ethanol 100%	2 min
Ethanol 100%	2 min
Xylol	2 min
Xylol	2 min
Xylol	2 min

Table 2. Haematoxylin eosin stain's steps:

Xylol	2 min
Xylol	2 min
Ethanol 100%	2 min
Ethanol 100%	2 min
Ethanol 70%	2 min
Distilled water	2 min
Distilled water	2 min
Distilled water	2 min
Harris' haematoxylin	15 min
Running water	30 s
Hydrochloric alcohol	4 dips
Running water	15 dips
Ammonia water	15 dips
Running water	10 min
Ethanol 96º	1 min
Eosin	5 min
Ethanol 96%	2 min
Ethanol 96%	2 min
Ethanol 100%	2 min
Ethanol 100%	2 min
Xylol	2 min
Xylol	2 min
Xylol	2 min

To finish the process the slides were covered with a coverslip with DPX mounting medium for observation under a microscope. Finally, after several weeks of work to have all the samples ready, they were individually observed and taken micrographs by microscope camera (OLYMPUS XC50). Micrographs of each individual section were taken at a final magnification of 20× and 40×. Then hepatocellular area and maximum and minimum hepatocyte length measures were taken with the program Image-Pro Plus 6.0 (Media Cybernetic, Maryland, U.S.A.).

Statistical analysis of the obtained results was done with the program IBM SPSS Statistics v.25. Data reported were analysed by analysis of normality and equality of variance with $P<0.05$. This analysis allowed to show if there was a relationship between variables and verified if there were significant differences between them. After this the Kruskall-wallis test was carried out to determine if there are significant differences by dietary couple.

Results

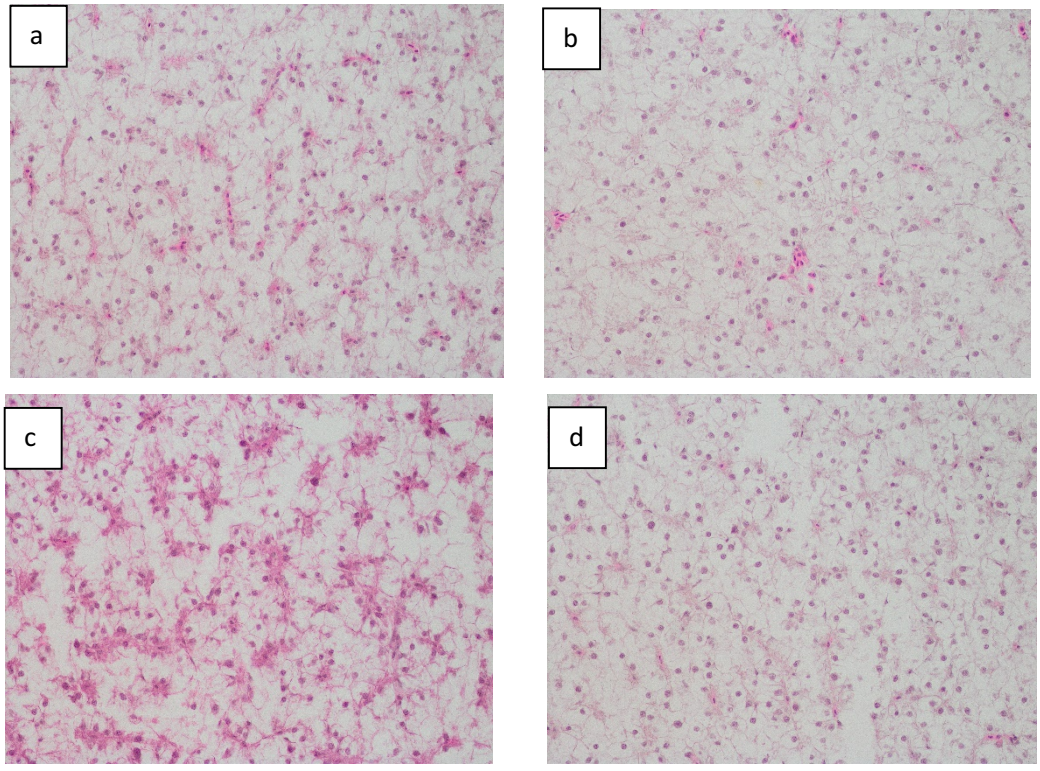
The data obtained showed the state of the liver tissue of both species after feeding the different banana flower levels. For tilapia, fish fed diets F0.2 and F3 presented the biggest hepatocellular area and largest maximum and minimum length, whereas fish fed diets C+ and F1 had the smallest hepatocellular area as well maximum and minimum length. For the hepatocellular area no significant differences were found between F1-C+. As for the maximum and minimum cell length, no significant differences were found between F1-C+ and F0.2-F0.5 (Table 3).

