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PhD thesis

Approach to Circular Economy through the inclusion of local agriculture by-products in fish feedstuff: consumer health risk assessment due to heavy metal accumulation and effects on fish health by study of liver and gut morphology

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Thesis title **"Approach to Circular Economy through the inclusion of local agriculture by-products in fish feedstuff: consumer health risk assessment due to heavy metal accumulation and effects on fish health by study of liver and gut morphology"**

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Directed by Dr. **RAFAEL GINÉS RUIZ**

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Las Palmas de Gran Canaria, in 17 March 2023.

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GINES RUIZ, RAFAEL FERMIN	CASTRO ALONSO, PEDRO LUIS	SELWAN MOHAMED HARB RABIA

To

My lovely husband ISLAM,

my handsome son ADAM,

my beautiful daughters

LAMAR and LUCINDA,

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"All praise is due to Allah, the Lord of the Worlds, the Beneficent, the Merciful"

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Abstract

The circular economy refers to an industrial economy that is restorative by intention, designed for reducing the carbon footprint and employing a more sustainable and eco-friendly use of resources. For this purpose, sustainability and efficiency must be applied to agricultural practices by improving management and reducing waste of native crops and revalorizing it into a series of new value chains. New economic models must adapt to the requirements of sustainability brought about by climate change. The recycling of materials that still are valuable in their end-of-life phase and closing production loops are one way to use resources more efficiently and minimize the impact on the environment. This new way of thinking will promote bio-based industries for the recovery, transformation and revalue of by-products from the primary and secondary sectors.

When formulating an aquafeed diet, the potentiality of each component must be evaluated not only from their productive responses but also from any synergic effects on the animal well-being, health and resistance. Moreover, possible contaminants related to plant growth or products and by-products processing should be addressed. The current study aimed to determine the metal content of Aloe vera and banana by-products as sustainable alternatives to standard ingredients in aquaculture diets, develop diets with varying levels of inclusion of these by-products in accordance with the circular economy concept, validate diets for fish, and assess the accumulation of pollutants as well as various risk management strategies and nutritional value. Also, assess the effects of adding banana meal to the diet on the growth, biochemistry, and histomorphology of the liver and intestine in juvenile tilapia.

In our study, two different plant by-products Aloe vera and banana crop by-products, as well as golden mullet *(Liza aurata*) and tilapia were evaluated (*Oreochromis niloticus*). For each of the by-products, four isocaloric and isoproteic diets were made: one of them was a commercial diet customized to the species being studied, and the other three contained increasing amounts of the raw material: 5, 10, and 20% of banana by-products and 2, 4, and 6% of Aloe vera by-product. All diets were analyzed both for biochemical composition and content in metals. risks arising from fish metal content has been measured using various parameters as Estimated Daily Intake (EDI), Maximum Safe Consumption (MSC_A), Target Hazard quotient (THQ), Hazard Index (HI) Carcinogenic risk of As (As- CR), the Value Selenium Health Benefit (Se HBV) and also the Nutritional Values has been evaluated.

In both trails it was found that in the various ratios of Aloe vera and banana by-product in diets, the content of all elements was less than the upper limits permitted. According to the different risk-benefit analyses used by national and international authorities, consuming *Liza aurata* and *Oreochromis niloticus* is generally safe. Although there were detectable levels of potentially dangerous inorganic As, they were below safety guidelines, and Se-HBV values showed benefits to human health.

In the third trail, the same technique by using the banana by-product with different inclusions in Nile tilapia diets but to evaluate the growth rate, proximal composition of the fish fillet and the effect on liver histology whence the level of vacuolization (steatosis), the presence of necrosis foci or pyknotic nuclei, nuclear pleomorphism, or the emergence of vascular alterations in the hepatic tissue and in the gut histology the height and width of the villi was measured. Moreover, the various fatty acids identification and quantitation was performed.

The finding of this study clarified that the banana ensure that the tilapia would grow and perform adequately which the consumption of banana by-products increased the rate of growth, as measured by weight and length, both during the experimental test and after. During histological study, the feeding conditions had no adverse impacts on growth or proximal composition, and neither the liver nor the gut seemed to have any unusual effects. Moreover, the presence of banana by-product had no effect on the lipid content of tilapia liver, although there was a slight change in the foregut and hindgut folds.

As a result, the banana by-product was shown to be an effective and environmentally safe way to feed Nile tilapia, which helps to reduce food loss and waste from a circular economy perspective. Our findings support the circular economy concept of employing Aloe vera and banana wastes as an alternative ingredient in fish feeding for *Liza aurata* and *Oreochromis niloticus* in order to lower the cost of the fish diets and ensure their safety for fish and humans.

Chapter 1. introduction

1.1. Circular Economy

The European Union and some national governments are pushing the Circular Economy (CE), a popular idea for long-term sustainable growth. It combines fuzzy concepts with certain elements of science from several technical domains (Korhonen, Honkasalo et al. 2018).

Since the beginning of industry, industrial companies have employed the original idea of materials cycles since it reduced harmful environmental impact, conserved energy, and was economically viable.

The CE is the most recent effort to imagine the sustainable fusion of economic activity and environmental wellbeing. The CE places a strong emphasis on process redesign and material recycling, which may assist in developing more sustainable business models, enhance ecosystem function, and enhance human well-being.

According to predictions, the world's population would increase further and reach 9.5 billion people by 2050. The overall protein demand will rise by an estimated 40–75% to go along with that expansion, with 72% of that demand coming from nations that are now classified as developing, with 70% of that population predicted to live in cities (Jobling 2012). The increased consumption of key natural resources (such as water, energy, and raw materials) to meet the growing needs will undoubtedly result in an unsustainable ecosystem (DeSA 2015). Therefore, Europe produces 1.3 billion tons of rubbish annually, of which 700 million tons are agricultural waste. Best projections indicate that by 2050, agricultural and food production will need to increase by two-thirds in order to feed an extra 2 billion people with acceptable nutrition, given the predicted one-third increase in world population (FAO, 2009). Increased temperatures and altered global precipitation patterns raise the likelihood of crop production losses and the spread of weeds and pests on agricultural land, which intensify the consequences of climate change on agricultural systems (Nelson, Rosegrant et al. 2009).

The aforementioned problems present a sizable potential for the development of a CE that handles the utilization of agricultural wastes, by-products, and coproducts using cutting-edge technology and successful business models. Although recycling has been emphasized, and waste effects have decreased, it has not been successful, since the amount of trash that is not recycled is growing (Preston 2012). The CE aims to provide a model with zero net environmental effects, ensuring that

waste creation and resource utilization are decreased (Murray, Skene et al. 2017). The solution to achieving sustainable development, which takes into consideration ongoing concerns about the depletion of natural resources and the degradation of environmental resources, is known as a CE. A business idea called the CE aims to reduce the number of resources used in the production process (Rekleitis, Haralambous et al. 2020).

The CE is a type of industrial economy that is intentionally restorative, with the goal of lowering carbon emissions and promoting resource use that is more sustainable and environmentally beneficial. To achieve this, agricultural practices must be improved to increase sustainability and efficiency by reducing waste and revalorizing local commodities through a number of new value chains. Climate change has forced new economic models to adapt to the demands of sustainability. Closing production loops and recycling materials that are still valuable in their end-of-life phase are two ways to use resources more efficiently and reduce environmental impact. This new way of thinking will encourage the recovery, transformation, and revaluation of by-products from the primary and secondary sectors through bio-based enterprises.

The principal sources of food are agricultural ecosystems (Aznar-Sánchez, Velasco-Muñoz et al. 2019) (Aznar-Sánchez, Velasco-Muñoz et al. 2020). These systems are not only essential for supplying raw resources like food, fiber, and other commodities, but they also consume the most freshwater globally (Velasco-Muñoz, Aznar-Sánchez et al. 2019) (De Corato 2020).

Sustainable agriculture comprises meeting human demands for feed, food, crops, fuel, and energy while simultaneously protecting the environment and preserving natural resources for present and future generations. In contrast to earlier and current economic systems, a bioeconomy's goal is to use all of the resources (water, land, and energy) available to sustain the expanding human population (Rekleitis, Haralambous et al. 2020).

Biological methods may be utilized to create new goods or enhance existing ones using a variety of agricultural wastes as suitable raw materials, providing a big potential for industry to innovate. Many agricultural material flows are perceived by the relevant observer as waste, despite the fact that they may potentially be beneficial resources for the agricultural system. Numerous agricultural wastes, often referred to as by-products, coproducts, or residues, are unavoidable by-products of the food production processes (e.g., manures, crop residues, leaves, peels).

The phrase "circular bioeconomy" is more explicitly used to describe the utilization of waste as an ingredient in aquafeed or as a standalone source of nutrients. It is suggested that the final driving

factor behind aquafeed will be circular bioeconomy (Colombo and Turchini 2021). A notable illustration of a circular bioeconomy is integrated aquaculture (IA), more especially integrated multitrophic aquaculture (IMTA). A non-fed plant or animal that fills a different ecological niche is grown using the waste products from a fed organism as food sources. Due to the fact that the amount of aquafeed provided is the same for the combined production of two species when using two producing species, production efficiency is boosted (Boyd, D'Abramo et al. 2020).

The conservation and improvement of natural capital, resource efficiency optimization, and system efficiency promotion are the three guiding concepts of the CE (MacArthur 2013) A CE strategy in agriculture strives to decrease waste, close nutrient cycles, restrict the use of external consumables in agricultural production, and recover agri-food by-products (Toop, Ward et al. 2017) (Muscio and Sisto 2020). Regarding the handling of agricultural waste, the bioeconomy may be described as "circular by nature" (MacArthur 2013) and "the renewable sector of the CE."

1.2. Overview of aquaculture

The world's increasing population expansion has caused an increase in the animal protein food gap. Increased demand for fisheries products from inland and marine sources, whose productivity is already being harmed by excessive fishing pressure, growing organic pollution, toxic contamination, habitat degradation, and climate change, is a result of excessive fishing pressure, growing organic pollution, toxic contamination, habitat degradation, and climate change (MASKE and SATYANARAYAN 2012). Aquaculture has so recently become well-known as one of the most major suppliers of animal protein.

The fastest-growing food-producing market is the aquaculture sector, which produces more than half of the world's fish, at a rate of 7-9 percent every year, compared to 2-4 percent for the other farmed animals (Galappaththi, Ichien et al. 2020). Around the world, aquatic products represent a key source of animal protein: 17% of animal protein consumed globally and 7% of all protein consumed comes from aquatic items (APROMAR 2021). Aquatic animal output was expected to have decreased slightly from the record-breaking year of 2018 when it reached 179 million tonnes to 178 million tons in 2020 (Figure 1.1). With a value of more than USD 265 billion, aquaculture output hit a record-high of 88 million tons in 2020 (FAO 2022).

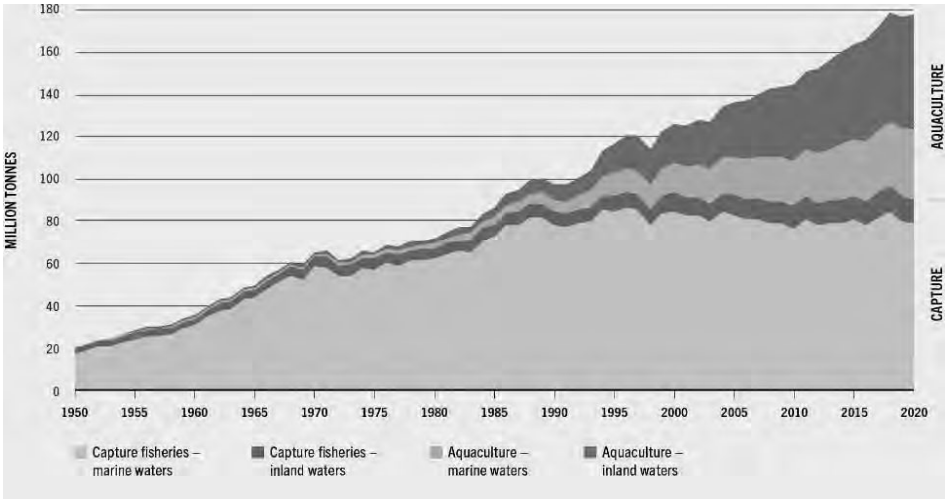

Figure 1.1. World capture fisheries and aquaculture production (Source: FAO, 2022)

Products from aquaculture play a crucial role in providing cheap, high-quality protein to people all around the world. Fish protein has a high nutritional value due to its well-balanced amino acid profile, ample amounts of polyunsaturated fatty acids (PUFA), and a range of vitamins and minerals (Edwards 1997). Additionally, fish meat is often soft since it has less connective tissue than other meats (such as cattle, mutton, pig, and fowl) and is easy for people of all ages to chew, especially children and the elderly (Tibbetts 2001).

In addition, aquaculture plays a crucial role in supplying millions of people with a means of livelihood worldwide. Millions of people throughout the world rely on the fishing and aquaculture sectors for their livelihood. About 14% of the 59.51 million people employed in the primary sector of catch fisheries and aquaculture in 2018 were women. 38.98 million persons were employed in fishing, compared to 20.53 million in aquaculture (FAO, 2020). Input and output processing are only two examples of the many aquaculture-related operations that many more people are involved in.

Approximately 156 million tons, or 88 percent, of the world's fisheries and aquaculture production in 2018, were used for direct human consumption. The production of fishmeal and fish oil accounted for the majority of the remaining 12% (22 million tons) of the total. 44% of the fish intended for human consumption were live and fresh (FAO, 2020).

Around the world, many different fish species are raised. The majority of cultured fish are herbivorous freshwater species, with silver carp (*Hypophthalmichthys molitrix*), forage carp

(*Ctenopharyngodon idellus*), and common carp (*Cyprinus carpio*) being the most popular. Nile tilapia (*Oreochromis niloticus*) and rohu (*Labeo rohita*) being the next most popular.

Among the most farmed species in industrialised nations include Atlantic salmon (*Salmo salar*), rainbow trout (*Onchorynchus mykiss*), catfish (*Ictalurus punctatus*), Japanese rookery (*Seriola quinqueradiata*), gilthead sea bream (*Sparus aurata*), black bocine (*Pagrus pagrus*), and European sea bass (*Dicentrarchus labrax*) (Pauly, Christensen et al. 1998).

1.2.1. Nile tilapia (*Oreochromis niloticus*)

Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758), has the following systematic position:

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Superclass: Gnathostomata
Class: Actinopterygii
Order: Cichliformes
Family: Cichlidae
Genus: Oreochromis
Species: O. niloticus

Tilapia, a huge tropical freshwater fish species with bilaterally compressed bodies and parental care, are a member of the Cichlidae family (Figure 1.2). The Nile tilapia, Oreochromis niloticus, is a deep-bodied fish with cycloid scales. Both the Middle East and central and northern Africa contain it (Boyd 2004). It is a tropical freshwater and estuarine species.

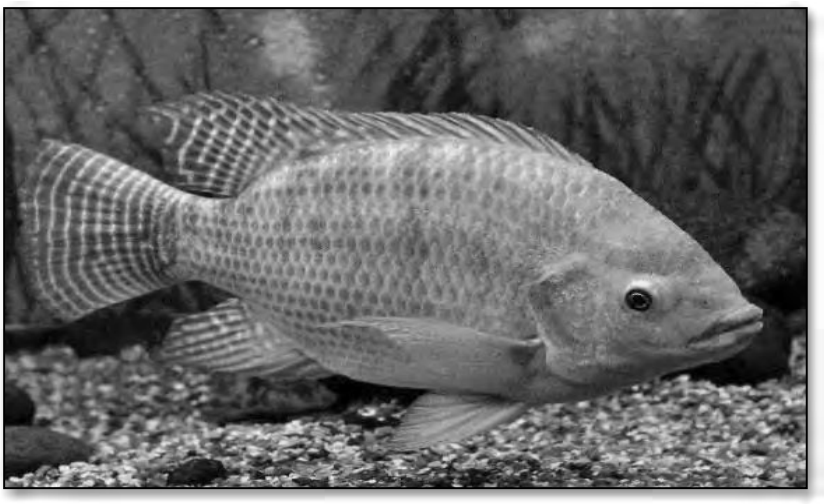

Figure 1.2. Nile tilapia, prototype specimen.

It is considered to be the most promising for fish farming due to its quick development in captivity and high-quality flesh (Furuya, Fujii et al. 2008). Due to its quick development rate, resistance to illness, and low trophic feeding levels, tilapia farming is widely practiced in tropical and subtropical regions. The second-largest farmed fish in the world, tilapias account for 125 percent of freshwater fish production and 107 percent of global fish farming (FAO, 2018). The output of farmed tilapia worldwide grew by 3.3% in 2020, breaking the 6 million tons barrier for the first time.

The success of Nile tilapia farming is primarily due to the fish's simplicity of culture and appealing qualities as food (Lucas, Southgate et al. 2019). These characteristics include ease of reproduction in captivity, resilience to crowding and unclean water, and low sensitivity to disease. According to (Hussain 2004). Nile tilapia can survive in water that is 12 to 35°C, pH 6.5 to 8.5, with dissolved oxygen levels between 2.0 and 8.0 mg l-1 and salt concentrations between 3 and 25 ppt.

Over 75 countries produce tilapia, making it one of the most widely consumed farmed fish worldwide, and output is increasing (Josupeit 2005). In 2009, salmon was surpassed by tilapia, which is now among the top five fish meals in America (SPC, 2011).

As a food fish, it may be prepared to suit a broad range of tastes and preferences because to its white flesh, neutral flavor, and firm texture. These qualities have led to the Nile tilapia being referred to as "aquatic chicken" (Pullin 1984). Due to their very adaptable and opportunistic dietary habits, adult *O. niloticus* are classified as omnivore (Jauncey 1998). They may consume a variety of things, such as detritus, periphyton, and phytoplankton (Beveridge, Baird et al. 2000).

1.2.2. Golden mullet (*Liza aurata*)

Golden mullet *Liza aurata* (Risso, 1810) has the following systematic position:

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Superclass: Gnathostomata
Class: Osteichthyes
Order: Perciformes
Suborder: Mugiloidei
Family: Mugilidae
Genus: Liza
Species: Liza aurata

With a wide range of distribution in tropical and temperate fresh, brackish, and coastal marine settings, mugilids are among the most widely distributed teleost fishes (Figure 1.3). The golden mullet (*Liza aurata*) lives in the Mediterranean, Black, and Caspian seas as well as along the Atlantic coast from Scotland, southern Norway, and Sweden to Morocco (Karapanagiotidis, Karalazos et al. 2014). It is a neritic species that prefers to stay along the coast, entering ports, estuaries, and lagoons, but very seldom venturing into freshwater. It occasionally consumes plankton, small benthic organisms, and trash, including insects.

Figure 1.3. Golden mullet, prototype specimen.

The golden mullet, along with other members of the Mugilidae family that inhabit coastal lagoons and estuaries, is a crucial species for the growth of artisanal fisheries in these areas (Katselis, Koukou et al. 2007). In tanks and reservoirs, it has been cultivated extensively or somewhat intensively for millennia. In 2010, 134.329 million tons were produced worldwide (Karapanagiotidis, Karalazos et al. 2014). Its focus on inexpensive items, with a high proportion of commodities and by-products of vegetable origin, which are challenging for higher trophic level animals to digest, is another factor in captive feeding.

Studies comparing diets with different levels of protein (ranging from 25% to 45%) have found that, despite the species' relatively slow growth rate, no significant differences in the growth of the groups fed the various percentages of protein were observed, despite a trend to the contrary. This is despite the paucity of information on these species' food utilization and growth (Karapanagiotidis, Karalazos et al. 2014).

In numerous experiments, the golden mullet and other Mugilidae have been used as a biomarker of environmental pollutants like cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), nickel (Ni), copper (Cu), zinc (Zn), or iron (Fe), allowing researchers to evaluate the state of conservation or health of various ecosystems under different conditions.

1.3. Nutrition and feeding for Sustainable Aquaculture.

Production from aquaculture has outpaced harvest from catch fisheries, demonstrating its value to the world's food security. A rising number of papers offer data and justifications underscoring aquaculture's potential for future expansion. To be able to achieve the objectives of sustainable development, this sector must overcome several obstacles. In this situation, nutritionally enhanced feeds from sustainable sources are essential for the growth of aquaculture since they not only have a lower impact on the environment but also benefit the natural capital (Mustafa 2022).

Fish and other aquatic animals require an adequate supply of nutrients, both in terms of quantity and quality, for growth, health, and reproduction, regardless of the culture system in which they are generated. Input supplies (feeds, fertilizers, etc.) must be guaranteed in order to satisfy the system's production objectives as well as the nutritional and energetic needs of the species being cultivated. One of the issues impeding the growth of aquaculture is the high cost of high-quality fish feed (Tiamiyu, Okomoda et al. 2015). Due to a growing demand for these traditional protein feedstuffs, such as soybean meal and fish meal, as a staple diet for people, a raw material for businesses, and a component in farm animals' feed, they may be scarce and expensive.

Fish has a high biochemical value for humans and is a nutrient-dense food that is high in proteins. Additionally, it is a great source of polyunsaturated fatty acids (PUFA), which are proven to lower the risk of psoriasis, other skin disorders, breast and colon cancer, and cardiovascular disease (Kaushik 2001). Highly unsaturated fatty acids (HUFA) present in marine fish oil have been demonstrated to be helpful in the treatment of inflammatory disorders and ischemic heart disease by modifying the arachidonic acid/prostaglandin pathways (Sargent and Harris 1992). Iodine, selenium, and fat-soluble vitamins (A and D) are among the micronutrients found in fish that are good for human health. Many underdeveloped countries across the world consume little fish whole, which adds calcium, phosphorus, and iron to the human diet.