Regarding general liver morphology, histological observations, the control diet presented a more regular morphology of hepatocytes, with better centrated nuclei. In this case, F1 (d) demonstrated closer figures to those of the fish fed the control diet (a) (Fig. 2).

Table 3. Morphometric values of hepatocytes from tilapia (*Oreochromis niloticus*)

	DIET				
	C+	**F0.2**	**F0.5**	**F1**	**F3**
HEPATOCELLULAR AREA	55.12 ± 16.47^{a}	100.4 ± 44.33^{b}	95.5 ± 51.67^{c}	61.28 ± 18.21^{a}	178.38 ± 56.53^{d}
MAX LENGTH	10.14 ± 1.8^{a}	12.97 ± 2.89^{b}	12.49 ± 3.19^{b}	10.52 ± 1.85^{a}	17.61 ± 3.66^{c}
MIN LENGTH	6.37 ± 1.41^{a}	8.68 ± 2.28^{b}	8.3 ± 2.74^{b}	6.77 ± 1.59^{a}	11.41 ± 2.36^{c}

*Different letters in same row denote significant different (P<0.05).



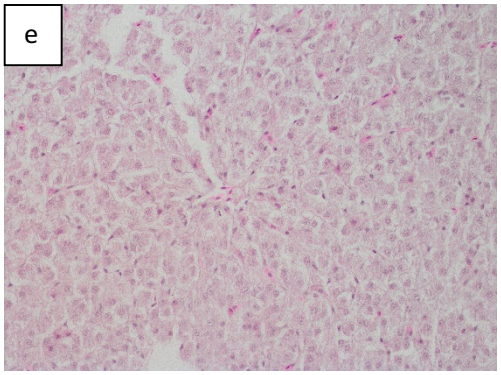


Fig 3. Hepatic samples of liver of *Oreochromis niloticus* stained with H&E evaluated by optic microscopy x40. Diet: Control (a), F02 (b), F05 (c), F1 (d) and F3 (e).

For sea bass fish fed diets C+ and BF3 presented the biggest hepatocellular area, whereas fish fed diets BF1 and BF6 had the smallest. Moreover, fish diets C+ and BF3 presented the largest maximum length too, whereas fish diets C+ and BF6 presented the smallest minimum length. As for the hepatocellular area no significant differences were found between BF1-BF6, equally for the maximum length. However, for the minimum length no significant differences were found between BF6-C+ (Table 4). The BF1 and BF6 diets showed less deformation in the hepatocytes, since they are smaller than those of the control diet (Fig. 4).

Table 4. Morphometric values of hepatocytes from sea bass (*Dicentrarchus labrax*)

	DIET			
	C+	**BF1**	**BF3**	**BF6**
HEPATOCELLULAR AREA	300.17 ± 120.41^{a}	218.12 ± 61.55^{b}	401.64 ± 195.10^{c}	243.99 ± 65.14^{b}
MAX LENGTH	22.81 ± 5.19^{a}	19.31 ± 3.9^{b}	26.27 ± 6.95^{c}	20.62 ± 6.07^{b}
MIN LENGTH	15.15 ± 3.69^{a}	13.02 ± 2.33^{b}	14.27 ± 2.55^{c}	15.2 ± 4.15^{a}

*Different letters in same row denote significant different ($P<0.05$).