We might be able to boost the nutritional value and benefits of the fish we eat thanks to advancements in aquaculture feed and nutrition. The nutritional content, color and appearance,

aroma and flavor, texture, and storage capacity of a crop can all be affected by the quality of the nutrients and feed used during production.

The availability of nutrients determines aquaculture production much like it does for other terrestrial agriculture methods (Tacon and Metian 2008). Nutritionally balanced diets are essential for enhancing fish health and raising production.

Protein and energy are essential components of fish diets for maintenance, growth, and reproduction. When given the proper amount and quality of food, fish can develop to their maximum potential. Cereals and fishmeal have historically been used as sources of protein and energy (El-Sayed 1999, Gatlin III, Barrows et al. 2007). However, they are frequently either beyond of reach or inimical to concerns about food security.

Complete fish feeds include a lot of protein, which is also the most expensive ingredient and makes up more than half of the total feed expenses in intensive aquaculture (Thompson, Muzinic et al. 2005). For fish to develop and reproduce or to restore depleted protein, they require the amino acids needed for tissue formation (maintenance). In fish tissue, protein is the most prevalent organic material, making up between 65-75% of the total weight on a dry matter basis (Wilson 2002). Dietary protein is therefore prioritized in the formulation of complete meals to prevent insufficiency, which can result in subpar growth and weight loss.

Despite not being necessary, carbohydrates are a cheap source of energy in fish diets (Kaushik 2001). When non-protein energy sources are insufficient, protein is used as a source of energy rather than growth (lipid and glucose). One of the favored sources of carbohydrates is cereal and its by-products. Cereals provide for the bulk of energy sources in diets due to their high carbohydrate content, especially for omnivorous and herbivorous fish (De Silva and Gunasekera 1989). Along with supplying energy, cereals may increase the protein and fat composition of meals. The majority of cereal carbohydrate (68–72%) is made up of starch, which gelatinizes during pelleting and increases the stability of pellets in water (Jauncey 1998, Hardy and Barrows 2003).

1.3.1. Use of Plant as an alternative feed ingredient

Fish and fishery products are excellent for a well-balanced diet and good health since they are rich in protein and minerals. The demand for premium fish feed has been steadily rising in recent years as a result of the aquaculture industry's quick development. The provision of high-quality fish feed has become the main goal of any aquacultures. Fish feed is crucial to the production and yield

outcome even though it makes up about half of the total production cost (Mzengereza, Msiska et al. 2014).

Because of their superior nutritional qualities (greater protein content, amino acid profile), animal proteins are preferred, but they are also more expensive. For example, fish meal is delectable, simple to digest, and rich in essential amino acids, fatty acids, energy, and minerals (Ogunji 2004). However, its supply is unstable because to dwindling fisheries catches (caused by overfishing, pollution, and climate change), extreme weather, growing demand from the quickly expanding feed industry, and other factors (Naylor, Goldburg et al. 2001).

In developing countries, grains provide about half of the daily caloric intake, making them scarce and costly. The supply of grain is also impacted by low yield in compared to a growing population. Forecasts indicate that rising nations won't be able to meet their demand for grains, which would cause major price increases. As a result, the trend is predicted to continue (Rosegrant, Paisner et al. 2001). The demand for cereals for ethanol production has changed as a result of the growth of the biofuel industry, and/or cereal acreage has decreased in favor of biofuel (Dickie 2007).

Therefore, it is crucial to design affordable, nutritionally full meals that can support expanding intensive and semi-intensive systems using locally available and affordable plant resources. Alternative protein sources for fish culture have been widely researched on a global scale in recent decades (Naylor, Hardy et al. 2009, Hardy 2010, FAO, 2010) due to the dwindling quantity and high cost of fish meal. Because they are more plentiful and less expensive than fish meal, plant components are frequently included in fish diets. Soybean meal, cassava leaf meal, sweet potato leaf meal, groundnut cake (Da, Lundh et al. 2012), pea, horsebean, and rapeseed plant protein concentrates (Lund, Dalsgaard et al. 2011)), as well as cottonseed, sunflower, and cornmeal. As prospective sources of protein for fish diets, jatropha kernel meal (Harter, Buhrke et al. 2011), cowpea (Olivera-Castillo, Pino-Aguilar et al. 2011) and lupin meal (Tabrett, Blyth et al. 2012) have all been investigated.

A lack of protein content, an imbalanced amino acid composition, and the presence of anti-nutritional agents are only a few of the challenges faced by plant components. Anti-nutrients have been defined as substances that disrupt the digestion of food and harm the health and productivity of animals either directly or by the release of their metabolic by-products into living systems (Makkar 1993). In order to protect themselves against predators, plants synthesis phytochemicals. Because of this, using plant components in fish feed without proper processing might seriously

endanger the fish's health. There are many different anti-nutritional variables at play, but they all have the ability to decrease fish feed consumption and nutrient digestion (Bandara 2018).

In addition, certain plant species can hyperaccumulate excess nutrients and/or metal toxins in harvestable root and shoot tissue from the growing substrate through the phytoextraction process. This holds true for organics, nonmetals, radionuclides and metalloids in soil, sediment, and sludge. It's possible for heavy metals to accumulate up in the soil and crops when sewage and industrial effluent are used to continuously irrigate agricultural land (Singh, Mohan et al. 2004, Marshall, Holden et al. 2007, Sharma, Kaushik et al. 2007), for that reason probably the inclusion of the by-products in diets increase the level of metals risk in fish and humans.

1.3.1.1. Aloe vera

Aloe vera, often known as Aloe barbadense, is a stemless succulent plant, wide-spread herb that can be found in tropical and subtropical areas. There are more than 360 species in the genus Aloe (Saljooghianpour and Javaran 2013), of which A. vera is regarded as the most well-known and bioactive herb (Mahdavi, Hajimoradloo et al. 2013), including more than 70 biologically active components. Bitter yellow latex and mucilaginous gel, two forms of exudates released by Aloe leaves, contain these bioactive substances (Kaithwas, Dubey et al. 2011, Thu, Mon et al. 2013). Aloe gel has been found to contain a variety of polysaccharides, including protein, pectin, cellulose, hemicellulose, glucomanna, acemannan, and mannose derivatives, as well as about 20 of the 22 essential amino acids that the body cannot synthesize and several vitamins, including A, B1, B2, B6, C, E, and folic acid, mineral (Ca, Mg, and Na), enzymes (lipase, amylase, carboxypeptidase and more), salicylic acid, lignin, saponins, fatty acids and hormones (Surjushe, Vasani et al. 2008, Adesuyi, Awosanya et al. 2012).

It is one of the many Aloe species that has been acclaimed to manage several health conditions in humans (Abdullah, Abdullah et al. 2003) and in some domesticated animals such as chickens (Akhtar, Hai et al. 2012), dogs (Altug, Yuksek et al. 2010) and cats (Harris, Pierce et al. 1991). In humans, A. vera has been used directly or as extracts to cure ailments such as cuts, minor burns, eczema, inflammation (Arunkumar and Muthuselvam 2009), constipation, gastrointestinal disorders, and immune system deficiency (Boudreau and Beland 2006). A number of health benefits associated with A. vera have been attributed to the polysaccharides contained in the gel of the leaves (Hamman 2008). Other beneficial A. vera phytoconstituents include glycoprotein,

amino acids, anthraquinones, antioxidants compounds, and vitamins A, E, and B12 (López-Cervantes, Sánchez-Machado et al. 2018).

As well it is a perennial plant belonging to the family Liliaceae, which lives in tropical and subtropical regions, and that contains more than 70 biologically active components (Langmead, Feakins et al. 2004) . These benefits have aroused the interest for its use in aquaculture, having shown some positive effects in fish such as the promotion of the survival against pathogens (Kim, Hwang et al. 1999), improvement of growth and immune response (Lu, Luo et al. 2013), hypocholesterolemia effects (Palermo, Cocci et al. 2013) , effects against oxidative stress (Kang, Kim et al. 2014) reduction in the burden of gill parasites (Dotta, de Andrade et al. 2014), activation of the not–specific immune response (Dotta, Ledic-Neto et al. 2015), effects on haemato-biochemical parameters (Gabriel, Qiang et al. 2015).

The Canary Islands is the leading European region in the cultivation and production of Aloe vera due to its particular climatic conditions. The production of Aloe vera in the Canary Islands is around 150 hectares per year (ALOVERIA, 2023) and the annual production is growing every year, helped by national and European funding to the sector.

Those by-products do not have any use, being a logistical problem to the producers that must eliminate them mainly by natural degradation on the land. As the need for a “turn” towards a sustainable production strategy in the global political agenda grows, taking advantage of by-products and promoting reusable biomass makes sense.

Aloe vera consists primarily of water and polysaccharides (pectin, cellulose, hemicellulose, glucomannan, acemannan, and mannose derivatives) and is composed of a long chain of acetylated mannose (Lee, Weintraub et al. 2000). The biological activity of Aloe vera’s polysaccharides has been reported widely (Tan and Vanitha 2004) and shown to act as an immunostimulant (Abdy, Alishahi et al. 2017). Acemannan has been reported to have antimicrobial properties, including antibacterial, antifungal, antiviral, and antiparasitic properties (Alishahi, Dezfuly et al. 2017). Aloe vera is recognized for its widespread use and reported healing powers (Krishnan 2006), alleviating pain and treating a variety of ailments (Shelton 1991). Therefore, Aloe vera has therapeutic qualities such anti-oxidant, anti-cancerous, immune-modulating, antibacterial, anti-septic, anti-inflammatory, and antimutagenic characteristics and effects that promote growth (Freyhof and Kottelat 2008), anti-hypersensitivity (Zanuzzo, Sabioni et al. 2017), and gastrointestinal health (Manaf, Daud et al. 2016) have been widely described.

According to (Quirós-Pozo, Ventura-Castellano et al. 2021) data, the grey mullet species could withstand up to 6% of the aloe by-product in their diets without any negative effects on their quality or growth characteristics. However, no improvement over the control fish could be seen. Also, (Gabriel, Qiang et al. 2015) indicated that dietary aloe supplementation could improve growth, feed utilization, and haemato-biochemical parameters of cultured tilapia.

1.3.1.2. Banana by-product

It is one of the first crops to be grown by humans in recorded history is the banana. This particular plant family's origins are in Southeast Asia, from India to Papua New Guinea (Arvanitoyannis and Mavromatis 2009, De Langhe, Vrydaghs et al. 2009). With an estimated gross production of 139 million tonnes, it is now the second largest fruit crop in the world due to its widespread cultivation and consumption in recent decades (FAO, 2010a).

The banana (Musa spp.) is the most consumed fruit in developed nations and is essential for food security in many tropical and subtropical nations (D'hont, Denoeud et al. 2012). With a contribution of over 17% to global fruit output, bananas are the second most popular fruit after citrus and are grown in more than 130 nations (FAO, 2010b).

According to (Caballero, Finglas et al. 2015, Singh, Singh et al. 2016), every portion of the banana plant has medicinal benefits.

Moreover, the banana has a better digestion than most other fruits and is very nutritive. Their widespread consumption is a result of their appealing texture, flavor, and sensory qualities. It is also a great source of dietary fiber, vitamin C, vitamin B6, and manganese (Kuyu and Tola 2018). It also has a high calorie count with little fat.

Banana peel, which can make up to 35% of the ripe fruit, is a common industrial and household food waste (Emaga, Robert et al. 2008). It is a good source of potassium, polyunsaturated fatty acids, vital amino acids, necessary dietary fiber and proteins (Emaga, Andrianaivo et al. 2007). Also, it contains bioactive substances such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, anthocyanins, and terpenoids. These substances have been shown to have a range of biological and pharmacological effects, including antibacterial, antihypertensive, antidiabetic, and anti-inflammatory activities (Pereira and Maraschin 2015).

The demand for items with organic substrates has significantly expanded in recent years. According to (Waldron 2009, Jayathilakan, Sultana et al. 2012), food waste and agro-food industry by-products contain a variety of functional and bioactive components, such as phenolics,

antioxidants, dietary fibers, flavonoids, anthocyanins, proteins, peptides, and enzymes. In the food chain, bioactive substances can be recovered and reused as supplements, fortifiers, or minor ingredients (Geoffrey 2008, Baiano 2014, Roselló-Soto, Galanakis et al. 2015).

Due to the need to cut off the entire plant so that the young suckers can replace the mother plant, banana farms generate a lot of waste both at the farming site (pseudo stem, leaves, and inflorescences) and at the processing sites (rachis and rejected bananas) where the fruit is packaged. For every tons of fruit picked, around four tons of trash are produced (Souza, Liebl et al. 2014).

This could aid in waste management while also providing a lucrative revenue for banana growers, strengthening the local economy (Oliveira, Cordeiro et al. 2007), especially in the Canary Islands where the banana crop is a crucial economic pillar. Previous research has demonstrated that many banana by-product fractions, such as banana peels (Mokbel and Hashinaga 2005, Sundaram, Anjum et al. 2011, Anal, Jaisanti et al. 2014, Devatkal, Kumboj et al. 2014, Okolie, Henry et al. 2016), fruit pulp (Bennett, Shiga et al. 2010), rhizome (Saravanan and Aradhya 2011, Kandasamy, Baggu et al. 2014), inflorescence (Padam, Tin et al. 2012, Schmidt, Prestes et al. 2015) and leaves (Karuppiah and Mustaffa 2013), contain chemicals that have considerable antibacterial and antioxidant activities.

However, the supply chain for the manufacturing of bananas might experience losses and waste of up to 35% (Sagar, Pareek et al. 2018). More than 353,443 tons of banana trash annually have been recorded during the last ten years, a rise. When banana waste naturally degraded, it released poisonous fumes into the air and gave off a bad smell (Housagul, Sirisukpoka et al. 2014). According to (Rattanapan and Ounsaneha 2020, Ounsaneha, Rattanapan et al. 2021), the supply chain for banana production in Thailand has a significant negative influence on the environment in terms of water and carbon footprints, as well as the amount of banana trash produced.

In order to better use banana by-products and meet the growing demand for raw materials supply across a variety of sectors, numerous studies have been conducted (Doran, Sen et al. 2005, Kuo, Hwang et al. 2006, Clarke, Radnidge et al. 2008, Emaga, Robert et al. 2008).

(Giri, Jun et al. 2016) shows that feeding on banana peel flour at 5% could enhance *Labeo rohita's* immunity and growth performance. Furthermore, (Mendez 2003) found that Nile tilapia fingerlings can consume up to 200 grammes of green banana meal per kilograms of feed without it impairing their ability to grow and utilize their feed.

1.4. Heavy metal accumulation in fish

Metals are a broad group of naturally occurring substances and pollutants that have been extracted from the earth and employed in human industry (Howard 2002). Natural trace elements called heavy metals can be found in water, soil, and the environment (Gaber 2007). They are formed from a variety of natural and human sources and are organic components of our environment. Metal pollution in fluvial environments can be brought on by direct air deposition, geologic weathering, and the release of agricultural, municipal, or industrial waste products.

In addition to natural sources, a number of manmade activities have contributed to the environment's metal concentrations. Despite the fact that some heavy metals are necessary for aquatic creature growth at very low concentrations, heavy metals are one of the most prevalent kinds of pollution in aquatic environments because of their significant influence on ecological quality (Järup 2003, Gaber 2007).

A heavy metal is any metallic chemical element with a specific gravity that is at least five times greater than that of water and a sufficiently high density (Järup 2003). Anthropogenic sources are acknowledged as the main causes of heavy metal contamination in aquatic systems, and heavy metal concentrations in aquatic ecosystems are continually growing (Linnik and Zubenko 2000, WHO, 2000).

Heavy metal pollution of the aquatic environment has become a worldwide issue and a reason for scientific worry due to the fact that heavy metals are indestructible and the most of them have negative effects on animals (MacFarlane and Burchett 2000, Censi, Spoto et al. 2006, Oronsaye, Wangboje et al. 2010). Aquatic meals may contain all of the essential fatty acids, proteins, carbs, vitamins, and minerals. Since fish is the most popular seafood, it plays a crucial role in the transfer of dangerous heavy metals to humans. Fish in particular have a tendency to accumulate heavy metals in their various organs, which can eventually enter the human metabolism through consumption and pose major health hazards. Fish toxicological and ecological research has drawn the majority of focus to hazardous metal determination (Sen, Shandil et al. 2011).

International organizations like the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) are working in a variety of ways to control the infection and transmission of diseases linked to food products, utilizing various regulatory mechanisms like the Hazard Analysis and Critical Control Point (HACCP), Codex Alimentarius, and the ISO 9000 series. Hazards from contaminated food might be biological, chemical, or physical. Foodborne

illness is mostly brought on by pathogens, heavy metal pollution, and allergies, all of which have long-term effects.

Rates of absorption, storage, and removal all affect the amount of metal that accumulates in fish tissues. It is projected that metals with high absorption rates, but low clearance rates will progressively accumulate in fish tissues. Non-essential metals build up at very low ambient quantities because fish cannot regulate their levels (ShaAto, Eneji et al. 2011). According to (Gaber 2007), tissue alterations can occur even at low trace metal concentrations, and it is challenging to repair fish tissue that has already been harmed by zinc (Zn).

Fish tissues have variable degrees of metal affinity, with the bulk collecting in the gills, liver, and kidney. Critical metal buildup, such as that of iron, zinc, copper, manganese, and cobalt, for instance, is organ-specific (Guallar, Sanz-Gallardo et al. 2002). Even at low ambient concentrations, copper has a great affinity for the liver and zinc for the gonads since these organs are where they carry out their main metabolic tasks (Jezierska and Witeska 2006). Although it may also be found in significant concentrations in the gills, digestive system, and spleen, cadmium is mostly retained in the kidneys and liver. The liver, kidneys, spleen, digestive tract, and gills are just a few of the organs that exhibit lead deposits. High concentrations of this metal have occasionally been found in bones. Although the liver, kidneys, and gastrointestinal tract may also be severely strained, the zinc concentrations in the gills are often the highest. Fish muscles are regularly evaluated for metal content since they are used as human food despite having low metal contents compared to other tissues. The gonads, bones, and brain are among the organs with high metal concentrations (Jezierska and Witeska 2006)).

Due to their long-term persistence, more than ten (10) heavy metals, including cobalt (Co), lead (Pb), mercury (Hg), arsenic (As), thallium (Tl), nickel (Ni), manganese (Mn), zinc (Zn), cadmium (Cd), and chromium (Cr), have a particular importance in ecotoxicology (Storelli, Storelli et al. 2005). While Pb, Ni, and Cd are dangerous even at very low concentrations, metals like Mn, Zn, and Cr are only dangerous at a certain threshold (Bury, Walker et al. 2003, Fernandes, Fontaínhas-Fernandes et al. 2008). When heavy metal levels in drinking water surpass the permitted limit, which varies depending on the element, toxicity is discovered.

1.4.1. Arsenic

Arsenic is a component of the earth's crust that is widely distributed in the atmosphere, water, and land. It is very toxic in its inorganic form (WHO, 2022). It is one among the most major heavy metals raising issues for both the environment and human health (HUGHES, POLISSAR et al. 1988). It has a semimetallic composition, is extremely toxic, and causes cancer (Singh, Kumar et al. 2007). Arsenic is the twenty-first most common element on earth, and both humans and animals are poisonous to its inorganic forms, such as arsenite and arsenate compounds. Because it primarily affects cells in the sulphydryl group, affecting cell respiration, enzymes, and mitosis, arsenic is a protoplastic toxin (Gordon and Quastel 1948).

The greatest threat that arsenic poses to human health is contaminated groundwater. Drinking water, crops irrigated with contaminated water, and food prepared with contaminated water are the sources of exposure. Fish, shellfish, pig, poultry, dairy products, and cereals can also contain arsenic, although the exposure from these foods is frequently much smaller than the exposure from contaminated groundwater. Most of the arsenic found in seafood is in its less harmful organic form. Inorganic arsenic, which is known to cause cancer, is the most prevalent chemical contaminant in drinking water worldwide. Arsenic can also be present in organic forms. Compared to inorganic arsenic compounds, which are present in water and shellfish, organic arsenic compounds are less harmful to health (WHO, 2022).

1.4.2. Lead

The Earth's crust contains naturally occurring lead, which is a dangerous metal. Its broad use has resulted in significant environmental contamination, human exposure, and public health problems in many parts of the world (WHO, 2022).

Lead is a bright, silvery metal with a hint of blue when it is dried. It starts to tarnish when exposed to air, producing a complex chemical mixture that changes depending on the environment. In contrast to other metals like zinc, copper, and manganese, lead is a particularly dangerous heavy metal that disrupts a number of physiological processes in plants and serves no biological function. Even at modest dosages, lead treatment was found to cause significant instability in plant ion absorption, which in turn causes significant metabolic changes in photosynthetic capacity and, ultimately, a severe inhibition of plant growth.

Lead exposure can have a major negative impact on children's health. When lead poisoning occurs in high amounts, the brain and central nervous system are affected, which can result in

unconsciousness, convulsions, and even death. Children who survive severe lead exposure may experience mental impairment and behavioral issues. It is now understood that lead exposure may cause a range of harm to many biological systems at low exposure levels with no immediate symptoms (WHO, 2022).

1.4.3. Mercury

Metallic mercury is a substance that occurs in nature. It is a sparkling silver-white, odourless liquid that, when heated, changes into an inert gas. The toxin mercury is extremely bio accumulative. Many studies are focusing on mercury dispersion in the aquatic environment since its presence has a detrimental effect on the marine ecology. Agriculture, municipal wastewater discharges, mining, incineration, and industrial wastewater discharges are all significant sources of mercury pollution caused by human activity (Chen, Xu et al. 2012).

Elemental and methylmercury both harm the central and peripheral nervous systems. Inhaling mercury vapor can be fatal and harm the nervous, digestive, and immune systems in addition to the lungs and kidneys. Inorganic salts of mercury are corrosive to the skin, eyes, and digestive system, and if eaten, they can harm the kidneys. When breathed, consumed, or absorbed via the skin, certain mercury compounds can result in neurological and behavioral issues. Some of the symptoms include tremors, insomnia, memory loss, neuromuscular effects, headaches, and cognitive and motor impairment. There have been instances of adverse renal reactions, including increased urine protein and kidney failure (WHO, 2017).

1.4.4. Cadmium

Cadmium is ranked as the sixth most dangerous heavy metal. As a by-product of the manufacture of zinc, it may be present in the environment or at work and expose people and animals. Heavy metal cadmium is quite toxic. At very low exposure levels, cadmium is dangerous and has both immediate and long-term negative effects on human health and the environment (WHO, 2019).

Cadmium is usually discovered in fruits and vegetables due to its high rate of soil-to-plant transfer (Satarug, Garrett et al. 2010). The very dangerous heavy metal cadmium is renowned for its detrimental effects on cellular enzyme systems, oxidative stress, and the creation of nutritional deficiency in plants (Irfan, Hayat et al. 2013).

The kidney is the organ that needs to be targeted the most. With a biological half-life of 10-35 years in humans, cadmium is mostly deposited in the kidneys. This accumulation may result in renal tubular failure, which raises the excretion of low-molecular-weight proteins in urine. Most

of the time, this is final. Kidney stones can form as a result of cadmium toxicity and issues with calcium metabolism. There is enough data to conclude that long-term occupational exposure to cadmium increases the risk of developing lung cancer (WHO, 2019).

1.4.5. Zinc

Zinc (Zn) has been identified as the nutrient that is most effective at reducing abiotic stress. One of the most crucial nutrients for animals, including fish, is zinc. Protein synthesis and transcription factors that bind Zn and are believed to be Zn dependent for their activity are critical for both processes. It also plays a significant part in biochemical processes necessary for maintaining life, such as cellular respiration, oxygen use by cells, DNA production, and RNA synthesis, as well as the preservation of cell membrane integrity and the sequestration of free radicals (Chan, Gerson et al. 1998, El Hendy, Yousef et al. 2001, Valko, Leibfritz et al. 2007).

One of the most crucial trace elements in the body, Zn is involved in the biological processes of many proteins and enzymes (Maity, Roy et al. 2008). Zn is a necessary trace element, but at a certain quantity, it is harmful to most species (Ho 2004). The definitive acute test is carried out first to determine the LC50 of the chemical to which organisms are exposed since the range-finding acute test is carried out to identify exposure concentrations (Di Giulio and Hinton 2008). Additionally, it controls energy, vitamin A, lipid metabolism, growth promotion, and immunomodulation. It also regulates protein synthesis (Tan and Mai 2001, Kumar, Krishnani et al. 2017, Kumar, Krishnani et al. 2017, Kumar, Krishnani et al. 2018, Kumar, Krishnani et al. 2018).

According to (Shay and Mangian 2000), zinc is also responsible for lactic dehydrogenase and alkaline phosphatase, development retardation, cutaneous lesions, reduced immunological function, and weight loss.

It is considered one of the heavy metals that is used in a variety of ways before ending up in a river or the ocean. Human operations include mining, purifying zinc, lead, and cadmium ores, burning coal, and burning garbage release too much zinc into the environment. Small amounts of zinc are necessary for proper growth and metabolism (Srivastava and Sharma 1996, Srivastava and Kaushik 2001, Shukla, Rathi et al. 2002), but if the level is more than what is necessary for health, it can become hazardous.

A common zinc-calcium transporter found in the chloride cells of fish gill epithelium is where zinc from water is at least partially absorbed (Hattink, De Boeck et al. 2006). The amount of calcium

in the water can affect how much zinc is absorbed by these carriers, and exposure to zinc can cause fish to absorb less zinc through these carriers (Hogstrand, Wilson et al. 1994, Hogstrand, Reid et al. 1995).

Through the fish's diet, zinc uptake also takes place in the digestive system (Bury, Walker et al. 2003, Pyle, Rajotte et al. 2005). Fish at the top of the food chain may be more susceptible to increased zinc consumption if a watershed has high levels of waterborne zinc because the smaller fish, they feed may have significant zinc buildup in their tissues (Koca, Koca et al. 2005).

1.4.6. Copper

Low quantities of copper naturally occur in the aquatic environment. Seawater typically, copper values are less than 1 ppb (Fowler, Nordberg et al. 2011). Urbanized locations and places close to copper mining and smelting operations are where elevated aquatic Cu concentrations are most common (Davis Jr, Welty et al. 2000, Eisler 2000). Because aquatic environments are the last recipients of industrial and urban effluent, storm water runoff, and atmospheric deposition, they are vulnerable to Cu pollution (Nriagu 1979, Davis Jr, Welty et al. 2000).

A heavy metal with a density greater than 5 g/cm3 and minimal chemical reactivity, copper is malleable and ductile. Additionally, copper is a necessary trace micronutrient that is crucial for survival in both humans and animals. It functions as a co-factor in vital enzyme activities associated to these processes (Lee, Prohaska et al. 2001, Stern 2010). Copper has a wide range of applications and is also a component of several enzymes, including lysyl oxidase, tyrosinase, and dopamine hydroxylase. It is linked to the synthesis of copper chelates and complexes of Cu proteins in its metabolic functions (Watanabe, Kiron et al. 1997).

1.4.7. Selenium

Aquatic animals require selenium (Se), an essential microelement, for enhanced performance and overall health (Pacitti, Lawan et al. 2016, Gobi, Vaseeharan et al. 2018). By secreting antioxidant enzymes and thyroid hormones, Se can shield the animal cell from oxidation, which is brought on by several stresses such as high density, transportation, poor water quality, and infectious diseases (Pacitti, Lawan et al. 2016).

Se already existed in fish meal, which is a significant component of fish diets, however further Se inclusion is strongly advised to meet the needs of specific fish species.

Fish and other animals require selenium, a vital trace element, for healthy growth and antioxidant defence (Watanabe, Kiron et al. 1997, Surai 2006, Khan, Zuberi et al. 2017). Se, in the form of

selenocysteine, which is regarded as the twenty-first of the sixty amino acids, is found in proteins and enzymes known as selenoproteins, and is primarily responsible for this function.

The non-metallic element selenium can be found in nature in a variety of forms, including selenite, selenate, and selenomethionine (Takayanagi 2001, Mechora, Stibilj et al. 2013, Iqbal, Atique et al. 2017). Water bodies and alluvial sediments are the main natural sources of this element for fish (Patterson, Paige et al. 2010, Younus, Iqbal et al. 2015).

Studies on fish have demonstrated that adding selenium to the diet can lessen oxidative stress that is brought on by handling (Rider, Davies et al. 2009), crowding (Küçükbay, Yazlak et al. 2009) heavy metal contamination (Lin and Shiau 2007, Alaa 2012, Penglase, Hamre et al. 2014), or consuming oxidised oil (Chen, Liu et al. 2013). Since this naturally occurring phenomenon occurs in most aquatic settings, particularly in intensive fish farming with large stocking densities, it is recognised as a major concern in aquaculture ((Van Raaij, Van den Thillart et al. 1996, Wu, You et al. 2016, Li, Wang et al. 2018).

Fish require selenium as a vitamin for sustained growth (Durigon, Kunz et al. 2019). It is a unique case of a nutritional paradox since it is poisonous at slightly greater doses but essential at smaller doses (Younus, Iqbal et al. 2015). In stress defences, it serves as an antioxidant (Lemly 1993).

As a result, it is acknowledged for its crucial physiological function and is necessary for the immune system and a variety of enzymes to function normally (Patterson, Paige et al. 2010); (Ramesh, Sankaran et al. 2014, Sarkar, Bhattacharjee et al. 2015, Iqbal, Atique et al. 2020)

Since 1967, numerous researches on a variety of fish species have shown that Se can mitigate against Hg toxicity (Kaneko and Ralston 2007, Copat, Vinceti et al. 2014, Ralston, Ralston et al. 2016). It has been suggested that Se can inhibit Hg methylation, aid in its demethylation or limit Hg absorption or bioaccumulation (Dang and Wang 2011). It is unreasonable to estimate the risk of exposure to MeHg without also taking into account how it interacts with selenium (Ralston, Ralston et al. 2016).

1.4.8. Manganese

Animals need manganese (Mn), a key micronutrient, for a variety of processes, including growth, immunity, reproduction, and bone building(Liang, Wang et al. 2015). Additionally, it functions as an enzyme cofactor or activator in the metabolism of lipids, proteins, and carbohydrates, including Mn superoxide dismutase (MnSOD)(Andreini, Bertini et al. 2008). The amounts of Mn typically present in water are reportedly insufficient to meet the metabolic needs of fish or aquatic

crustaceans, despite the fact that they can absorb Mn from the water. As a result, their diet is thought to be the main source of Mn (Zhao, Mao et al. 2017).

Mn is a transition metal that is necessary for life and serves as a cofactor for metalloenzymes. It is one of the minerals whose quantity in marine or terrestrial plant ingredients significantly varies. As a result, Mn not only participates in a number of enzyme complexes with antioxidant properties, such as the MnSOD (Holley, Bakthavatchalu et al. 2011), but also affects the metabolism of carbohydrates, lipids, and proteins (Lall 2002).

Mn is a mineral that is abundant in bone, but it is also present in high amounts in the liver, muscle, kidney, gonadal tissues, and skin, where it is concentrated greater in the mitochondria (Lall 2002).

1.4.9. Iron

In the human body, iron plays a crucial role in numerous biochemical processes such as oxygen transport, energy synthesis, and cell proliferation. However, it has been observed that iron deficiency causes nutritional issues in close to 5% of the world's population.

Although iron is prevalent in the crust of the earth and also naturally occurs in aquatic ecosystems, the concentration of iron in aquatic environments is found to be higher than desired due to human activity.

Due to their complicated speciation, which is influenced by redox potential, dissolved oxygen, pH, and organic matter, reducing their accumulation and impacts is difficult (Vuori 1995).

Iron is found in two oxidation states in aqueous ecosystems: reduced ferrous ion (Fe II) and oxidised ferric ion (Fe III). Ferrous ions oxidise to ferric ions in oxygen-rich fresh water. Ferric ions are insoluble and violently precipitate as hydroxides and oxyhydroxides in fluids with a pH higher than 6.5 (Hem 1985, Kimball, Walton-Day et al. 2007).

1.4.10. Cobalt

A crucial nutrient required in the production of vitamin B12 is cobalt (Banerjee and Ragsdale 2003). It also functions as an enzyme cofactor for transferases, hydratases, and dehydrogenases (Nelson, Lehninger et al. 2008). In aquatic conditions, high cobalt concentrations can damage DNA and fragment chromosomes (Figgitt, Newson et al. 2010).

Chronic cobalt exposure in zebrafish led to DNA damage in sperm, increased expression of genes involved in DNA repair in the testes, and decreased reproductive success (Reinardy, Syrett et al. 2013). Furthermore, cobalt can lead to oxidative stress (Kubrak, Husak et al. 2011), apoptosis (Cai,

Zhu et al. 2012) and aberrant growth in fish (Dave and Xiu 1991, Saeedi Saravi, Karami et al. 2 09, Atamanalp, Kocaman et al. 2010, Cai, Zhu et al. 2012, Kubrak, Rovenko et al. 2012).

1.5. Objectives

The principal goal of this research is to raise the importance of determine the content of metals in Aloe vera and banana by-products as sustainable alternatives to conventional ingredients in aquaculture diets.

As specific objectives:

1- Analyze the level of metals in products of the primary sector that can be used as raw materials to incorporate into fish diets, elaborate diets with different levels of inclusion of these by-products under the concept of circular economy, and validate diets for fish and evaluate the accumulation of pollutants.
2- Determine the content of heavy metals in golden mullet (*Liza aurata*) fed Aloe vera by-product and evaluate different risk approaches and the nutritional value.
3- Determine the content of heavy metals in tilapia (*Oreochromis niloticus*) fed banana by-product and evaluate different risk approaches and the nutritional value.
4- Evaluate the effects on growth, biochemistry and histomorphology of liver and gut in juvenile tilapia after the inclusion of banana meal in the diet.

Chapter 2. Methodology

2.1. Processing raw material and experimental diets

Two different plants by-products were tested in our study, Aloe vera by-product and banana crop by-products. Aloe vera raw ingredients were supplied by Aloe Canarias S.L. (Canary Islands, Spain), and banana by-products were supplied by local Canarian producers of banana cultivars.

The by-products had received in Bio factory of the Pilot Plant of Products and Processes of GIA-ECOAQUA in Taliarte. This had been treated, sanitized, dried, sterilized and grind for later inclusion as raw material in fish feed: lyophilization in the lyophilizer and drying in an oven at a temperature below 40ºC.

Four isocaloric and isoproteic diets were developed for each one of by-products: one of them was a commercial diet of the specie under study, and other three including increasing percentages of the raw material, 5, 10 and 20 % of banana pseudostem by-products and 2, 4, 6 % of Aloe vera by-product. All diets were analyzed both for biochemical composition and content in metals.

2.2. Experimental design and fish preparation and sampling

Experiments were conducted following the recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) category C certificate and following the European Economic Community animal experimentation guidelines directive of 24 November 1986 (86/609/EEC). The feeding trial was carried out in the experimental aquaculture facilities of the Technological Science Park Foundation (FCPCT) of the University of Las Palmas de Gran Canaria. Two different species were used in our study, golden mullet (*Liza aurata*) and tilapia (*Oreochromis niloticus*).

Total of 192 juvenile golden mullets with a mean weight of 8.93 ± 1.88 g were randomly distributed in twelve 0.2 m^3 cylindrical-conical fiberglass tanks in a flow-through system. Three tanks were used per diet.

Concerning tilapia, one hundred and ninety-two fish (5 g initial body weight) had been distributed among 12 glass tanks of 50 l of fresh water using the closed system in this experiment, (16 fish/tank, each diet fed in triplicate). Fish had fed over the experimental diets three times per day 6 days per week, until apparent satiation, for almost 3 months. During the trial period oxygen and temperature parameters were measured once per day, using an oximeter (Oxy Guard-Guard-handy

beta, Zeigler Bros, Gardners, USA). Feed intake had been determined daily and all fish individually weighed monthly. Conversion index (CI), and specific growth rate (SGR) calculated. Sampling was made monthly to measure the parameters of weight, total size and furcal size, two fishes of every tank were collected for the heavy metal's determination. The final sampling of the trial was carried out after 80 days, when the fish doubled its weight. Samples of raw materials, by-product of banana tallow and Aloe vera, processed diets and samples of tissues were taken at the beginning and end of the experiment.

2.3. Metals Determination

The Clinical and Analytical Toxicology Service (SERTOX) laboratories of the Universidad de Las Palmas de Gran Canarias were in which this assignment was completed. The data were analyzed using Agilent Mass Hunter Data software using an Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) to measure the concentration of the various components (version 4.2). Every sample was examined in triplicate.

The samples from each tank were homogenized separately. Three 1-gram subsamples of each homogenate were digested with the aid of a microwave digester (Ethos Up, Milestone SRL, Italy). Briefly, the 1 g-subsamples were weighed into the digestion vessels, and 50 µL of the internal standard solution (Sc (scandium), Ge (germanium), Rh (rhodium), and Ir (iridium) at a stock concentration of 20 mg/mL each) was added. Then, 2.5 mL of concentrated sub-boiling HNO3 (65%) and 7.5 mL of Mili-Q water were added to each sample. All samples were digested according to the following program: Step 1: 1800 – 100 – 5 power (W) – Temperature (ºC) – time (min); Step 2: 1800 – 150 – 5; Step 3: 1800 – 200 – 8 and Step 4: 1800 – 200 – 7. After cooling, the entire digests were transferred into conic bottom polypropylene tubes and diluted up to 15 mL with Mili-Q water. Finally, an aliquot of each sample was taken for the analysis. Reagent blanks were prepared similarly to the samples and were included every 14 samples in the analytical batch. The entire/complete procedure was validated prior to its use in the analyses of samples. Recoveries obtained ranged from 87 to 118% for toxic and essential elements. Linear calibration curves were found for all elements (regression coefficients ≥ 0.998). Instrumental LODs and LOQs were calculated as the concentration of the element that produced a signal that was three and ten times higher than that of the averaged blanks, respectively. The sample LOQs were calculated by

multiplying the instrumental LOQ by the dilution factor suffered by the sample during the digestion procedure (1:10 v:v).

2.4. Risk assessment and nutritional assessment analysis

To determine the risk factors of the two species, golden grey mullets (*Liza aurata*) and tilapia (*Oreochromis niloticus*), consumption different approaches were used.

2.4.1. Estimated Daily Intake (EDI)

Estimated Daily Intake (EDI; µg/kg bw/day) for toxic and essential metals was calculated according to the following equation (Onsanit, Ke et al. 2010, Kalantzi, Black et al. 2013, Copat, Vinceti et al. 2014):

$$EDI = [\ C \times AvC] / BW$$

where C (µg/g ww of fish or diet) is the average metal concentration in the edible part of the fish and diet, AvC is the seafood ingestion rate considering 31.13 g/person/day as global consumption of fish in Spain and 9.94 g/person/day as the higher amount of fish consumption in Spain and bw is the adult body weight of the general population. An AvC of 31.13 g/day of fish in Spain and an average BW of the general population of 70 kg were assumed (FAO, 2005–2012).

These EDI values were compared with the parallel Reference Doses (RfD) as established by the (USEPA 2017). The RfD is an estimation (with an uncertainty up to one order of magnitude) of the daily exposure of the population due to ingestion which is unlikely to produce any (noncarcinogenic) effects, even in vulnerable population groups during a lifetime (USEPA, 2012). These Reference Doses are 0.3 µg/kg bw/d for Co, 20 µg/kg bw/d for Ni, 40 µg/kg bw/d for Cu, 300 µg/kg bw/d for Zn, 700 µg/kg bw/d for Fe, 1500 µg/kg bw/d for Cr, 0.3 µg/kg bw/d for As ,1.0 µg/kg bw/d for Cd, 0.1 µg/kg bw/d for Hg, 0.2 µg/kg bw/d for U (USEPA, 2017). Regarding Pb, since an RfD value was not set by USEPA in 2017, a previously established value by USEPA (2014) was used, that is 3.57 µg/kg bw/d.

2.4.2. Maximum Safe Consumption (MSC)

Because of the possible toxicity from a single element, the Maximum Safe Consumption (MSC, kg fish ww/day) specifies a maximum limit for safe daily consumption of fish. For metals and

elements with established RfD according to the following equation (Metian, Warnau et al. 2013, Kalantzi, Pergantis et al. 2016), MSCA was assessed:

$$MSC = [BW \times RfD] / C$$

where C (μg/g ww of fish) is the mean metal concentration in different parts of the fish and diets, BW is the adult body weight of the general population (70 kg; FAO, 2005–2012) and RfD is the reference dose.

2.4.3. Target Hazard Quotient (THQ) and Hazard Index (HI)

The Target Hazard Quotient (THQ) indicates the noncarcinogenic risk level associated with pollutant exposure (USEPA, 2011). THQ value lower than 1 signifies the absence of any harmful effects on human health. THQ was calculated as follows:

$$THQ= (EF*ED* AvC*C) / (RfD*BW*AT)$$

where EF is the exposure frequency (365 days/year); ED is exposure duration (70 years); AvC is the seafood ingestion rate considering 31.13 g/person/day as global consumption of fish in Spain and 9.94 g/person/day as the higher amount of fish consumption in Spain when this amount is safe for consumers in the majority of health risk parameters the lower amount will be safer (Marcos, Rubio et al. 2016), C is metal concentration (mg/kg, wet weight); RfD is the reference dose of the metal. BW is body weight (adults 70 kg). AT is averaging time, which is given by EF × ED.

2.4.4. Hazard Index (HI)

Furthermore, the total THQ, also known as the hazard index (HI), was used by adding up all of the target hazard quotients (THQ) for the determined metals in order to assess the human health risk from exposure to multiple pollutants and the combined or interactive effects of those effects as well as to describe the additive effect among the elements (Li, Huang et al. 2013). In this manner HI was determined as follow:

$$HI = THQ (element 1) + THQ (element 2) + THQ (element n)$$

2.4.5. Carcinogenic risk of As

Consuming As much as possible throughout one's life raises the risk of getting cancer because it is one of the chemicals regarded to be most likely to do so. As a result, the possibility of developing cancer from As was calculated using the following equation:

$$As\text{-}CR = [\ EDI \times CSF\ ] / 1000$$

where As-CR is dimensionless, EDI (µg/kg bw/day) is the Estimated Daily Intake and CSF is the oral cancer slope factor for inorganic As, equal to 1.5 (mg/kg/day)-1 as set by USEPA, 2012. The exposure duration 56 years and the exposure frequency were assumed to be 365 days per year. Lower levels than 1 in 10,000 and 1 in 1,000,000, respectively, are safe even though the tolerable risk thresholds for carcinogens vary from 10-4 to 10-6. However, going above these refers to a chance of developing cancer in terms of carcinogenic risk (Nadal, Ferré-Huguet et al. 2008, Vieira, Morais et al. 2011, Copat, Arena et al. 2013, Kalantzi, Pergantis et al. 2016).