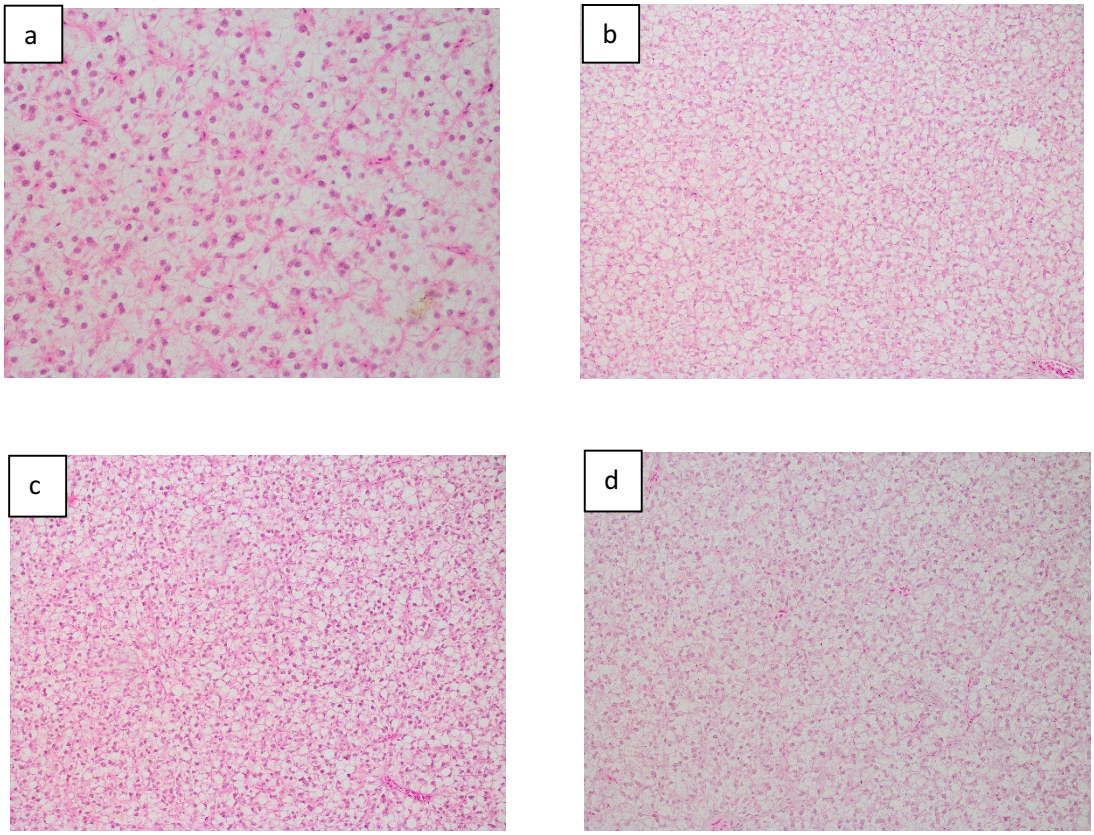


Fig 4. Hepatic samples of liver of *Dicentrarchus labrax* stained with H&E. Seen with optic microscopy x20. Diet: Control (a), BF1 (b), BF3 (c) and BF6 (d).

Discussion

Fish growth is highly dependent on feed composition and quality (Oniszczuk *et al.,* 2016). Many studies are currently under way to test the efficacy of herbal supplementation in the feeding of fish to control fish diseases and produce healthy fish. In conclusion to some of those studies, herbal food supplements favour growth, minimize stress, improve immunity and prevent various infections in fish, so this will help produce healthy fish for human consumption (Shakya, 2017).

An example where the use of herbs gives positive results in growth is Ji *et al.,* (2007). In this study the effects of dietary medicinal herbs on growth and some non-specific immunity were investigated in juvenile red sea bream (*Pagrus major*). Some medicinal herbs and their mixture in diets induced a higher growth performance than fish fed with the diet control. It was concluded that juvenile bream have a greater survival, weight gain, nutritional efficiency and immune activity in a 0.5% dietary addition of a mixture of four herbs. On the other hand, there are studies that have reflected a negative

effect on fish growth due to the inclusion of plants. In Mundheim *et al.,* (2004) the effect on fish (*Salmo salar* L.) growth performance was assessed by replacing fishmeal with a mixture of plant protein sources. It was concluded that a greater dietary inclusion of the mixture of plant proteins negatively affected the digestibility of nutrients and as a result the growth of fish was reduced.

Based on the results obtained we have that for sea bass the diet that presents the best growth of fish is BF6. This diet has the highest percentage of growth and also the best value of FCR (Feed Conversion Ratio). Looking at the amount of fat present in the livers it is also observed that the diet with the lowest percentage is BF6. Having these results one might think that a more functional liver would be related to better fish growth. These results are consistent with Ji *et al.,* (2007) which says that herb blend diets that showed greater weight gain and feed efficiency also had a slightly low lipid content. These changes could indirectly indicate that herbs promote the use of cellular lipids and fatty acids and metabolism as an energy source, resulting in good growth performance. Also these results are consistent to Yılmaz *et al.,* (2012) who says that the thyme diet improved growth and nutrient utilization in the sea bass (*Dicentrarchus labrax*).