2.4.6. Selenium Health Benefit Value

Calculating the Se-Hg balance was used to estimate Hg toxicity because Se is known to decrease Hg toxicity. Thus, the selenium health benefit value (Se-HBV) and the selenium to mercury molar ratio were computed. Se-dependent health benefits and protection from mercury toxicity are provided by diets higher in selenium than mercury, whereas foods higher in mercury are linked to health risks. Because of this, the existence of Se molar excess (a Se to Hg ratio greater than 1) shows that consuming fish is safe. In a similar manner, positive Se-HBV ratios denote health advantages, whereas negative Se-HBV ratios denote health risks. The Se-HBV was calculated using the following equation (Kaneko and Ralston 2007) using mean concentrations of Se and Hg:

2.5. Nutritional value

Additionally, this study examined many metals, some of which are critical minerals in the suggested human nutritional requirements. Regarding the nutritional evaluation, the Recommended Dietary Allowance (RDA) for each element, with the exception of Mn, was compared to the estimated daily intake of trace elements from the consumption of golden mullet fed with Aloe vera by-products of varying concentrations. Due to a lack of sufficient scientific data

to determine an average demand, this element was considered to have an adequate intake (AI) (Authority 2017).

2.6. Sampling and growth parameters

Sampling was made monthly to measure the parameters of weight, total size and furcal size. The final sampling of the trial was carried out after 80 days, when the fish doubled its weight. Samples of raw materials, by-product of banana pseudo stem and processed diets were taken for the biochemistry analysis. Samples of tissues were also taken at the beginning and end of the experiment. Conversion index (CI), and specific growth rate (SGR) calculated. In each monthly sampling, two fish had slaughtered and used for contaminants analysis monthly and at the end of the experiment.

2.7. Biochemistry analyses

2.7.1. Proximal composition

Samples of raw materials and by-products of aloe vera and banana and processed diets were taken for the biochemistry analysis. Samples of whole fish and muscles were also taken at the beginning and end of the experiment and then stored at -80°C. Proximate composition was conducted following standard procedures (Gaithersburg 1984). Ash content of the samples was determined by combustion in a muffle furnace at 600 °C for 12 h. Crude Protein content were determined by the Kjeldahl technique (N × 6.25), which is based on the measurement of the total nitrogen present in the samples. To determine the percentage of moisture by thermal dehydration to constant weight at 105 °C an amount of each sample was weighed, reweighed after 24 hours, repeating this measurement at least once more time after another hour on the stove. Total lipids content is that described by (Folch, Lees et al. 1957), by which a mixture of chloroform-methanol (2: 1 v / v) with 0.01% butylhydroxytoluene (BHT) is used, and fatty acid methyl esters obtained by transmethylation (Christie, Kwon et al. 2003) then separated by gas-liquid chromatography.

2.7.2. Fatty acid analyses

The total lipids were trans-esterified with 1% sulfuric acid in methanol following the methodology of (Christie 1982). A dilution was made in hexane, and the separation, identification and

quantification of the different fatty acids was carried out through gas chromatography, following the protocol described by (Izquierdo 1989).

2.8. Histology analyses

During sampling, tissues were segmented to allow a faster penetration of alcohol in histology cassettes. A Histokinette 2000 (Leica, Nussloch, Germany) was used for sample dehydration by gradually increasing alcohol grades from 70° to 100° and ending with xylene and paraffin. 3 µm thick slices of the paraffin block were prepared using a Leica RM 2135 microtome (Leica, Nussloch, Germany) and fixed to a slide. Haematoxylin–eosin staining (Martoja and Martoja-Pierson 1970) was employed for optical evaluation by pair evaluators to determine signs of hepatopancreas damage, including signs of inflammation and presence of eosinophyls, accumulation of fat, and bile duct obstruction, using a 0-4 scale, where 0 was absence of observation and 3 was widespread presence in the tissue.

Measurements were acquired using the ImageJ software, and visual evaluations were carried out using an optical microscope (Olympus Optical, CX41, PA, USA) and an integrated camera (Olympus DP50, Olympus Optical Co. LTD, Shinjuku-ku, Tokyo, Japan) (Schneider, Rasband et al. 2012).

2.8.1. Liver

The hepatocyte nucleus and the total area of the hepatocyte were intersected by measuring the greatest and lowest diameters in the liver. the number and size of goblet cells, the lamina propria's breadth, the length and width of the villi of the gut.

2.8.2. Foregut

The assessment of the intestine has been carried out following two different methodologies: measuring the values of villus height, width of the villi, and width of the lamina propria through specific programs. On the other hand, the evaluation under the microscope and assignment of an intensity scale for the rest of the selected parameters.

As with the liver, after a first general visualization, the parameters to be assessed, which are detailed below:

-Height and width of the villi.

-Width of the lamina itself.

-Necrosis, Inflammatory infiltrate, Eosinophilic granules, Presence of parasites, Fusion of the villi and Vacuolization.

The parameters of height and width of the villi and the width of the sheet own were calculated by measurements using the Image-pro plus program, from photos taken with the Olympus Cell Sens program. The other parameters were evaluated following the scale assessment system. intensity from 0 to 3.

2.8.3. Hindgut

In the posterior intestine sections, in addition to evaluating all the parameters described for the foregut, an assessment was performed by intensity scale from 0 to 3, of the abundance of goblet cells.

2.9. Statistical analysis

The results were expressed as mean ± standard deviation (SD). Normality and homogeneity of the variance of all the variables were evaluated using the Kolmogorov-Smirnoff test and Levene test respectively. The homogeneity of variances has been determined with the Levene test ($p \leq 0.05$). The analysis of variance was performed using Kruskal-Wallis test for metals and histological results, while a General Linear Model was made for growth and biochemical parameters. The means were compared by Duncan post-hoc tests ($p \leq 0.05$). All statistical analyses were conducted by IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp.).

Chapter 3. Determination of heavy metals from Aloe vera by-product in golden mullet (*liza aurata*); a consumer health risk assessment

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3.1. Abstract

Heavy metals have become subject of concern in the recent years because of its potency to cause cardiovascular diseases and other toxic health effects. Therefore, this research was assumed to investigate the level of toxicity in terms of heavy metals accumulation in the fish samples and its benefits and risk for human consumers health and also evaluate the partial replacement of plant sources by canarian *Aloe vera* diets as a pure product or like a by-product on toxicological effects on the golden mullet (*Liza aurata*) fillet and whole body. In this study risks arising from fish metal content has been measured using various parameters as Estimated Daily Intake (EDI), Maximum

Safe Consumption (MSC_A), Target Hazard quotient (THQ), Hazard Index (HI) Carcinogenic risk of As (As- CR), the Value Selenium Health Benefit (Se HBV) and also the Nutritional Values has been evaluated. The results showed that all heavy metal levels in the fish tissue and diets were below the confirmed safe limits for consumption. In case of diets, it is obvious that with the exception of As, Hg, and Se, the presence of heavy or essential metals in both whole fish and raw fillet in golden grey mullet given experimental diets revealed that the whole fish had the highest concentration. Thus, it can be concluded that Aloe vera product and by-product were in safety limits for fish and also for humans through food chain. Various risk and benefit assessment measures established by national and international authorities concluded that *Liza aurata* use was mostly safe.

3.2. Introduction

Increasing competition for traditional ingredients in the feed sector has expanded the use of agro-industrial by-products as an essential ingredient in aquaculture diets (Masoudi, Bojarpour et al. 2010). In order to achieve sustainable aquaculture, new sources of alternative ingredients, including less expensive plant-derived ingredients, need to be introduced as part of safe aquafeed processing (Higgs, Dosanjh et al. 1995).

Plant by-products are of particular interest due to their high availability and proximity, as they are widely cultivated worldwide. These ingredients could be available to most of the world's aquaculture farmers (Obirikorang, Amisah et al. 2015) and would therefore ensure sustainability and contribution to the circular economy.

The circular economy, which promotes the elimination of waste and the continual safe use of natural resources, involves industrial processes and economic activities that are restorative by design, allowing the resources used in those processes and activities to retain their maximum value for as long as possible. In this way, waste generation is reduced by recapturing "waste" as a resource for manufacturing new materials and products. Circular economy and industrial ecology are considered leading concepts for eco-innovation, which emphasize a "zero waste" environment and economy where waste can be used as raw materials (Kasapidou, Sossidou et al. 2015).

The Canary Islands are the most important European area for *Aloe vera* cultivation, with an annual production that increases every year due to its unique climatic conditions. Like other crops, the

Aloe vera plant generates a large amount of waste, so the optimization of its treatment contributes fundamentally to the implementation of the circular economy for efficient use of resources and the reduction of environmental impact.

Aloe vera is a perennial plant that belongs to the Liliaceae family, which lives in tropical and subtropical areas, and comprises more than 70 biologically active components (Langmead, Feakins et al. 2004). It is one of the most common plants in the geographical area of the Canary Islands and has been historically known as a food product on the islands in the past. This plant by-product has been used as an alternative dietary component in other species (Dotta, de Andrade et al. 2014, Gabriel, Qiang et al. 2015). However, this type of by-product would not be of the first choice as an ingredient to feed fish of the higher trophic level, as it is difficult for these species to digest. Therefore, a species from the lower part of the aquatic trophic chain, the golden grey mullet, was chosen for this study.

The golden mullet (*Liza aurata*) is a cosmopolitan species that which usually lives in tropical and temperate waters around the world. It may graze directly on food that has spread from the thin water film to the bottom mud, or it may use the food chains of plants and detritus as a source of energy. It is a small food species that depends on low-cost products and intensive use of plant-based products and by-products. It is also one of the most commonly disseminated species in the geographical area of the Canary Islands and was widely consumed and appreciated in the islands in the past.

On the other hand, *Aloe vera* waste is an efficient by-product for removing heavy metals from aqueous media (Giannakoudakis, Hosseini-Bandegharaei et al. 2018). Accordingly, its use as a fish feed ingredient may pose a risk. Fish can absorb heavy metals from the food they consume, and these can accumulate in large amounts in different tissues and cause toxicological effects in critical organs (Seymore 2014). Therefore, certain edible fish have been studied to determine the harmful effects they may have on human health (Begum, Amin et al. 2005). According to different authors, fish are effective bioindicator species for determining the level of contamination of aquatic environments caused by various elements (Chovanec, Hofer et al. 2003, Lamas, Fernández et al. 2007, Milošković, Dojčinović et al. 2016).

Due to the trophic level of the fish in the aquatic food chain, it can bioaccumulate contaminants in its tissues and participate in the biomagnification of contaminants by serving as food for other

predatory fish (Gobas, Wilcockson et al. 1999, Noël, Chekri et al. 2013, Subotić, Višnjić Jeftić et al. 2013, Djikanović, Skorić et al. 2016, Milošković, Dojčinović et al. 2016). Therefore, bioconcentration and biomagnification rates are influenced by the amount and type of contaminants (Watanabe, Kiron et al. 1997, Carline, Barry et al. 2004) as well as by other variables, such as water quality, age, scale type, and nutritional status of the fish (Watanabe, Kiron et al. 1997, Carline, Barry et al. 2004). In the case of elements, the amount and their distribution in each tissue or organ of the fish are determined by the function and physiological role of the tissue, as well as by the type of exposure (dietary or through the aquatic environment) (Uysal, Emre et al. 2008, Kalantzi, Pergantis et al. 2016).

Human exposure to contaminants occurs mainly through diet (Castro-González and Méndez-Armenta 2008, Nadal, Ferré-Huguet et al. 2008, Olmedo, Hernández et al. 2013), and there are numerous studies that indicate that fish and seafood are significant exposure sources of essential and toxic elements to consumers (Dural, Lugal Göksu et al. 2006, Castro-González and Méndez-Armenta 2008, Herreros, Iñigo-Nuñez et al. 2008, Nadal, Ferré-Huguet et al. 2008, Storelli 2008, Uysal, Emre et al. 2008, Yildirim, Gonulalan et al. 2009, Renieri, Alegakis et al. 2014, Aydın and Tokalıoğlu 2015, Rodríguez-Hernández, Camacho et al. 2016, Copat, Grasso et al. 2018). Toxic metals such as As, Hg, Pb, and Cd can adversely affect the brain, kidneys, lung, liver, and developing fetus, have hematological and immunological effects, induce skeletal damage, cardiovascular and neurological disorders, carcinogenesis, cognitive and behavioral developmental impairment, and chronic or acute diseases, among many other issues (Ikem and Egiebor 2005, Castro-González and Méndez-Armenta 2008, Vieira, Morais et al. 2011, Afonso, Lourenco et al. 2013, Copat, Arena et al. 2013, Renieri, Alegakis et al. 2014, Copat, Grasso et al. 2018, USEPA/USFDA, 2017). Because of their toxicity, international organizations such as the World Health Organization (WHO) and the European Food Safety Authority (EFSA) have set permissible limits of heavy metals in foods.

Taking into account the above, the objectives of this study were (1) to determine the concentration of toxic metals and trace elements in muscle and in whole fish tissues of the golden grey mullet species (*Liza aurata)* fed with *Aloe vera* by-products, and (2) to calculate the risk/benefit ratio for the human consumer exposed to these elements through the consumption of golden grey mullet fed with *Aloe vera* by-products.

3.3. Materials and methods

3.3.1. Diets

Three experimental diets were formulated to include graded levels of an *Aloe vera* by-product remaining after processing the plant: diet BP2 with 2% of inclusion, diet BP4 with 4%, and diet BP6 with 6%. In addition, another experimental diet was supplemented with 2% of pure product of *Aloe vera* before processing (diet P2). The raw ingredients were supplied by Aloe Canarias S.L. (Canary Islands, Spain) and included in the diets after lyophilizing. Diets were isoproteic (40% of protein) and isolipidic (15% of lipids) and balanced in macro and micronutrients. The ingredients and chemical composition are shown in Table 3.1.

3.3.2. Experimental design

Experiments were conducted following the recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) category C certificate and following the European Economic Community animal experimentation guidelines directive of 24 November 1986 (86/609/EEC). The feeding trial was carried out in the experimental aquaculture facilities of the Fundación Parque Científico y Tecnológico de la Universidad de Las Palmas de Gran Canaria. A total of 192 juvenile golden mullets with a mean weight of 8.93 ± 1.88 g were randomly distributed in twelve 0.2 m^3 cylindrical-conical fiberglass tanks in a flow-through system. Three tanks were used per diet. Water dissolved oxygen and temperature ranged between 6.4 and 6.8 ppm and 20.1 and 21.3 °C, respectively. A 12L:12D photoperiod was automatically controlled. Fish were hand-fed to apparent satiation twice a day, six days a week, for 90 days. At the end of the trial, fish were sacrificed following anaesthesic procedure. Ten fish per tank were analysed, five as whole fish and five only the edible part, i.e., the fillet.

Table 3.1 Ingredients (g/kg) and chemical composition (% dry basis) of the experimental diets.

	Diets			
Ingredients	**P2**	**BP2**	**BP4**	**BP6**
Fish meal[a]	**200**	**200**	**200**	**200**
Blood meal[b]	**30**	**30**	**30**	**30**
Ulva meal[c]	**100**	**100**	**100**	**100**
Rapeseed meal[d]	**80**	**80**	**80**	**80**
Corn meal[e]	**50**	**50**	**40**	**30**
Wheat gluten[e]	**60**	**60**	**60**	**60**
SPC[f]	**200**	**200**	**200**	**200**
Wheat meal[e]	**50**	**50**	**40**	**30**
Fish oil[a]	**85**	**85**	**85**	**85**
Soy lecitin[g]	**10**	**10**	**10**	**10**
Aloe product[h]	**20**	**-**	**-**	**-**
Aloe by-product[h]	**-**	**20**	**40**	**60**
Vitamin mix[i]	**20**	**20**	**20**	**20**
Mineral mix[j]	**20**	**20**	**20**	**20**
Ca(H2PO4)2[k]	**10**	**10**	**10**	**10**
CMC[l]	**5**	**5**	**5**	**5**
Composition				
Lipids	**14.13**	**14.95**	**15.39**	**14.23**
Protein	**40.50**	**41.16**	**39.58**	**39.46**
Ash	**10.92**	**11.63**	**11.61**	**11.73**
Moisture	**7.06**	**6.27**	**6.91**	**7.05**

[a] Fish meal and fish oil from South America (supplied by Skretting, Spain);
[b] Blood meal (supplied by Dibaq, Spain);
[c] Ulva meal (supplied by Puerto Muiños S.L., Spain);
[d] Rapeseed 0.0 (supplied by Dibaq, Spain);
[e] Corn meal, wheat meal, and wheat gluten (supplied by Capisa S.A., Spain);
[f] Soy protein concentrate (Sopropeche, France);
[g] Soy lecitin (92% fat; Korott S.L., Alicante);
[h] Aloe y Aloe by-product (supplied by Aloe Canarias S.L., Spain);
[i] Vitamin premix containing (mg kg-1 o IU/kg of feed): thiamine 40 mg, riboflavin 50 mg, pirydoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, folic acid 10 mg, cyanocobalamine, 0.5 mg, choline chloride 2700 mg, Mio-inositol 2000 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2000 IU, etoxyquine 100 mg, retinol acetate 5000 IU. Vitamin E (DL-alpha-tocopherol acetate) 250 mg;
[j] Mineral premix containing (g/kg de dry feed): calcium orthoformate 1.60 g, calcium carbonate 4 g, ferrous sulfate 1.5 g, magnesic sulfate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminum sulfate 0.02 g, zinc sulfate 0.24 g, copper sulfate 0.20 g, manganese sulfate 0.08 g, potassium iodate 0.02 g;
[k] Sigma-Aldrich, Munich, Germany; [l] carboxymethylcellulose (sodium salt, Sigma-Aldrich, Munich, Germany).

3.3.3. Determination of elements

An Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) was used to determine the concentration of the different elements and the data were processed with Agilent Mass Hunter Data software (version 4.2). All samples were analysed in triplicate.

Samples from each tank were homogenized separately. Three 1-gram subsamples of each homogenate were subjected to a microwave digestion procedure. (Ethos Up, Milestone SRL, Italy). The 1 g-subsamples were weighed into the digestion vessels, and 50 µL of the internal standard solution (Sc (scandium), Ge (germanium), Rh (rhodium), and Ir (iridium) were added at a stock concentration of 20 mg/mL each). Then, 2.5 mL of concentrated sub-boiling HNO_3 (65%) and 7.5 mL of Mili-Q water were added to each sample. All samples were digested according to the following program: Step 1: 1800 - 100 - 5 power (W) - Temperature (ºC) - time (min); Step 2: 1800 - 150 - 5; Step 3: 1800 - 200 - 8 and Step 4: 1800 - 200 - 7. After cooling, the complete digests were transferred to polypropylene conical bottom tubes and diluted to 15 mL with Mili-Q water. Finally, an aliquot of each sample was taken for analysis. Reagent blanks were prepared similarly to the samples, and every 14 samples were included in the analytical batch. The entire procedure was validated prior to its use in the sample analyses. The recoveries obtained ranged from 87 to 118% for toxic and essential elements. Linear calibration curves were found for all elements (regression coefficients ≥ 0.998). Instrumental LODs and LOQs were calculated as the concentration of the element that produced a signal that was three and ten times higher than that of the averaged blanks, respectively. The sample LOQs were calculated by multiplying the instrumental LOQ by the dilution factor suffered by the sample during the digestion procedure (1:10 v:v).

3.3.4. Risk assessment and nutritional assessment analysis

3.3.4.1. Target hazard quotient (THQ)

The Target Hazard Quotient (THQ) represents the risk level (noncarcinogenic) due to pollutant exposure (USEPA, 2012). A THQ value below 1 indicates no adverse effect on human health. The THQ was calculated as follows(Chien, Hung et al. 2002).

$$THQ= (EF*ED* AvC*C) / (RfD*BW*AT)$$

Where EF is the exposure frequency (365 days/year); ED is the exposure duration (56 years); *AvC* is the seafood intake rate, considering 31.13 g/person/day as the overall fish consumption in Spain and 9.94 g/person/day as the highest amount of fish consumption in Spain (Marcos, Rubio et al. 2016). C is the metal concentration in the edible part of the fish (mg/kg, wet weight); RfD is the reference dose, defined as an assessment (with an uncertainty of up to an order of magnitude) of the daily exposure of the population to intake of noncarcinogenic effects except in susceptible population groups over a lifetime(USEPA 2012); BW is the adult body weight of the general population (70 kg; (FAO, 2010); AT is averaging time, which is given by EF × ED. These reference doses are 0.3 µg/kg bw/d for Co, 40 µg/kg bw/d for Cu, 300 µg/kg bw/d for Zn, 700 µg/kg bw/d for Fe, 0.3 µg/kg bw/d for As,1.0 µg/kg bw/d for Cd, 0.1 µg/kg bw/d for Hg, 140 µg/kg bw/d for Mn and 5.0 µg/kg bw/d for Se (USEPA 2017). Since the (USEPA 2017) has not set an RfD value for Pb, a previously defined value by the (USEPA, 2014), 3.57 µg/kg bw/d was used.

3.3.4.2. Hazard Index (HI)

In addition, to assess the risk to the human health exposure to more than one pollutant and their additive effect, the total THQ, also called Hazard Index (HI), was calculated by summing all the THQs of the metals determined (Li, Huang et al. 2013). As a result, the HI was calculated as follows:

$$HI = THQ\ (element\ 1) + THQ\ (element\ 2) + THQ\ (element\ n)$$

3.3.4.3. Estimated daily intake (EDI)

The estimated daily intake (EDI) (µg/kg bw/day) for toxic and essential metals was calculated according to the following equation (Onsanit, Ke et al. 2010, Kalantzi, Black et al. 2013, Copat, Vinceti et al. 2014).

$$EDI = [\ C \times AvC] / BW$$

Where C (mg/kg ww of fish) is the average metal concentration in the edible part of the fish, AvC is the average consumption of g fish per day and BW is the body weight with an estimated average BW of the general population of 70 kg (FAO, 2010). The AvC in Spain is calculated to be between 9.94 and 31.13 g/day. These EDI values were compared with the Reference Dose (RfD) established by the (USEPA 2017).

3.3.4.4. Maximum Safe Consumption (MSCA)

Due to the potential toxicity of an individual element, the Maximum Safe Consumption (MSCA, kg fish ww/day) provides a maximum limit for safe daily consumption of fish. Therefore, MSCA metals and elements with established RfD were assessed using the following equation (Metian et al., 2013), (Kalantzi, Pergantis et al. 2016).

$$MSC = [BW \times RfD] / C$$

Where C (mg/kg ww of fish) is the mean metal concentration in different parts of the fish and diets, BW is body weight (70 kg adults), and RfD is the reference dose.

3.3.4.5. Carcinogenic risk of As

The As element is considered to be one of the elements likely to cause cancer. This increased possibility of developing cancer over a lifetime is due to the accumulation of doses through dietary intake. Therefore, the risk of developing cancer due to As was estimated by the following equation (Sofoulaki, Kalantzi et al. 2019).

$$As\text{-}CR = [EDI \times CSF] / 1000$$

Where As-CR is dimensionless, EDI is the estimated daily intake (μg/kg bw/day), and CSF is the oral cancer slope factor for inorganic As equal to 1.5 $(mg/kg/day)^{-1}$ as established by USEPA 2012). The exposure duration is 56 years, and the exposure frequency is assumed to be 365 days per year. Acceptable risk levels for carcinogens range from 10^{-4} to 10^{-6}, whereby levels below 1 in 10,000 and 1 in 1,000,000, respectively, are considered safe. However, exceeding these levels implies a probability of developing cancer associated with the carcinogenic risks of food (Nadal, Ferré-Huguet et al. 2008, Vieira, Morais et al. 2011, Copat, Arena et al. 2013, Kalantzi, Pergantis et al. 2016).

3.3.4.6. Selenium Health Benefit Value

Hg toxicity was determined by calculating the Se-Hg balance, as Se is known to reduce Hg toxicity. As a result, the selenium-to-mercury molar ratio and the selenium health benefit value (Se-HBV) were calculated. Foods with more Se than Hg protect against mercury toxicity and provide Se-dependent health advantages, while foods with more Hg than Se are associated with mercury health hazards. Therefore, the presence of Se molar excess (a ratio of Se to Hg greater than one) suggests

the safety of fish intake. Similarly, positive Se-HBV ratios suggest health benefits, whereas negative Se-HBV ratios indicate health hazards. The Se-HBV was estimated using mean concentrations of Se and Hg according to the following equation (Kaneko and Ralston 2007):

$$Se\ HBV = (Se/Hg\ molar\ ratio \times total\ Se) - (Hg/Se\ molar\ ratio \times total\ Hg)$$

3.3.5. Nutritional value

This study also analyzed different metals, some of which are essential minerals in the recommended human nutritional requirements. As for the nutritional assessment, the estimated daily intake of trace elements through the consumption of golden mullet fed with Aloe vera by-products of different concentrations was compared with the Recommended Dietary Allowance (RDA) established for each element except for Mn. This element was regarded as Adequate Intake (AI) as there is insufficient scientific evidence to derive an average requirement (Meyers, Hellwig et al. 2006).

3.3.6. Statistical analysis

The results were expressed as mean ± standard deviation (SD). Differences were assessed by the non-parametric Krskal-Wallis test, considering $p<0.05$ as the level of statistically significant differences. All statistical analyses were conducted by IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp.).

3.4. Results

3.4.1. Metals content

Regarding toxic elements, As was present in fillet at a higher concentration than in whole fish in all treatments and increased with higher concentrations of *Aloe vera* products and by-products in the diets (246.57, 270.89, 301.12 and 360.58 ng/g ww in fillet of fish fed with diets P2, BP2, BP4, and BP6 respectively), with statistically significant differences in the BP6 diet (Figure 3.1). As for Hg concentrations, a trend towards higher concentrations was also observed in fillet than in whole fish, although statistical significance was not reached in either case. The slight increase in Hg content in the diets did not lead to the same trend in the variation of Hg content, in both whole fish and fillet (Figure 3.2). In contrast to As and Hg, Cd concentrations were higher in whole fish than

in fillet, although our results indicated that the Cd content in both muscle and whole fish was the lowest of all the elements studied (0.16, 0.15, 0.11and 0.14 ng/g ww in the fillet of fish fed with diets P2, BP2, BP4, and BP6 respectively) with no statistically significant differences between groups (Figure 3.3). We also found that Pb concentrations in the fillets were lower than in the whole fish, which in turn were higher than those found in the diets (7.39, 4.39, 2.91and 4.67 ng/g ww in fillet of fish fed with diets P2, BP2, BP4, and BP6 respectively) (Figure 3.4).

Table 3.2 Ingredients (g/kg) and chemical composition (% dry basis) of the experimental diets.

	Diets			
Heavy metals	**P2**	**BP2**	**BP4**	**BP6**
Toxic elements				
Arsenic	**1841.32**	**2057.77**	**2177.46**	**2255.42**
Mercury	**122.82**	**132.11**	**135.09**	**137.77**
Cadmium	**459.84**	**490.33**	**495.97**	**500.34**
Lead	**228.54**	**239.75**	**285.56**	**329.45**
Essential elements				
Iron	**5.55E5**	**4.89E5**	**4.91E5**	**5.19E5**
Cobalt	**1.53E4**	**1.53E4**	**1.57E4**	**1.65E4**
Copper	**3.30E4**	**3.94E4**	**4.11E4**	**5.46E4**
Zinc	**1.10E5**	**1.06E5**	**1.12E5**	**1.22E5**
Selenium	**1.90E3**	**2.15E3**	**2.23E3**	**2.37E3**
Manganese	**5.04E4**	**4.48E4**	**4.79E4**	**5.12E4**

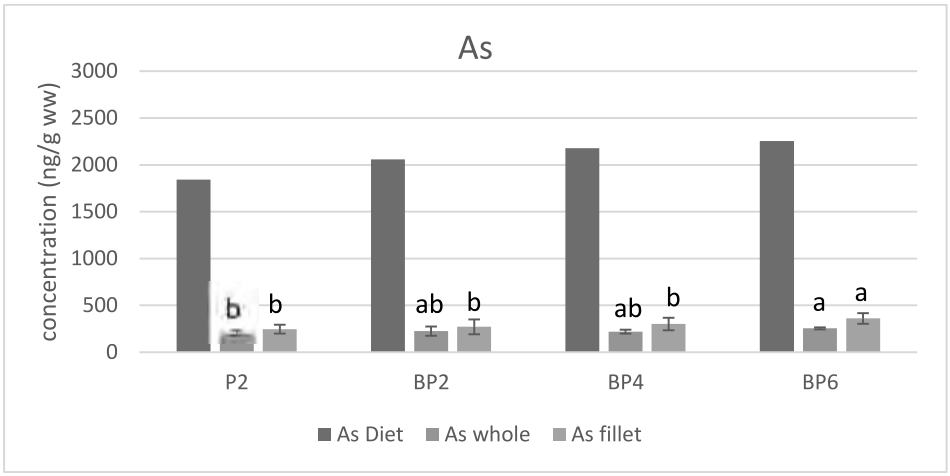

Figure 3.1. Total arsenic concentration (ng/g ww) in diets, whole fish, and fillet of golden mullet. Different letters in the same group denote statistically significant differences among dietary treatments (P < 0.05).

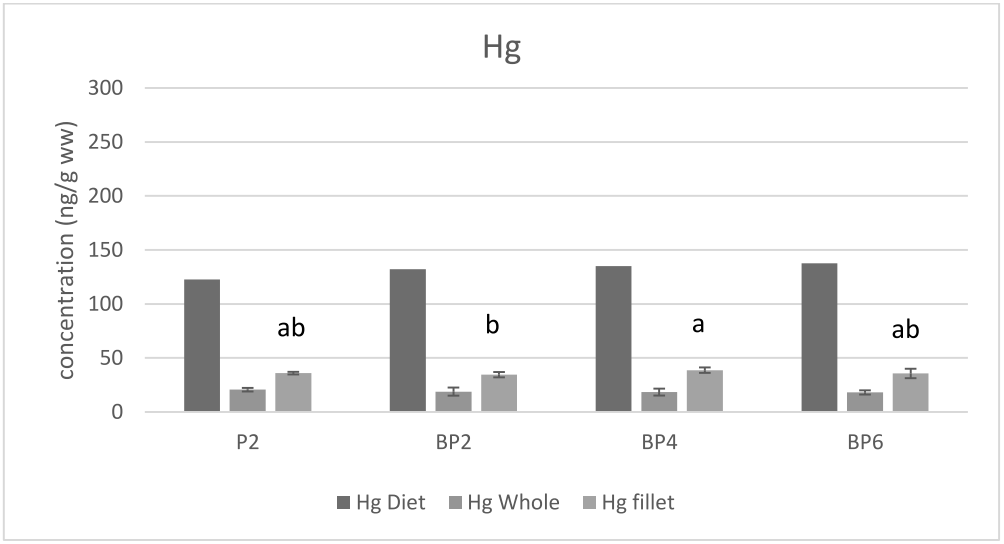

Figure 3.2. Total mercury concentration (ng/g ww) in diets, whole fish, and fillet of golden mullet. Different letters in the same group denote statistically significant differences among dietary treatments (P < 0.05).

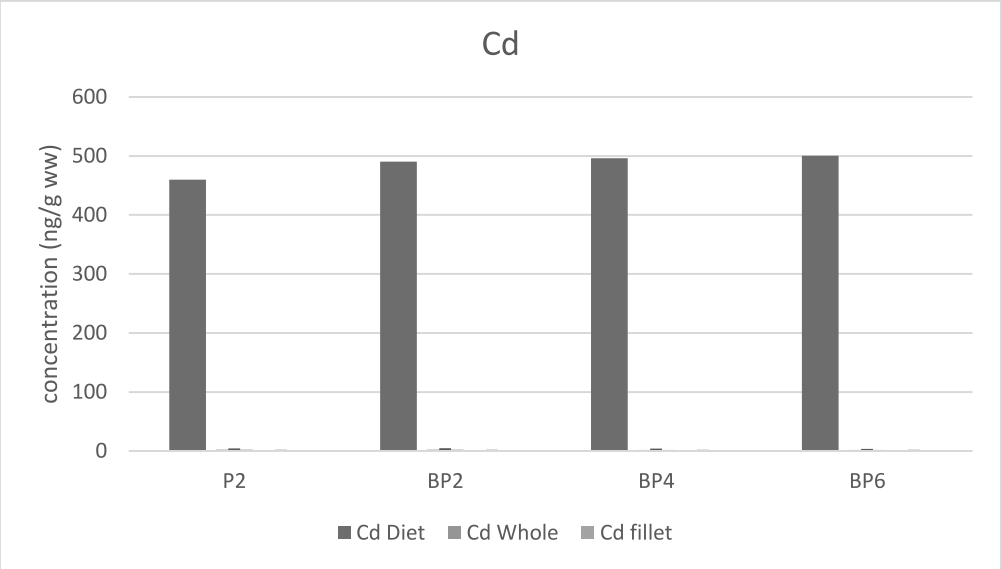

Figure 3.3. Total cadmium concentration (ng/g ww) in diets, whole fish, and fillet of golden mullet.

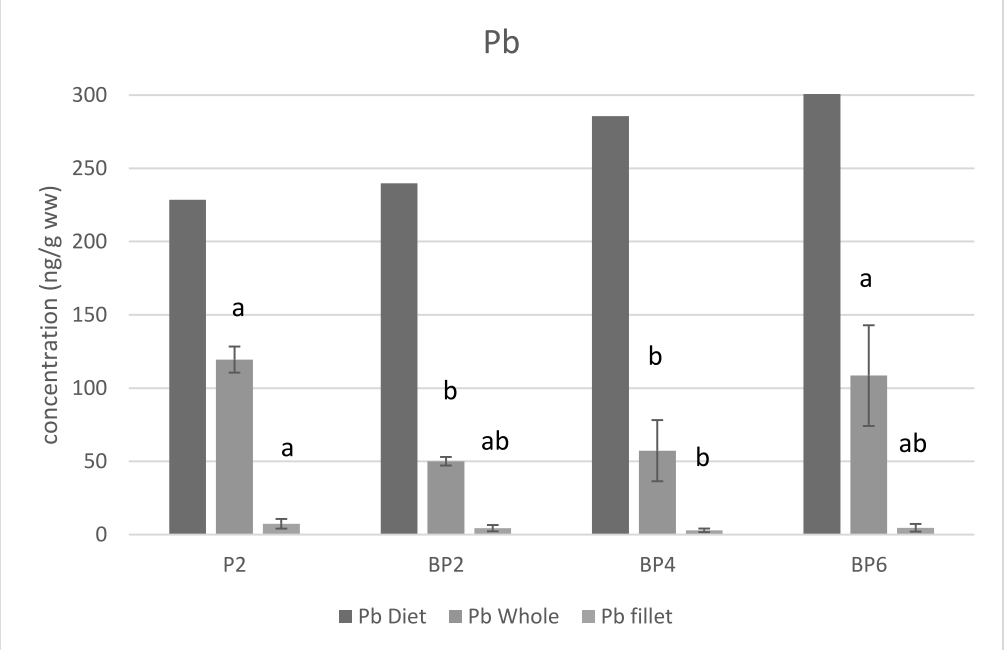

Figure 3.4. Total lead concentration (ng/g ww) in diets, whole fish and fillet of golden mullet . Different letters in the same group denote statistically significant differences among dietary treatments ($P < 0.05$).

As for the essential elements (Table 3.3), in general, the highest levels were found in the whole fish, except in the case of Se, where the highest level was found in fillet. In the case of whole fish, no homogeneous relationship was found for all elements between the type of diet and the concentration of trace elements in the fish. Thus, for Cu, Zn, and Mn the highest content was in those fish fed with BP4, while for Co and Se, the highest value was in BP2 group and finally, for Fe, the diet P2 yielded the highest concentration. On the other hand, in the case of raw fillet, Fe, Co, and Cu had the same descending order as BP4>BP2>P2>BP6. The BP4 diet also gave the highest concentration of Se in fillet, while Zn and Mn had the highest content in BP6 group. For Fe, Cu and Mn, we found no statistically significant differences in accumulation in either whole fish or fillets. We only found differences for Co and Zn accumulation in whole fish, and Se in fillets.

Table 3.3 Contents of essential heavy metals (ng/g ww) in whole fish and raw fillet in golden mullet fed experimental diets.

	Diets			
Heavy metals	**P2**	**BP2**	**BP4**	**BP6**
Whole fish				
Iron	**4550.73±628.51**	**3312.25±141.33**	**3381.68±1122.58**	**3883.14±1365.64**
Cobalt	**32.55±5.45^{ab}**	**37.07±3.95^{a}**	**29.48±7.29^{b}**	**33.43±4.34^{ab}**
Copper	**1336.41±349.20**	**1619.01±416.91**	**1717.89±578.13**	**1630.39±400.61**
Zinc	**4087.27±331.84^{bc}**	**4920.79±641.55^{a}**	**4586.58±600.16^{ab}**	**3772.66±303.83^{c}**
Selenium	**76.68±23.13**	**83.62±18.66**	**84.45±25.19**	**83.55±9.85**
Manganese	**257.03±62.42**	**242.76±23.36**	**304.88±93.12**	**289.53±70.01**
Raw fillet				
Iron	**1364.03±469.32**	**1479.75±389.10**	**1558.25±456.50**	**1215.31±431.69**
Cobalt	**15.53±2.05**	**15.54±5.25**	**17.42±1.78**	**16.38±1.49**
Copper	**266.59±83.95**	**269.11±70.71**	**297.72±106.37**	**216.17±69.80**
Zinc	**2214.43±593.13**	**2110.15±428.57**	**2346.11±418.19**	**2473.87±863.16**
Selenium	**85.72±17.24^{ab}**	**73.32±16.22^{b}**	**91.97±20.31^{a}**	**68.34±10.28^{b}**
Manganese	**75.31±25.92**	**68.94±32.38**	**70.19±16.85**	**80.23±31.84**

Values expressed in mean ± SD. (n = 15). Different letters within a line denote significant differences among dietary treatments ($P < 0.05$).

3.4.2. Estimated Daily Intake

The Estimated Daily Intake (EDI) for the elements that were determined in our study is shown in Tables 3.4 and 3.5. The EDI for Pb and Cd elements was below the established Reference Dose (RfD). However, in the case of As and Hg, the values were higher than the established RfD, not as high compared to the higher amount of fish consumption performed in our study, as mentioned above. For the essential elements (Table 3.5), the EDI was higher than the established RfD, except for Mn and Cu.

3.4.3. Maximum Safe Consumption

The maximum safe consumption (Tables 3.4 and 3.5) indicates that a person weighing 70 kg would have to consume between 37 and 614 kg of golden mullet to be exposed to dangerous amounts of Cd, Mn, and Pb. This is due to the low amount of these metals in the raw fillet. However, in the case of As, Hg, and Zn, whose concentrations in the fillets are higher, the safe consumption of this type of fish fed with *Aloe vera* by-products would drop to 58-995 g. Therefore, health risks are not excluded if a 70 kg person consumes more than 58 g of golden mullet per day.

Table 3.4 Target Hazard Quotient (THQ), Estimated Daily Intake (EDI) (µg/kg BW/day) for both consumptions (ng/g WW) (x $^{10-1}$), and Maximum Safe Consumption (MSC) (kg fish ww/day), based on the Contents of heavy metals of golden mullet fed experimental diets.

Element	Treatment	THQ 9.94	THQ 31.13	EDI 9.94	EDI 31.13	MSC
As	P2	1.167	3.655	0.350	1.097	0.085
	BP2	1.282	3.212	0.385	1.205	0.078
	BP4	1.425	3.571	0.428	1.339	0.070
	BP6	1.707	4.276	0.512	1.604	0.058
Hg	P2	0.511	1.279	0.051	0.160	0.195
	BP2	0.490	1.229	0.049	0.154	0.203
	BP4	0.550	1.378	0.055	0.172	0.181
	BP6	0.506	1.269	0.051	0.159	0.196
Cd	P2	0.000	0.001	0.000	0.001	432.099
	BP2	0.000	0.001	0.000	0.001	457.516
	BP4	0.000	0.000	0.000	0.001	614.035
	BP6	0.000	0.001	0.000	0.001	496.454
Pb	P2	0.003	0.007	0.010	0.033	37.910
	BP2	0.002	0.004	0.006	0.020	63.839
	BP4	0.001	0.003	0.004	0.013	96.319
	BP6	0.002	0.004	0.007	0.021	59.983

Table 0.5 Target Hazard Quotient (THQ), Estimated Daily Intake (EDI) (mg/kg BW/day), for both consumptions (ng/g WW) (x $^{10-1}$), and Maximum Safe Consumption (MSC) (kg fish WW/day) based on the Contents of essential metalsof golden mullet fed experimental diets.

Element	Treatment	THQ 9.94	THQ 31.13	EDI 9.94	EDI 31.13	MSC
Mn	P2	0.001	0.002	0.107	0.335	130.125
	BP2	0.001	0.002	0.098	0.307	142.146
	BP4	0.001	0.002	0.100	0.312	139.613
	BP6	0.001	0.002	0.114	0.357	122.150
Fe	P2	0.048	0.121	1.937	6.066	2.053
	BP2	0.053	0.132	2.101	6.581	1.892
	BP4	0.055	0.139	2.213	6.930	1.797
	BP6	0.043	0.108	1.726	5.405	2.304
Co	P2	0.073	0.184	0.022	0.069	1.352
	BP2	0.074	0.184	0.022	0.069	1.351
	BP4	0.083	0.207	0.025	0.078	1.204
	BP6	0.078	0.194	0.023	0.073	1.282
Cu	P2	0.009	0.024	0.379	1.186	10.503
	BP2	0.010	0.024	0.382	1.197	10.405
	BP4	0.011	0.026	0.423	1.324	9.405
	BP6	0.008	0.019	0.307	0.961	12.953
Zn	P2	0.105	0.263	3.144	9.848	0.948
	BP2	0.100	0.250	2.996	9.384	0.995
	BP4	0.111	0.278	3.331	10.433	0.895
	BP6	0.117	0.293	3.513	11.002	0.849
Se	P2	0.024	0.061	0.122	0.381	4.083
	BP2	0.021	0.052	0.104	0.326	4.773
	BP4	0.026	0.065	0.131	0.409	3.806
	BP6	0.019	0.049	0.097	0.304	5.121

3.4.4. Risk Assessment

As shown in Table 3.4 for toxic elements, the THQ values for both consumptions, mentioned above, were well below 1 in all diets, indicating that they do not pose a risk to the consumer. For As and Hg, the values were higher than the other metals but also under the hazard values. The results shown in Table 3.5 also indicate that the THQ was less than 1 for all the elements, although in this case it is logical, since these are the essential elements.

HI values increased significantly with higher concentrations of *Aloe vera* in the diets but were also below 1 in all cases. The two approaches used to assess the exposure of the Spanish population to heavy metals through fish consumption indicate that using *Aloe vera* by-products as an ingredient for the diet of golden mullet is safe for human consumption (Table 3.6), regardless the concentration employed.