As for Tilapia the diet with better growth parameters is control, also having the lowest percentage of fat in the liver. These results are consistent with Obaroh (2014) which says that for *Oreochromis niloticus* liver in this work showed increase in alteration of liver and other organs as the level of inclusion of *Azadirachta indica* leaf extract increases and therefore obtaining better results in fish fed with diet control. In the study of Mostafa *et al.,* (2009) the use of fenugreek flour (FKSM), as a natural feed additive in diets for tilapia fry (*Oreochromis niloticus*) was evaluated. Individuals fed the diet containing 1 % FKSM had significantly higher growth parameters (body weight, weight gain, and specific growth rate) than those fed the control diet. On the other hand, these results contrast with Metwally, (2009) who says that the highest growth in *Oreochromis niloticus* was observed in fish fed with diets containing garlic, as well as lower levels in percentage of fat in tissues. Also according to Ergün *et al*., (2008) the inclusion of 5% of Ulva in the diet of tilapia (*Oreochromis niloticus*) improved growth, food efficiency, nutrient utilization and composition.

Looking at the results we see that there are different results if we compare both species. For Sea bass the best result is obtained with a BF6 level in the diet, while for Tilapia the best results are observed with the control diet. The differences between the two species may be due to their feeding. On the one hand we have the sea bass that is omnivore and on the other the tilapia that is herbivore.

The failure to obtain negative results can be considered an important advance, since we are using a by-product that comes from another industry and therefore could serve to reduce the use of other flours that can compete with human consumption, such as corn flour.

Conclusions

To conclude, it is determined that for sea bass (*Dicentrarchus labrax*) the best dietary level is BF6, as this has less deformation of the hepatocytes and better growth parameters of the fish. However, for tilapia we observed that the diet control gets better results than those that have inclusion of banana flower.

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Memoria

- Descripción de las actividades realizadas:

Desde el primer día me integré en el GIA (Grupo de investigación en Acuicultura), donde desarrollé mi actividad entre las instalaciones de selección del PCTM (Parque Científico Tecnológico Marino) y el edificio del SABE (Servicio de Acuicultura y Biotecnología de Alta Especialización), donde fui asignado al laboratorio de histología.

En el laboratorio de histología bajo la supervisión de una técnico cualificada empecé a tallar, tarea que consistía en realizar cortes de intestino anterior, posterior y de hígado, meterlos en casets y fijarlos en formol, para su posterior deshidratación mediante un procesado. Seguidamente las muestras se fijaron en parafina y se realizaron los cortes, colocando cada uno en portaobjetos. Finalmente se realizaron dos tipos de tinciones de las muestras con hematoxilina eosina y azul alcian para su observación al microscopio. Al terminar la observación procedí a sacar fotos de las muestras de intestinos e hígado con la cámara de microscopía, las cuales fueron analizadas mediante un programa de tratamiento de imágenes (Image-Pro Plus) para tomar una serie de medidas.

A su vez en las instalaciones de selección alimentaba a los ejemplares de lubina, dando las tres tomas diarias de lunes a viernes, pesando al final del día el pienso sobrante, dejando preparado el necesario para las tomas del día siguiente y desaguando para mantener los tanques limpios,

- Formación recibida:

En el laboratorio se me explicó el funcionamiento de los aparatos que iba a manipular durante el desarrollo de mi actividad.

Aprendí a realizar los bloques de parafina, a realizar los cortes de los mismos y la forma correcta de hacer las tinciones. Por último se me explicó uso del programa de tratamiento de imágenes.

En selección se me enseñó la forma adecuada de alimentar a los ejemplares de cada tanque y a desaguar para el mantenimiento de los tanques.

- Nivel de integración e implicación dentro del departamento y relaciones con el personal:

Dentro de la dinámica del Parque Científico me sentí muy integrado, gracias especialmente a todas las personas que me encontré durante la duración de las prácticas. Mi intención siempre fue la de estar los más implicado posible ayudando siempre en lo

que hiciera falta, así como todos siempre estaban dispuestos a ayudarme y hacerme lo más sencilla posible mi estancia en las instalaciones.

- Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFG:

Como aspectos positivos destaco el poder adquirir nuevos conocimientos e integrarme en la dinámica de un parque científico, desarrollando actividades que realmente me apasionan y la disposición para ayudar siempre de todo el personal con el que pude tratar durante mi estancia en el parque y su cordialidad.

- Valoración personal del aprendizaje conseguido a lo largo del TFG:

En general me llevo una valoración muy positiva de todo el aprendizaje adquirido a lo largo de la realización del TFG y la satisfacción de haber trabajado en cosas que realmente me apasionan. Finalmente agradecer la ayuda prestada a mi tutora Lidia y a todo el personal del parque, especialmente a Sara, mi cotutora, quien fue la persona que estuvo conmigo día a día para enseñarme y ayudarme en todo.