Table 3.6 Hazard Index values (HI) for heavy and essential metals, for both consumptions (ng/g ww) of golden mullet fed experimental diets.

treatment	HI. 9.94	HI. 31.13
P2	0.17	0.49
BP2	0.18	0.44
BP4	0.20	0.50
BP6	0.22	0.55

3.4.4.1. Carcinogenic risk of As

The As Carcinogenic Risk (As-CR) of the detected As was below the highest established acceptable risk level of 10^{-4} (Table 3.7). This suggests that if a person of 70 kg ingests 9.94g or 31.13g of these fish each day, 365 days per year and 56 years, the risk of developing cancer would not be of concern as all the values are within the permitted limits.

3.4.4.2. Selenium Health Benefit Value

The Se-HBV values were not only found to be positive but ranged from 3.28 to 5.54, indicating beneficial effects on human health (Table 3.7). All diets tested had a molar ratio of Se to Hg ranging from 4.81 to 6.08, showing that different concentrations of *Aloe vera* in the diets had different molar values of Se. In terms of Hg toxicity, this confirms safe ingestion. The values of Se-HBV and free Se to free Hg ratio were ordered as follows BP4>P2>BP2>BP6, which indicates that the diet BP4 had the highest value of Se-HBV and free Se to Hg ratio (19.83 and 3.28 respectively) and that the lowest values corresponded to the diet BP6.

Table 3. 7 The carcinogenic risk of arsenic (As-CR) for both consumptions (ng/g ww) (x $^{10-1}$) and Selenium Health Benefit values (mole) of golden mullet fed experimental diets.

Treatment	As-CR 9.94	As-CR 31.13	Se HBV
P2	0.001	0.002	5.536
BP2	0.001	0.002	3.992
BP4	0.001	0.002	5.831
BP6	0.001	0.002	3.280

3.4.5. Nutritional value

As shown in Table 3.8, the RDA in Se, Zn, and Cu is adequately fulfilled at both low and high intake levels, 9.94 or 31.13 g fish/day, respectively. However, in the case of Fe, the consumption rate of 9.94 g fish/day does not meet the nutrient requirements of women. Moreover, the EDI values of Mn with the lowest fish consumption were insufficient to meet the AI in both men and women. However, the higher fish consumption provided sufficient Mn in women, whereas in men, it was only achieved with diets P2 and BP6.

Table 3.8 Estimation of the contribution of the Estimated daily intake to the daily reference intakes established for each element in all the experimental diets.

element	treatment	EDI 9.94 WW (mg/day)	EDI 31.13 WW (mg/day)	RDA or AI (F) mg/day	RDA or AI (M) mg/day
Mn	P2	0.75	2.34	1.8	2.3
	BP2	0.69	2.15		
	BP4	0.70	2.19		
	BP6	0.80	2.50		
Fe	P2	13.56	42.46	18	8
	BP2	14.71	46.06		
	BP4	15.49	48.51		
	BP6	12.08	37.83		
Co	P2	0.15	0.48	NA	NA
	BP2	0.15	0.48		
	BP4	0.17	0.54		
	BP6	0.16	0.51		
Cu	P2	2.65	8.30		
	BP2	2.67	8.38		

	BP4	**2.96**	**9.27**	**0.9**	**0.9**
	BP6	**2.15**	**6.73**		
Zn	**P2**	**22.01**	**68.94**	**8**	**11**
	BP2	**20.97**	**65.69**		
	BP4	**23.32**	**73.03**		
	BP6	**24.59**	**77.01**		
Se	**P2**	**0.85**	**2.67**	**0.055**	**0.055**
	BP2	**0.73**	**2.28**		
	BP4	**0.91**	**2.86**		
	BP6	**0.68**	**2.13**		

3.5. Discussion

3.5.1. Metals content

Differences in heavy metals levels among fish species are based on feeding patterns (Subotić, Višnjić Jeftić et al. 2013) with the food being primary source of heavy metal accumulation (Ferreira, Caetano et al. 2008). In controlled fish production, the different heavy metal content of commercial feeds is the cause of alterations in heavy metal concentrations in farmed fish tissues (Cretì, Trinchella et al. 2010) along with variations in the assimilation capacity of different species (Kalantzi, Pergantis et al. 2016, Marengo, Durieux et al. 2018) and the duration of exposure (Amlund, Francesconi et al. 2006). Thus, dietary uptake is a defining component in establishing a model for metal bioaccumulation (Luoma and Rainbow 2005) although both essential and toxic tend to show a process of bio dilution in the food chain rather than biomagnification, with a lower concentration on a wet weight basis compared to levels in consumed food (Kelly, Ikonomou et al. 2008). The addition of *Aloe vera* by-products to experimental diets has resulted in slight variations in the concentration of heavy metals, which increase according to the level of inclusion, except for Fe and Mn, which have the highest concentrations in diets that include *Aloe vera* before being processed. However, differences due to increased levels of heavy metals in the experimental diets have only been found for As and Pb accumulations. As increases both in whole fish and fillet, while Pb increases only in whole fish. The low concentration of heavy metals in our experimental diets compared to other commercial fish feeds (Psoma, Pasias et al. 2014) may explain the lack of variation between treatments.

The contents of the essential heavy metals analysed were lower in muscle than in whole fish, except for Se, which was quite similar in both muscle and whole fish. These elements preferentially

accumulate in active metabolic tissues according to the physiological functions of each element in fish metabolism (Canli and Atli 2003, Basim, Khoshnood et al. 2016).

As for toxic heavy metals, their accumulation in muscle depends on the affinity towards proteins (Ramos-Miras, Sanchez-Muros et al. 2019, Kontas, Alyuruk et al. 2022) as they have no biological function in fish (Marengo, Durieux et al. 2018). Thus, both As and Hg showed higher concentrations in muscle than in whole fish. In the case of As, a concentration in golden mullet muscle almost ten times higher than our results has been reported (Usero, Izquierdo et al. 2004). Dietary Cd and Pb retention is generally low in whole fish but accumulates to a lesser extent in muscle (Amlund, Francesconi et al. 2006). The low concentrations of Cd are notable for their low absorption in the gastrointestinal tract (Ciardullo, Aureli et al. 2008), even though their dietary content is higher than that of Hg and Pb.

After being fed for three months on dietary *Aloe vera* by-products, farmed golden mullet show a relationship between dietary As and Pb content and their accumulation in fillet and whole fish. In other species, such as Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*), two months fed on a diet with added As have been sufficient to detect significant increases in the concentration of this heavy metal in muscle (Amlund, Francesconi et al. 2006). It is possible that even less time may be needed to detect Pb accumulation in rainbow trout (*Oncorhynchus mykiss*) (Alves, Glover et al. 2006).

3.5.2. Risk assessment

THQ values above 1 as a health risk associated with exposure to potentially toxic elements are of concern (Zheng, Wang et al. 2007), indicating that potential long-term non-carcinogenic effects cannot be excluded (Li, Huang et al. 2013). There were no THQs above 1 associated with human consumption of golden grey mullet fed with *Aloe vera* by-product, indicating no health risk. They were always less than 0.5 for the consumption of 9.94g g fish per day, indicating safe levels of dietary intake of heavy metals through fish consumption.

There is no hazard for HI values below 0.1, while the hazard is considered low for values between 0.1 and 1.0 (Kalogeropoulos, Karavoltsos et al. 2012). In this study, HI Index was below 1 and ranged from 0.17 to 0.22 for the lowest consumption of 9.94 g fish/day and from 0.49 to 0.55 for the highest consumption of 31.13 g fish/day, with a gradual increase in HI value in the different

diets. The high HI value of 0.5 in the case of 31.13 g fish consumption due to the THQ arsenic being higher than 2, was mainly responsible for this increase, but it is considered safe. As the HI is below 1, no adverse health effects are expected from the exposure described in the assessment (Storelli 2008). In this context, the risk to consumers must be considered according to the amount of seafood intake per day (Prato, Biandolino et al. 2019).

3.5.2.1. Carcinogenic risk of As

Although As may have a non-carcinogenic implication considering its inhibition of cellular respiration in mitochondria, chronic exposure to As promotes changes in DNA methylation patterns with proliferative cell effects (Dissanayake, Chandrajith et al. 2009), mainly related to lung, kidney, bladder, and skin cancer (Castro-González and Méndez-Armenta 2008). The primary source of exposure for humans is the diet, mainly seafood, as it can contain several times the amount of As than other foods (Uneyama, Toda et al. 2007). However, marine organisms accumulate As over a wide range, even with considerable variations within the same fish species (Afonso, Lourenco et al. 2013). Moreover, As exists in both organic and inorganic forms, the latter having a toxic effect (Varol, Kaya et al. 2017). Fortunately, up to 90% of this element is present as organoarsenic compounds in fish muscle in the non-toxic form of arsenobetaine (Bosch, O'Neill et al. 2016). The assumption that inorganic As is 3% of total As (Copat, Arena et al. 2013) shows that the potential risk is overestimated (Sofoulaki, Kalantzi et al. 2019). This study showed that As concentrations, assuming 3% of the total is inorganic, did not exceed the recommended levels even in fish fed with the diet BP6, which showed the highest As accumulation in muscle due to the maximum inclusion of *Aloe vera* by-products.

3.5.2.2. Selenium Health Benefit Value

Se is considered to be an effective agent for reducing both the bioaccumulation of Hg (Dang and Wang 2011) and moderating its toxic effects (Peterson, Ralston et al. 2009). Due to an excess of Se molarity, Se/ Hg balance study indicate it is safe for human consumption. Although the interactions between selenium and mercury, and their molar ratios in seafood, are essential to determine the risks associated with dietary mercury exposure, focusing solely on mercury concentration is insufficient. Furthermore, the high positive Se-HBV readings suggest favorable effects (3.28 – 5.83), which had the lowest Se-HBV value in the diet BP6 while reaching the

highest value in diet BP4. Some studies on various fish species have shown that Se can reduce Hg toxicity (Copat, Vinceti et al. 2014, Ralston, Ralston et al. 2016). It has been noted that whereas Hg concentration cannot predict MeHg toxicity, Se concentration and Hg/Se molar ratio are significantly and inversely associated (Ralston, Ralston et al. 2008). Depending on their relative levels, the presence of heavy metals in different foods, constitutes a severe health risk. In addition, elemental mercury and methylmercury are toxic to the central and peripheral nervous system (FAO and WHO, 2002). It has been argued that risk assessment based solely on MeHg exposure without considering its interaction with Se is inaccurate (Ralston, Ralston et al. 2016).

The Hg/Se molar ratio has been identified as the most useful Hg toxicity assessment criterion for decreasing Hg bioaccumulation as long as interactions between Se and Hg are possible (Copat, Vinceti et al. 2014). In this study, the molar ratio of Se/Hg was higher than of Hg/Se, indicating expected health benefits such as improved immune function, antioxidant tone, and anticancer effects. However, our values were lower than those reported in other species (Olmedo, Hernández et al. 2013, Copat, Vinceti et al. 2014, Sofoulaki, Kalantzi et al. 2019) although not due to a high presence of Hg but rather because of the low accumulation of Se in muscle conditioned by its availability in experimental diets.

3.5.3. Nutritional Value

The nutritional assessment of the golden mullet showed that this species can be considered rich in essential metals such as Fe, Cu, Zn, and Se, while for Mn, is reasonably good. Thus, the inclusion of *Aloe vera* by-products in the diet slightly affected the metal content in the fillet, but not to the extent of decreasing the nutritional value. Compared to the wild *Liza aurata*, our farmed fish had lower contents of all metals analysed (Usero, Izquierdo et al. 2004). This is probably because wild fish were caught in polluted areas with high concentrations not only in sediments but also in the water column. In fact, a very similar species in terms of feeding habits, such as the flathead grey mullet (*Mugil cephalus*), caught in other areas of the Mediterranean, showed metal contents reasonably similar to those obtained in our study (Canli and Atli 2003). Most of the differences in mineral content of farmed fish fillets compared to their wild counterparts depend on the dietary mineral concentrations (Alasalvar, Taylor et al. 2002). Despite this, our results showed slight differences in the nutritional value of golden grey mullet farmed batches, not being able to establish a relationship between muscle content of essential minerals and the feed concentration, as reported

in other studies (Yildiz 2008). Compared to other farmed species such as European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*), golden mullet reared on experimental diets showed higher concentrations of Se and Zn and lower Mn and Cu in the edible part (Olmedo, Hernández et al. 2013).

3.6. Conclusions

The concentration of all elements was below the maximum limits allowed in the different ratios of *Aloe vera* in diets. The different risk-benefit assessment measures established by national and international authorities indicate that the consumption of *Liza aurata* is mainly safe. The detected amounts of potentially hazardous inorganic As were below safety standards and Se-HBV values indicated positive effects on human health.

In conclusion, the consumption of golden mullet from the locality does not pose a health risk, and the RDA values meet healthy nutritional requirements.

Chapter 4. Heavy metal And Trace Elements Content in Farmed Nile Tilapia (*Oreochromis Niloticus*) Fed with Banana By-products

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4.1. Introduction

The food gap in animal protein requirement had greatly mounted as the world's population has grown. As a result, aquaculture has recently gained attention as a reliable source of animal protein and had since become the fastest-growing food-producing sector, accounting for more than half of global fish production (Galappaththi, Ichien et al. 2020), therefore, it is often considered a viable choice for bridging the gap between fish output and consumption (FAO 2012).

Over the last 2 decades, the expanded aquaculture has coincided with an increase in the aquafeed industry, which recognized including plant by-products in formulated diets as a key-determining step for the cost-effectiveness of the sector (Paul, Barman et al. 2013). The qualitative and quantitative variability of dietary components influences fish digestibility, growth performance,

biochemical composition, hematocrit, and enzyme activity (Lundstedt, Melo et al. 2004). The presence of anti-nutrients in botanical waste constrains its use in aqua feeding, as do alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haemagglutinin, sapogenin, mimosine, cyanoglycosides, as well as low protein content, amino acid, fatty acid, and micronutrient imbalances (Wee and Wang 1987, Abowei, Ekubo et al. 2011). Since feeding cost accounts for 40-60% of total production costs, any dietary modifications of plant-derived resources must initially verify cost- effectiveness while ensuring that the fish's growth is not jeopardized to maintain the final product value as a human nutrient. Thus, the ultimate goal is to incorporate plant products with growth-promoting properties, immune-system tonics, hunger stimulants, antibacterial activity, and anti-stress properties in an eco-friendly, cost-effective, and sustainable manner to improve fisheries-horticulture coordination and build a robust interaction between agriculture and aquaculture.

The circular economy (CE) is a regenerative and renewable industrial model that replaces the end-of-life notion, encourages energy consumption, eliminates the use of hazardous chemicals that limit reuse, and aims to eliminate waste products through better design (Ghisellini, Cialani et al. 2016). In the same context, The World Health Organization (WHO) promotes the use of medicinal herbs and plant wastes to replace or reduce the use of chemicals as part of the global movement to return to nature. Various research has been conducted on non-traditional raw components, including sweet potato peels (Omoregie, Igoche et al. 2009), banana peels (Zargaran Hoseini and Chelemal Dezfulnejad 2018, Alzate Acevedo, Díaz Carrillo Á et al. 2021), calabash seeds (Kaur and Shah 2017), and papaya seeds (Kang 2007).

Agricultural by-products contain a wide variety of bioactive components, including phenolics, antioxidants, dietary fibers, flavonoids, anthocyanins, proteins, peptides, and enzymes, which can be reused as supplements, fortifiers, or minor ingredients (Galali, Omar et al. 2020). Corn meal (CM) as a main source of highly digestible carbohydrates for both omnivorous and herbivorous fish (Stone 2003, Kaushik, Panserat et al. 2022) exerts a protein-sparing effect (García-Meilán, Ordóñez-Grande et al. 2014, Sadiku and Orire 2020) that necessitates a pre-designed utilization pattern to maximize the fish protein content while minimizing nitrogenous residues (Nguyen, Hilmarsdóttir et al. 2022). Many studies in *O. niloticus* feeding have shown that including fruits such as mango or whole banana meal in the fish diet, can be a good substitute for CM (Felix e Silva, Copatti et al. 2020, Souza, Souza et al. 2020).

Bananas (Musa spp.), the world's fourth most consumed food after wheat, maize, and rice (Zou, Tan et al. 2022), are essential for food security in many tropical and subtropical countries. Cultivating and manufacturing bananas yield waste of up to 35% (Padam, Tin et al. 2014). More than 350 thousand tonnes of banana trash have been recorded annually over the last ten years, and the natural degradation of bananas emits poisonous fumes into the air (Zou, Tan et al. 2022). Many studies identified the benefits of adding banana by-products to fish feeding. Promoted growth, improved physiological function, boosted immunity, and increased anti-hypothermal stress were reported in prawns fed with diets containing banana blossom powder at 10-20 g/kg (Mapanao, Rangabpai et al. 2022). In the same context, others recommended banana wastes as an additive in easy-to-digest fish feeds (Paz, Monzón et al. 2020).

The Nile tilapia (*Oreochromis niloticus*) is one of the most widely used species in tropical and subtropical intensive farming. *O. niloticus* is regarded as the most promising for fish farming due to its rapid growth in captivity, high disease resistance, low trophic feeding levels, and high-quality meat. Tilapia is the most popular cultured fish in East Africa, whereas it is the world's second most important cultivated fish after carp (Dan and Little 2000, El-Sayed 2006). It has multiplied its contribution to global aquaculture production from 28,000 tonnes in the 1970s (Halwart and Moehl 2006) to more than 6.5 million tonnes in the 2017s (Mathiesen 2015).

O. niloticus is omnivorous from the post-larva to adulthood and easily accepts a wide variety of foods (Boscolo, Hayashi et al. 2001). Although there are minor differences between tilapia species, the fish size greatly impacts the nutrient requirements (El-Sayed and Teshima 1992), a well-balanced prepared feed is essential to enhance tilapia culture since it provides a high yield and rapid growth at a low cost.

The proclivity of heavy metals to bioaccumulate in aquatic habitats and their ecological persistence (Tofighy and Mohammadi 2011) raise concerns about their toxicity, devoting attention to a rigorous assessment of heavy metal levels in food (Ashraf, Maah et al. 2012). Exhaustive research has shown that fish can uptake heavy metals through respiration, adsorption, and consumption of contaminated water or food. Accumulation of significant levels of metals in fish various tissue posing a possible risk of metal-related diseases if consumed by humans, thus, determining the metal content of fish is crucial in terms of human health (Resma, Meaze et al. 2020).

This study aims to (1) determine the concentration of toxic metals and trace elements in the muscles of *O. niloticus* fed with banana by-products, and (2) calculate the risk/benefit ratio for the human consumer of exposure to these elements through the consumption of Nile Tilapia.

4.2. Materials and Methods

4.2.1. Experimental design and fish preparation and sampling

In this experiment, 192 *O. niloticus* (5 g initial body weight) were distributed among 12 glass tanks of 50 L of fresh water (16 fish/tank). Using a closed system at the Technological Science Park Foundation (FCPCT) facilities in Taliarte, Telde. The fish were fed the experimental diets three times per day, six days per week, for three months, until apparent satiation. During the experiment, oxygen and temperature parameters were measured using an oximeter (Oxy Guard-Guard-handy beta, Zeigler Bros, Gardners, USA). During the feeding period, the average water temperature was 20.1±1.22o C, with an average oxygen saturation of 6.4±0.37 mg/L. The experiment's final sample was taken after 80 days when the fish had doubled in weight. At the start and end of the experiment, raw materials, a by-product of banana pseudo-stem, processed diets, and fish tissues were collected.

4.2.2. Processing raw materials and experimental diets

The banana crop by-products were received in the Bio factory of the Pilot Plant of Products and Processes of (GIA-ECOAQUA) in Taliarte. The raw materials' biochemical composition, and heavy metal content were tested before being treated, sanitized, sterilized, and lyophilized using a lyophilizer then finally dried in an oven at a temperature below 40°C to be eventually included in fish feed. 4 isocaloric and isoproteic diets were prepared: one was a commercial diet of the species under study, and the other 3 included varying percentages of raw material obtained during the banana pseudo-stem processing (5, 10, and 20%). Ingredients used and biochemical composition of diets tested are shown in the Table 4.1.

Table 4.1 Ingredients (g/kg) and chemical composition (% dry basis) of the experimental diets.

	Diets			
Ingredients	**Control**	**5**	**10**	**20**
Fish meal[a]	**210**	**210**	**210**	**210**
Corn meal[b]	**200**	**150**	**100**	**-**

Soy meal[b]	**260**	**260**	**260**	**260**
Wheat meal[b]	**90**	**90**	**90**	**90**
Corn gluten[b]	**70**	**70**	**70**	**70**
Wheat gluten[b]	**60**	**0**	**0**	**60**
Linseed oil[b]	**0**	**0**	**0**	**60**
Banana by-product[c]	**-**	**50**	**100**	**200**
Vitamin mix[d]	**10**	**10**	**10**	**10**
Mineral mix[e]	**20**	**20**	**20**	**20**
Ca(H2PO4)2[f]	**5**	**5**	**5**	**5**
CMC[g]	**15**	**15**	**15**	**15**
Composition				
Lipids	**12.72**	**14.76**	**10.2**	**10.87**
Protein	**40.61**	**38.25**	**37.8**	**33.09**
Ash	**8.42**	**9.21**	**10.03**	**12.17**
Moisture	**6.65**	**.8**	**6.79**	**8.25**

[a] Supplied by Skretting, Spain.
b Supplied by Capisa S.A., Spain.
c Supplied by banana local producers.
d Vitamin premix containing (mg kg-1 o IU/kg de pienso): tiamine 40 mg, riboflavin 50 mg, piridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, folic acid 10 mg, cianocobalamine, 0.5 mg, choline chloride 2700 mg, Mio-inositol 2000 mg, ascorbic acid 5000 mg, menadione 20 mg, colecalciferol 2000 IU, etoxiquine 100 mg, retinol acetate 5000 IU. Vitamin E (DL-alpha-tocopherol acetato) 250 mg.
e Mineral premix containing (g/kg de dry feed): calcium orthofosfate 1.60 g, calcium carbonate 4 g, ferrous sulfate 1.5 g, magnesic sulfate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminum sulfate 0.02 g, zinc sulfate 0.24 g, copper sulfate 0.20 g, manganese sulfate 0.08 g, potassium iodate 0.02 g;
f Sigma-Aldrich, Munich, Germany.
g Carboxymethylcellulose (sodium salt, Sigma-Aldrich, Munich, Germany).

4.2.3. Metals Assay

Metal contents were assayed in the laboratories of the Clinical and Analytical Toxicology Service (SERTOX) of the Universidad de Las Palmas de Gran Canaria. An Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) was used to determine the concentration of all elements and the data were processed using Agilent Mass Hunter Data software (version 4.2). Each sample was analyzed in triplicate. A sample from each tank was collected and homogenized separately. A microwave digester was used to digest three (1-gram) subsamples of each homogenate (Ethos Up, Milestone SRL, Italy). Subsamples were weighed into the digestion vessels before adding 50 µL of the internal standard solution of Scandium (Sc), Germanium (Ge), Rhodium (Rh), and Iridium (Ir) at concentrations of 20 mg/mL each). All sample is then treated with 2.5 mL of concentrated sub-boiling HNO_3 (65%) and 7.5 mL of Mili-Q water. Then, all are digested using the following

protocol: Step 1: [1800–100–5 power (W)– Temperature (C)– time (min)]; Step 2: [1800–150–5]; Step 3: [1800–200–8] and Step 4: [1800– 200–7].

After cooling, all digested samples were transferred to conic-bottom polypropylene tubes and dilute them with Mili-Q water to a volume of 15 mL. Finally, an aliquot of each sample was collected for analysis. Reagent blanks were prepared the same as the samples and all 14 samples were included in the same analytical batch. The entire procedure was validated before being used in the sample analyses.

The values obtained for toxic and essential elements ranged between 87-118%, with all elements having linear calibration curves (regression coefficients ≥0.998). The concentration of the element that produced a signal three and ten times higher than that of the averaged blanks were used to calculate instrumental LODs and LOQs. The sample LOQs were calculated by multiplying the instrumental LOQ by the sample's dilution factor during the digestion procedure (1:10 v/v).

4.2.4. Risk Assessment and nutritional assessment analysis

Various approaches were used to determine the risk factors associated with fish consumption.

4.2.4.1. Estimated daily intake (EDI)

Estimated Daily Intake (EDI; g/kg bw/day) for toxic and essential metals was calculated using the following equation (Onsanit, Ke et al. 2010, Kalantzi, Black et al. 2013, Copat, Vinceti et al. 2014),

$$EDI = \frac{[C \ x \ AvC]}{bw}, \ where:$$

C (µg/g w/w of fish or diet) is the average metal concentration in the fish's edible parts and diet, (AvC) is the daily average consumption of Grams fish of 31.13 g/day as the average fish consumption in Spain and 9.94 g/individual/day as the safest amount of fish consumption in Spain for consumers regarding the majority of health risk parameters (Marcos, Rubio et al. 2016). Bw is the average adult body weight of the general population (Mathiesen 2015). All EDI values were compared to the USEPA's parallel Reference Doses (RfD) (USEPA 2005). The RfD is an estimate (with uncertainty up to one order of magnitude) of the population's daily exposure due to ingestion that is unlikely to1 cause any carcinogenic effects, even in vulnerable populations, over a lifetime (USEPA 2005). These Reference Doses are as follows: Cobalt (Co) [0.3 g/kg bw/d], Nikal (Ni) [20 g/kg bw/d], Cupper (Cu) [40 g/kg bw/d], Zinc (Zn) [300 g/kg bw/d], Ferrous (Fe) [700 g/kg bw/d], Chromium (Cr) [1500 g/kg bw/d], Arsenic (As) [0.3 g/kg bw/d], Uranium (U) [1.0 g/kg bw/d] (Usepa 2011). In the case of Lead (Pb), because the USEPA did not establish an RfD value in 2017, a previously established value by the USEPA (2014), 3.57 g/kg bw/day, was used.

4.2.4.2. Maximum Safe Consumption (MSC)

Since fish content of metals may possess potential toxicity, the Maximum Safe Consumption (MSCA, kg fish w/w/day) specifies the safe daily limit for fish consumption. MSCA was calculated for metals with established RfD according to the following equation (Tacon and Metian 2013, Kalantzi, Pergantis et al. 2016):

$$MSC = \frac{[bw\ x\ RfD]}{C}\ ,\ where:$$

where C (µg/g w/w of fish) is the mean metal concentration in various parts of the fish and diets, bw is the adult body weight of the general population (70 kg) and RfD is the reference dose (Mathiesen 2015).

4.2.4.3. Target hazard quotient (THQ)

The target hazard quotient (THQ) indicates the risk of carcinogenicity associated with pollutant exposure (Usepa 2011). THQ values less than 1 indicate the absence of any harmful effects on human health. THQ was calculated as follows:

$$THQ = \frac{(EF\ x\ ED\ x\ AvC\ x\ C)}{(RfD\ x\ bw\ x\ AT)}\ ,\ where:$$

EF is the exposure frequency (365 days/year); ED is exposure duration (70 years); AvC is the fish consumption rate, C is the metal concentration (mg/kg, wet weight); RfD is the reference dose of the metal. BW is body weight (adults 70 kg). AT is the average time, which is given by (EF × ED).

4.2.4.4. Hazard Index (HI)

The total THQ, also known as the hazard index (HI), was calculated by adding all of the target hazard quotients (THQ) for the determined metals to assess the human health risk resulting from additive or interactive effects of those metals (Li, Huang et al. 2013). HI was calculated as follows:

$$HI = THQ\ (element\ 1) + THQ\ (element\ 2) + THQ\ (element\ n)$$

4.2.4.5. Carcinogenic risk of Arsenic (As)

Because chronic consumption of (As) is thought to increase the risk of cancer, we used the following equation to calculate the probability of developing cancer from As:

$$As(CR) = \frac{[\text{EDI x CSF}]}{1000},\ \text{where:}$$

As(CR) is dimensionless, EDI (g/kg bw/day) is the Estimated Daily Intake, and CSF is the oral cancer slope factor for inorganic (As), which is set by the USEPA at 1.5 $(mg/kg/day)^{-1}$ (2012). The exposure duration was set to 56 years, and the frequency of exposure was assumed to be 365 days per year. Carcinogen tolerable risk thresholds range from 10^{-4} to 10^{-6}, with lower levels considered

safe, however, exceeding these levels refers to a higher risk of developing cancer (Vieira, Morais et al. 2011, Kalantzi, Pergantis et al. 2016)

4.2.5. Nutritional value

To determine the nutritional value of the diet and fish, the mean concentrations of important elements are compared with the minimal dietary needs that should be met for each element. The European Union has established nutrient reference values (NRVs) for daily recommended vitamin and mineral intakes. If 100 g of food contains 15% of the NRVs, it is considered to contain a significant amount of vitamins and minerals (Authority 2017).

4.2.6. Statistical analysis

The results were expressed as mean ± standard deviation (SD). Differences were assessed by the non-parametric Krskal-Wallis test, considering $p<0.05$ as the level of statistically significant differences. All statistical analyses were conducted by IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp.).

4.3. Results

4.3.1. Metals content in diets

Diet 3 (D3) has always had the highest concentration of all heavy and essential elements in diets. For heavy metals, Arsenic had the highest value followed by Cd, Pb, and Mercury (Hg) respectively. (As) showed non-statistically significant differences in all diets with the highest value in D3 (D3>control>D1>D2). (Cd) and (Pb) also demonstrated non-significance variation (D3>D1>control>D2) and (D3>D2>control>D1), respectively. Hg had the lowest concentrations among all heavy metals with statistically significant variation in D1. see Table 4.2.

When it comes to essential metals, (Fe) had the highest value and Selenium (Se) had the lowest. (Fe), (Co), and (Cu) concentrations remarkedly varied in diets (D3>Control>D2>D1). While (Zn) and (Se) showed the highest levels in D3 and the lowest in D2, Manganese (Mn) had the lowest value in the control diet. Except for Fe, which showed statistically significant differences in D2, all essential elements showed no statistically significant differences.

4.3.2. Metal contents in raw fillet

Although, non-significant variable metal accumulation was identified in the raw fillet, metal accumulation in raw fillets was lower compared to the diets, in contrast to diet content, raw fillet demonstrated lower Cd concentration. (As) accumulation was the highest among all heavy metals

with the highest value in D1 and the lowest value in D2, followed by (Hg), and (Pb) with the same sequence of accumulation [D1>D3>D2>control], while (Cd) concentrations were the lowest among all with the highest value in D2 and the lowest in D3. For the essential elements, (Zn) was the highest value and (Co) was the lowest as shown in Table 4.3.

Table 4.2 Contents of heavy metals (ng/g bw), toxic and essential, in the experimental diets.

	Control	D1	D2	D3
Toxic elements				
As	**2779.55**	**2739.16**	**2459.79**	**3107.95**
Cd	**916.46**	**935.42**	**847.57**	**1056.41**
Hg	**302.63^{a}**	**306.66^{b}**	**281.84^{ab}**	**341.78^{ab}**
Pb	**466.96**	**438.10**	**445.80**	**598.57**
Essential elements				
MN	**50414.76**	**52159.00**	**53359.66**	**73210.66**
Fe	**598363.48^{a}**	**534794.95^{a}**	**552997.26^{b}**	**685031.76^{ab}**
Co	**21315.15**	**18063.89**	**18897.38**	**27028.60**
Cu	**43869.07**	**39018.08**	**42387.28**	**59419.32**
Zn	**150569.02**	**142966.85**	**142755.73**	**161884.45**
Se	**4468.15**	**4101.17**	**3583.03**	**4617.92**

Table 4.3 Contents of heavy metals (ng/g ww), toxic and essential in raw fillet in Nile tilapia fed experimental diets.

	Control	D1	D2	D3
Toxic elements				
As	**231.28±25.15**	**279.64±59.84**	**202.25±50.12**	**253.96±19.10**
Cd	**0.27±0.19**	**0.24±0.21**	**0.29±0.26**	**0.22±0.17**
Hg	**51.58±2.19**	**60.50±2.47**	**54.75±1.79**	**56.63±2.01**
Pb	**4.14±0.49**	**14.14±4.29**	**7.49±1.01**	**11.33±4.11**
Essential elements				
Mn	**29.34±3.40**	**31.45±4.85**	**52.28±6.18**	**41.13±5.20**
Fe	**1378.37±52.30**	**1224.91±169.09**	**1357.58±10.27**	**1431.57±285.68**
Co	**13.18±1.11**	**13.61±1.45**	**13.18±1.14**	**14.25±3.04**
Cu	**173.24±53.78**	**145.87±22.51**	**159.42±10.51**	**161.88±21.63**
Zn	**2211.86±247.57**	**1989.25±156.70**	**2231.44±200.30**	**2061.64±109.28**
Se	**84.57±3.21**	**91.69±3.74**	**78.27±2.73**	**89.13±0.92**

4.3.3. Risk Assessment

Based on the average consumptions (31.13 and 9.94 g/individual/day), THQ values of toxic elements were significantly below 1 in all diets except for (As) and (Hg) which had values greater than 1 and were the highest levels among all metals (Table 4.4). The same is copied with trace elements, as the THQ values in both average consumptions were below 1 in all by-product strengths (Table 4.5).

Although varying strengths of banana by-products in the diets caused distinct HI values, they were all <1 indicating that using different strengths of banana by-products as a feed for Nile Tilapia is acceptable for humans based on the average fish consumption by the Spanish population (Table 6).

Table 4.4 Target Hazard Quotient (THQ), Estimated Daily Intake (EDI) for both consumptions, and Maximum Safe Consumption (MSC), based on the contents of heavy metals of Nile tilapia fed experimental diets.

Element	Treatment	THQ 9.94	THQ 31.13	EDI 9.94	EDI 31.13	MSC
AS	Control	1.095	3.428	0.328	1.029	0.091
	D1	1.324	4.145	0.397	1.244	0.075
	D2	0.957	2.998	0.287	0.899	0.104
	D3	1.202	3.765	0.361	1.129	0.083
Hg	Control	0.732	2.294	0.073	0.229	0.136
	D1	0.859	2.690	0.086	0.269	0.116
	D2	0.777	2.435	0.078	0.243	0.128
	D3	0.804	2.519	0.080	0.252	0.124
Cd	Control	0.000	0.001	0.000	0.001	258.770
	D1	0.000	0.001	0.000	0.001	292.447
	D2	0.000	0.001	0.000	0.001	240.173
	D3	0.000	0.001	0.000	0.001	319.346
Pb	Control	0.001	0.005	0.006	0.018	67.552
	D1	0.005	0.016	0.020	0.063	19.809
	D2	0.003	0.008	0.011	0.033	37.398
	D3	0.004	0.013	0.016	0.050	24.714

4.3.3.1. Estimated Daily Intake (EDI)

Tables 4.4 and 4.5 display the EDI values for metals and elements identified in our experiment. MSC values for (Pb) and (Cd) were found to be below the established RfD values. Regarding (As) and (Hg), they showed EDIs statistically non-significant higher values compared to the standard RfD, which is of no impact when implying the large average fish consumption in our study. Except for (Mn) and (Cu), all other elements had values that are lower than the specified RfD, with a considerable increase in (Fe) and Zn and a very minor increase with (Co) and (Se).

4.3.3.2. Maximum Safe Consumption (MSC)

Due to the extremely low metal content in the raw fillet, a person weighing 70 kg can safely consume very large quantities (ranging from 19 to 319 kg per day) of fish fillets containing (Cd), (Mn), and (Pb) daily. Meanwhile, an individual of the same weight consuming daily 75-941 gm of Nile tilapia fillets containing (As), (Hg), and (Zn) is not protected from health hazards (Tables 4.4 and 4.5).

Table 0.5 Target Hazard Quotient (THQ), Estimated Daily Intake (EDI) for both consumptions, and Maximum Safe Consumption (MSC) based on the contents of essential metals of Nile tilapia fed experimental diets.

Element	Treatment	THQ 9.94	THQ 31.13	EDI 9.94	EDI 31.13	MSC
Mn	Control	0.000	0.001	0.042	0.130	334.055
	D1	0.000	0.001	0.045	0.140	311.565
	D2	0.001	0.002	0.074	0.233	187.449
	D3	0.000	0.001	0.058	0.183	238.258
Fe	Control	0.049	0.153	1.957	6.130	2.031
	D1	0.043	0.136	1.739	5.447	2.286
	D2	0.048	0.151	1.928	6.037	2.062
	D3	0.051	0.159	2.033	6.366	1.956
Co	Control	0.062	0.195	0.019	0.059	1.594
	D1	0.064	0.202	0.019	0.061	1.543
	D2	0.062	0.195	0.019	0.059	1.593
	D3	0.067	0.211	0.020	0.063	1.474
Cu	Control	0.006	0.019	0.246	0.770	16.163
	D1	0.005	0.016	0.207	0.649	19.195
	D2	0.006	0.018	0.226	0.709	17.564
	D3	0.006	0.018	0.230	0.720	17.296
	Control	0.105	0.328	3.141	9.836	0.949

Zn	D1	0.094	0.295	2.825	8.846	1.056
	D2	0.106	0.331	3.169	9.924	0.941
	D3	0.098	0.306	2.928	9.168	1.019
Se	Control	0.024	0.075	0.120	0.376	4.138
	D1	0.026	0.082	0.130	0.408	3.817
	D2	0.022	0.070	0.111	0.348	4.472
	D3	0.025	0.079	0.127	0.396	3.927

Table 0.6 Hazard Index values (HI) for heavy and essential metals, for both consumptions (ng/g ww) of Nile Tilapia, fed experimental diets.

Treatment	HI. 9.94	HI. 31.13
Control	0.21	0.65
D1	0.24	0.76
D2	0.20	0.62
D3	0.23	0.71

4.3.3.3. Carcinogenic risk of (As)

All [As (CR)] values were below the maximum declared acceptable risk level (10^{-4}), indicating a very unlikely risk of developing cancer if a 70 kg person consumed 9.94g or 31.13g of these fish every day for 56 years, 365 days a year (Table 4.7).

Table 4.7 The carcinogenic risk of arsenic (As-CR) for both consumptions and Selenium Health Benefit values of Nile tilapia fed experimental diets.

Element	Treatment	As-CR 9.94	As-CR 31.13	Se HBV
As	control	0.0005	0.0015	3.421
	D1	0.0006	0.0019	3.233
	D2	0.0004	0.0013	2.594
	D3	0.0005	0.0017	3.311

4.3.3.4. Selenium Health Benefit Value

The (Se-HBV) reported positive values ranging between 2.59 to 3.31, which indicates a good impact on human health. All of the examined diets had Se/Hg molar ratios ranging from 3.62 to 4.18, indicating that each banana raw material diet formula had a different Se molar value, which confirms safe consumption regardless of Hg toxicity. The free Se/Hg ratio and Se-HBV values

were the highest in D3 [D3>D1>D2], free Se/Hg ratio, and free (Se) values (3.31 and 2.59), respectively (Table 4.7).

4.3.4. Nutritional value

The investigated fish would constitute just a minor fraction of the total daily intake for the requirements assessed if the consumption of each ingredient was taken into account. Even the element Fe, which was found in higher concentrations than other elements, would only comprise 8 to 18 of the diet, which can only be satisfied by 31.13 g of fish each day. Table 4.8 demonstrates that both low and high consumption amounts of fish 9.94 or 31.13 g per day, respectively can satisfy the RDAs for Se, co, Zn, and Cu which were well covered except in the case of Mn in both concentrations wasn't cover the RDA values for men and women and it was the lowest EDI values regarding the other elements.

Table 0.8 Estimation of the contribution of the Estimated Daily Intake to the daily reference intakes established for each element in all the experimental diets.

Element	Treatment	EDI 9.94 WW (mg/day)	EDI 31.13 WW (mg/day	RDA or AI (F) mg/day	RDA or AI (M) mg/day
Mn	Control	0.29	0.91	1.8	2.3
	D1	0.31	0.98		
	D2	0.52	1.63		
	D3	0.41	1.28		
Fe	Control	13.70	42.91	18	8
	D1	12.18	38.13		
	D2	13.49	42.26		
	D3	14.23	44.56		
Co	Control	0.13	0.41	NA	NA
	D1	0.14	0.42		
	D2	0.13	0.41		
	D3	0.14	0.44		
Cu	Control	1.72	5.39	0.9	0.9
	D1	1.45	4.54		
	D2	1.58	4.96		
	D3	1.61	5.04		
Zn	Control	21.99	68.86	8	11
	D1	19.77	61.93		
	D2	22.18	69.46		
	D3	20.49	64.18		

Se	Control	0.84	2.63	0.055	0.055
	D1	0.91	2.85		
	D2	0.78	2.44		
	D3	0.89	2.77		

4.4. Discussion

Human food must contain reasonable concentrations of trace elements required for normal metabolic activity and growth, whereas heavy metal-contaminated food is hazardous to human health due to its ability to accumulate in the body tissues and the inability of human systems to clear it (Balali-Mood, Naseri et al. 2021). Tracking the levels of heavy metals in food is a pivotal step in providing safe food sources for humans, particularly in fish which accounts for a large proportion of human food in many parts of the world, in addition to its ability to store heavy metals by extracting them from contaminated water or food (Javed and Usmani 2011).

In our study, *O. niloticus* in a predesigned farming module was used with a variety of concentrations of prepared Banana by-products as a feeding source to estimate the trace elements levels as well as the heavy metals-related toxicity, and compare our estimates to the standard permissible levels – RfD – assigned by the USEPA's. Our findings showed that Nile tilapia cultivated with varying concentrations of banana by-products was safe for human consumption in terms of the heavy metals and trace elements contained in the fish's fillet. The low heavy metal content (Cd, Pb, and Mn) in raw fillets allows for a safe daily intake of 19 to 319 kg and 75-941 gm of fillet containing (As, Hg, and Zn) by 70 kg adults. Our findings provide clear evidence that farmed fish crops may be a safe alternative to wild crops that are shown to be highly contaminated with high levels of metals in numerous studies. (Ayanda, Ekhator et al. 2019) found heavy metals contamination in *Oreochromis niloticus*, *Malapterurus electricus*, *Parachanna obscura*, and *Chrysichthys nigrodigitatus* in Ogun River, Nigeria. Another study on wild 11 fish species in the Amazon found risky levels of (Pb), (Hg), and (Cd) above the RfD, suggesting the necessity of educating local populations on safe fish consumption (Viana, Kummrow et al. 2023).

Essential elements were found in higher concentrations compared to toxic elements [Zn> Fe> As> Cu>Se> Hg> Mn> Co> Pb> Cd], most probably due to their higher tendency to bioaccumulate and the planned uptake by fish systems, as they play numerous functional and regulatory roles in the fish's enzymatic activity, protein synthesis, and immune response. Meanwhile, toxic metal's lack of biological significance and higher excretion rate by the body systems drove the lower levels

detected in the fish fillets (Xie, Qian et al. 2020). (El-Batrawy, El-Gammal et al. 2018) replicated the same with wild *O. niloticus* fish.

4.4.1. Toxic Metal content

Heavy metal levels in fish fillets ranked as follows (As> Hg> Pb> Cd). When the estimated MSC values of the aforementioned metals were compared to the standard RfD recommended by the USEPA in fishery products, it is remarkably noticed that a very high daily intake of the fish fillets was required to reach the maximum allowable levels. (As) accumulation was the highest, followed by (Hg), and (Pb), while (Cd) concentrations were the lowest among all. According to the calculated MSC values of the four heavy metals, a 70 kg adult can safely consume very large amounts (ranging from 19 to 319 kg per day) of fish fillets daily. All diets showed [As (CR)] values remarkedly below (10^{-6}), indicating a very unlikely risk of developing cancer if a 70 kg person consumed 9.94g or 31.13g of these fish every day for 56 years, 365 days a year.

The herbivorous nature of tilapia and the herbal origin of the diet (Hashim, Song et al. 2014), in addition to its clearance by the body detoxification and excretory systems, and low tissue-proteins binding affinity (Osman 2012, Xie, Qian et al. 2020) suggest low metal levels in the edible parts.

4.4.2. Essential elements content

The trace element concentrations (ng/g) in edible fish parts were Zn (1989-2061)>Fe (1224-1431)>Cu (145.8-161.9)> Se (78-89)>Mn (31-52) >Co (13.1-14.2). Zinc is essential for protein synthesis, and immune response, whereas, excessive (Zn) intake (>11 mg/day) causes severe gastrointestinal upset (Igic, Lee et al. 2002, Roohani, Hurrell et al. 2013), (Zn) is also essential for bone and scale growth, and the digestibility of the fish. Although there are debates about the dietary (Zn) requirement of Tilapia species, some researchers believe that 30 mg/kg dietary (Zn) is the optimal requirement. Our experimental diet formulas contained a range of 140-160 mg/Kg of (Zn) supplements allowing for safe consumption of fish fillets in terms of (Zn) content (MSC=0.9-1.1 kg/day). (Li and Huang 2016) reported superior growth and survival in tilapia fed on a diet supplemented with 127 mg/kg (Zn).

Iron (Fe) content in our experimental diets was 5-fold the optimal dietary (Fe) requirements for tilapia recommended by (Makwinja and Geremew 2020) (120 mg iron/kg diet), in our case of *O. niloticus*, this refutes others hypothesis that iron uptake of 200 mg/kg could be lethal to fish (Eid, Arab et al. 2017). The estimated iron MSC values of the fish fillets remained significantly below the RfD recommended by the USEPA in fishery products. (Armstrong, Dewey et al. 2017)

recognized fish as a (Fe) supplement to human. While, (Wheal, DeCourcy-Ireland et al. 2016) measured (Fe) and non-haem (Fe) concentrations in fish, shrimp and prawn and considered them as a good sources of Fe for human requirements.

Cupper copied the same safe levels (MSC=17-19 kg/day). Contrarily, (Cu) levels exceeding the maximum allowable concentrations were identified in fish farms using different types of vegetables in Bangladesh (Chakraborty, Chandra Ghosh et al. 2022), most likely due to prior contamination of the plant fed. Over-consumption of (Cu) > 0.5-3 mg/day is related to many life-threatening diseases and malignancies in humans (Rankins and Pugh 2012), thus, (Cu) supplementation to fish must be cautiously adjusted to meet nutritional demands while avoiding toxicity.

Another study suggested that 1.0 mg of organic selenium/kg of diet as an optimal level to promote the growth and immunity of Nile tilapia (Wangkahart, Bruneel et al. 2022). The maximum (Se) value in our experimental diets was 4.6mg/kg of diet, however, this high diet-(Se) levels resulted in safe MSC of the fillets (~4 kg/day). Nile tilapia seleno-methionine diets reported toxicity levels between 6.3–14.7 mg Se/kg in diet (Lee, Nambi et al. 2016). While it was suggested that (Se) requirement range of 0.57 mg/kg for all tilapia species. We used a (Se) content of 4.6 mg/Kg, which yielded (Se-HBV) values of (2.6 - 3.3), indicating high safety for humans. In the same context, all examined diets had Se/Hg molar ratios of (3.6 - 4.2) indicating safe consumption regardless of Hg toxicity. Selenium residues is a main component of many antioxidant enzymes and proteins, such as glutathione peroxidases, thioredoxin reductases and selenoprotein P, while fish products are considered as an essential source of this trace element (Combs Jr and Combs 1986, Himeno and Imura 2000).

Manganese, naturally found in nuts and seafood is an essential enzymatic cofactor involved in many physiological functions (Aschner and Aschner 2005, Kern, Stanwood et al. 2010). Whereas, (Mn) high intake (> 0.09 and 0.2 μg/m^{-3}) may lead to irreversible neurological disorders and organ failures (Keen, Ensunsa et al. 2013). It was reported that control-comparable (Mn) concentrations in the fish fillets among all diet formulas, which allow safe high daily consumption (MSC=178-311 kg/day). Other finding reported that significantly variable (Mn) contents in farmed *Oncorhynchus mykiss* using different diet formulas (Emadi, Samavat et al. 2015), while (Al-Kahtani 2009) recommended a 13 to 15 mg/kg (Mn) supplement for Tilapia for optimal fed.

Cobalt levels in *O. niloticus* diets that exceed the allowable limits (96.14 mg/L) are lethal to both fish and humans (Rai, Ullah et al. 2015). The maximum contents of (Co) in our experimental diets and fish fillets were 27 and 0.014 mg/kg, respectively, allowing for MSC of 1.4-1.6 kg daily of fish fillets. Many studies recommended a 0.05-1.5 mg/kg (Co) supplement in a fish diet to maintain an optimal growth rate (Shiau and Su 2003, Al-Ghanem 2011). (Co) content was the lowest among all trace elements detected in the fish fillets. A human daily intake of 5-8 g cobalt is crucial for vitamin B12 synthesis, the production of red blood cells, the metabolism of fats and carbohydrates as well as the synthesis of proteins (Czarnek, Terpiłowska et al. 2015)

4.4.3. Human health risk assessment

THQ, EDI, HI, and MSC were used in this study to assess the risk of fish consumption in humans. In terms of heavy metals, all THQ values ranged from (0.005-4.1), while MDIs ranged from (0.02-1.24 mg/kg/day) with the lowest values belonging to (Cd) and (Pb), implying safe consumption of fish fillets (MSC: 75-319 kg/day) based on the maximum average daily consumption tolerable by the Spanish population (31.13 g/individual/day), a similar finding is reported by (Liu, Xu et al. 2020) when studied freshwater fish collected from different locations. Meanwhile, comparable values of trace elements, THQs (0.001-0.331), and EDIs (0.06-9.8 mg/kg/day) were detected, implying safe consumption of fish fillets (MSC: 0.9-343 kg/day). Generally, all THQ and MDI values that emerged from our study were significantly comparable to the same values found in many other studies suggesting safe final edible parts consumption (Wei, Zhang et al. 2014, Zohra and Habib 2016, Ju, Chen et al. 2017). Hazard index (HI) values for all experimental diets were less than one for both consumption patterns (HI 9.94: 0.2-0.24) and (HI 31.13: 0.62-0.76), respectively. This confirms the safety of consuming cultured O. *niloticus* at the various concentrations of banana by-products studied.

4.4.4. Nutritional value

It is evident that the inclusion of banana by-products in the tilapia diet had a minor impact on the fillet's metal content, but not enough to reduce its nutritional value, where the nutritional analysis of the farmed Nile tilapia revealed that this species can be regarded as a rich source of necessary metals like Fe, Cu, Co, Zn, and Se, counter to Mn which had the lowest values. Our results coincide with the results of (Jim, Garamumhango et al. 2017) who determined the content of some elements in the wild tilapia meat with no effect on its nutritional value. It is also in concordance with the finding of (Ngugi, Oyoo-Okoth et al. 2017). who observed that the main elements like Fe, Zn,

Mn, and Cu were well available in the whole body of the farmed *Oreochromis niloticus* feed with amaranth leaf protein concentrates (ALPC) with different inclusion in the diet. Nile Tilapia fed on experimental diets revealed higher concentrations of Se and Zn in the edible part when compared to other farmed species like European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) (Olmedo, Hernández et al. 2013). Another study replicated the same, revealing that the nutritional value in grey mullet muscle was well covered when fed with Aloe vera by-product with different concentrations (Rabia, Luzardo et al. 2022).

4.5. Conclusion:

Using plant-derived by-products remains a reliable option for replacing conventionally expensive fish feeds in order to bridge the demand-production cost gap while also encouraging circular economy tactics. Since the heavy metal content of fish edible parts is highly dependent on diet composition, tracking metal levels in edible tissues is pivotal to estimating the potential human health risk using EDI, THQ, and HI indices. Toxicity levels of heavy metals and trace elements in *O. niloticus* fed on variable concentrations of banana by-products were substantially lower than the permissible limit for human consumption.

Chapter 5. Effect of Dietary Banana by-product Supplementation on Growth Performance, proximate composition and liver and gut morphology of farmed Nile Tilapia (*Oreochromis niloticus*) .

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5.1. Introduction

Animal protein gap in diets widens as world population grows. As a result, aquaculture has recently attracted more attention as one of the most significant sources of animal protein. More than half of the world's fish are produced through aquaculture, which is the food sector with the fastest growth (Galappaththi, Ichien et al. 2020). Aquaculture is therefore considered to be the only practical option for bridging the existing gap between fish output and consumption.

Quality fish feed is increasingly in demand because of the aquaculture industry's recent rapid rise. Fish feed consumes around 50% of the production costs and it is crucial to the production and yield outcome (Mzengereza, Msiska et al. 2014), which has sparked research on feeding fish with alternative foods that satisfy animals' nutritional needs while maintaining the quality of their diet. Through the global trend to return to nature, the World Health Organization promotes the use of therapeutic herbs and plants to replace or decrease the use of chemicals. Numerous researches have been conducted on non-traditional raw materials, including a variety of plant and animal by-products such papaya and calabash seeds, banana peels, and sweet potato peels.

Understanding the nutrient composition of the fish encourages others to identify the food requirements and the opportunities of using more economic available resources and plant-derived

by-products as food source for the purpose of achieving maximum growth with enriched nutritional value when reared in the laboratory, it was concluded that 45% protein and 400 kcal/100 g are required for maximum growth (El-Sayed and Teshima 1992). (Kubiriza, Akol et al. 2018) concluded significant growth in *O. niloticus* with fed diets containing either fresh water shrimps *(Caridina nilotica)* or Sardine *(Rastrineobola argentea)* or a combination of the two. While supplying 50% of the fish's dietary protein requirement, as corn products, improved the fatty acid composition (Herath, Haga et al. 2016), adding many plant-derived by-products to the fed demonstrated improved fish's mean weight. The most common plant-derived by-products were maize bran, sunflower seeds, rice bran and wheat pollard, soybean, cottonseeds, taro leaves, and banana by-products (Mmanda, Mulokozi et al. 2020). As a consequence, there is increased interest and awareness around the world in valuing existing resources, particularly recycling nutrients and by-products to promote a circular economy in aquaculture (Boyd and Ellison 2007, Arvanitoyannis and Kassaveti 2008, Alonso, Bond et al. 2010, Caballero, Finglas et al. 2015, Lopes, El-Basyoni et al. 2015, Stevens, Newton et al. 2018).

The European Commission endorsed "Blue Growth," a green economy strategy used in the maritime and coastal sectors. By-products and wastewater effluents have the ability to be recycled into aquaculture systems and make up significant portions of the industry's output (Newton, Prowse et al. 2014, FAO, 2018, Smárason, Alriksson et al. 2019).

Although Nile tilapia (*Oreochromis niloticus*) farming offers a reliable source of protein due to its ease of farming and rapid growth rate in tropical and semi-tropical environments, feeding remains the most significant barrier to commercial breeding since it accounts for the lion's share of overall expenditure. *O. niloticus* commercially known as mango fish, *nilotica*, or *boulti* (Zein, el-Bedawey et al. 1985), is a cichlid fish of the tilapia species that are indigenous to north Africa and the Levant region. (Dunz and Schliewen 2013). The fish's wide range of trophic and ecological adaptation and high capability to withstand harsh conditions of extreme temperature, food shortage, pollution, and salinity enables it to occupy a variety of tropical and sub-tropical freshwater niches, it has been introduced into more than 50 countries, primarily for farming purposes, as we speak, it is among one of the most widely cultured species in aquaculture and stock enhancements (Canonico, Arthington et al. 2005).

O. niloticus feeding habits are highly seasonal, habitat-dependent, and fish size-dependent. It is primarily a herbivore where plant sources are favorable in ideal circumstances, but it embraces an

omnivorous feeding behavior during its early life and dry seasons, where food requirements are most likely met by macrophytes, insects, phytoplankton, and detritus (Tesfahun, Temesgen et al. 2018). Small fishes (11.5 cm total length - TL) prefer animal origin foods, whereas larger groups (> 15 cm TL) primarily feed on phytoplankton, detritus, and macrophytes (El-Naggar, Khalaf Allah et al. 2019).

The nutritional composition of *O. niloticus*, particularly the lipid and total fatty acid content, was discovered to vary depending on geographical location and feeding habits. (Zenebe, Ahlgren et al. 1998, Martin, Carter et al. 2000, Ahmed, Jan et al. 2022). *O. niloticus* high nutritional content makes it one of the most valuable food sources due to its high protein and fat content. Many studies have been conducted to landscape the nutrient composition of the fish in variable environments, which includes determining the moisture, protein, fat, and ash contents, that account for more than 95% of the total fish body. Another study discovered that *O. niloticus* edible portion accounts for approximately 36% of its total weight and consists of 79.5% moisture, 18.8% crude protein, 0.36% fat, and 1.4% ash, whereas the protein part consists of 66% myosin nitrogen, 25% non-myosin nitrogen and 8.5% insoluble protein nitrogen; further electrophoreses analysis of muscle proteins revealed lysine, 10.6%, histidine, 1.7%, arginine, 5.8%, threonine, 3.8%, methionine, 1.1%, valine, 5.4%, phenylalanine, 3.2% and leucine/isoleucine, 12.4% (Khalil, Moustafa et al. 1980). In the same context, these findings reported that *O. niloticus* in different environments had 1.7–21 and 1.6–9.3% lipid and fatty acids content of the dried weight (DW) of the fish, respectively. numerous fatty acids were identified, among which saturated fatty acids ranged from 5.3–30 mg. gg−1 DW, monounsaturated fatty acids from 1.3–30 and polyunsaturated fatty acids from 6.8–29 mg. g−1 DW (Zenebe, Ahlgren et al. 1998).

Banana agriculture, which is one of the most important crops in tropical countries, yields tons of underutilized by-products such as leaves, inflorescence, pseudo-stems, and rhizomes, all of which contain many bioactive ingredients that may serve as a potential nutritional source in fish industry (Padam, Tin et al. 2014, Paz, Monzón et al. 2020, Gupta, Baranwal et al. 2022). Designing an evidence-based feeding regimen based on recapturing banana plant-derived by-products effectively promotes the ultimate goals of circular economy which aim to maintain a sustainable fed source while reducing environmental pollution and direct financial costs.

After banana harvesting there were a lot of wastes, 80% of the overall biomass is made up of these wastes (Padam, Tin et al. 2014). These residues can be used to solve the food security issue (Campos, Gómez-García et al. 2020).

The resistant starch (RS) and nonstarch polysaccharides that make up the DF of the starchy fruit known as the banana are rich in indigestible chemicals (Ovando-Martinez, Sáyago-Ayerdi et al. 2009). Banana pulp has a starch concentration similar to that of maize endosperm and white potato pulp when it is unripe, or between 70% and 80% of its dry weight. There are a lot of compounds or nutrients are present in the pulp of ripe banana such as: 20 g/100 g fresh weight (FW) of carbohydrates, 2 g/100 g FW of fibre, and 4.10 to 5.55 mg/100 g (dry weight) of potassium are all present in bananas (Goswami and Borthakur 1996). The presence of various compounds in banana suggests that the banana possess various properties and may be useful as an additive by-product to the fish feed.

In the current study we sought to identify the opportunities of integrating banana by-products as a food additive in *O. niloticus* cultivation in order to establish a new circular economy model for the use of banana residues, and the impact of the feeding regimen on the biochemical composition of the fish and the histology of gut and liver.

3.2. Material and methods

5.2.1. Experimental design and fish preparation

One hundred and ninety-two tilapia (*Oreochromis niloticus*) (5 g initial body weight) were distributed among 12 glass tanks of 50 l of fresh water using the closed system in this experiment, in the facilities of the Technological Science Park Foundation (FCPCT) of Taliarte, Telde (16 fish/tank, each diet fed in triplicate). Fish had fed over the experimental diets three times per day 6 days per week, until apparent satiation, for 3 months. During the trial period oxygen and temperature parameters were measured once per day, using an oximeter (Oxy Guard-Guard-handy beta, Zeigler Bros, Gardners, USA), the average water temperature determined during the feeding period was 20.1 ± 1.22 ^{o}C, with an average oxygen saturation of 6.4 ± 0.37 mg / l. Feed intake had been determined daily and all fish individually weighed monthly. Conversion index (CI), and specific growth rate (SGR) calculated. In each monthly sampling, two fish had slaughtered and used for contaminants analysis monthly and at the end of the experiment.

5.2.2. Processing raw material and experimental diets

The banana crop by-products had received in Bio factory of the Pilot Plant of Products and Processes of GIA-ECOAQUA in Taliarte. This had been treated, sanitized, dried, sterilized and grind for later inclusion as raw material in fish feed: lyophilization in the lyophilized and dried in an oven at a temperature below 40ºC. Prior to this, these raw materials analyzed both in biochemical composition and content of pesticides and heavy metals.

Four isocaloric and isoproteic diets were developed (table 5.1): one of them was commercial diet of the specie under study, and other three including increasing percentages of the raw material of banana tallow obtained in the processing phase (5,10 and 20 %). All diets were analyzed both for biochemical composition.

Table 0.1 Ingredients (g/kg) and chemical composition (% dry basis) of the experimental diets.

	Diets			
Ingredients	**Control**	**5**	**10**	**20**
Fish meal[a]	**210**	**210**	**210**	**210**
Corn meal[b]	**200**	**150**	**100**	**-**
Soy meal[b]	**260**	**260**	**260**	**260**
Wheat meal[b]	**90**	**90**	**90**	**90**
Corn gluten[b]	**70**	**70**	**70**	**70**
Wheat gluten[b]	**60**	**60**	**60**	**60**
Linseed oil[b]	**60**	**60**	**60**	**60**
Banana by-product[c]	**-**	**50**	**100**	**200**
Vitamin mix[d]	**10**	**10**	**10**	**10**
Mineral mix[e]	**20**	**20**	**20**	**20**
Ca(H2PO4)2[f]	**5**	**5**	**5**	**5**
CMC[g]	**15**	**15**	**15**	**15**

[a] Supplied by Skretting, Spain.
[b] Supplied by Capisa S.A., Spain.
[c] Supplied by banana local producers.
[d] Vitamin premix containing (mg kg-1 o IU/kg de pienso): tiamine 40 mg, riboflavin 50 mg, piridoxine 40 mg, calcium. pantothenate 117 mg, nicotinic acid 200 mg, folic acid 10 mg, cianocobalamine, 0.5 mg, choline chloride 2700 mg, Mio-inositol. 2000 mg, ascorbic acid 5000 mg, menadione 20 mg, colecalciferol 2000 IU, etoxiquine 100 mg, retinol acetate 5000 IU. Vitamin E (DL-alpha-tocopherol acetato) 250 mg.
[e] Mineral premix containing (g/kg de dry feed): calcium orthofosfate 1.60 g, calcium carbonate 4 g, ferrous sulfate 1.5 g. magnesic sulfate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminum sulfate 0.02 g, zinc sulfate 0.24 g, copper sulfate 0.20 g, manganese sulfate 0.08 g, potassium iodate 0.02 g.
[f] Sigma-Aldrich, Munich, Germany.
[g] Carboxymethylcellulose (sodium salt, Sigma-Aldrich, Munich, Germany).

5.2.3. Sampling and growth parameters

Sampling was made monthly to measure the parameters of weight, total size and furcal size. The final sampling of the trial was carried out after 90 days, when the fish doubled their weight. Samples of raw materials, by-product of banana pseudo-steam and processed diets were taken for the biochemistry analysis. Samples of tissues were also taken at the beginning and end of the experiment. The different parameters are FW, Final Weight (g); FI, Feed Intake (g/fish/day); FCR, Feed Conversion Ratio = Feed intake (g) / Weight increase (g); SGR, Specific Growth Rate = ((Ln Final weight – Ln Initial weight)/ nº days) x100; and specific growth rate (SGR) calculated. In each monthly sampling, two fish had slaughtered and used for contaminants analysis and at the end of the experiment.

5.2.4. Biochemistry analyses

5.2.4.1. Proximal composition

Samples of raw materials, products and by-products of banana and processed diets were taken for the biochemistry analysis. Samples of whole fish and muscles were also taken at the beginning and end of the experiment and then stored at -80°C. Proximate composition was conducted following standard procedures (Gaithersburg 1984). Ash content of the samples was determined by combustion in a muffle furnace at 600 °C for 12 h. Crude Protein content were determined by the Kjeldahl technique (N × 6.25), which is based on the measurement of the total nitrogen present in the samples. To determine the percentage of moisture by thermal dehydration to constant weight at 105 °C an amount of each sample was weighed, reweighed after 24 hours, repeating this measurement at least once more time after another hour on the stove. Total lipids content is that described by (Folch, Lees et al. 1957), by which a mixture of chloroform-methanol (2: 1 v / v) with 0.01% butylhydroxytoluene (BHT) is used, and fatty acid methyl esters obtained by transmethylation (Christie, Kwon et al. 2003) then separated by gas-liquid chromatography.

5.2.4.2. Fatty acid analyses:

The total lipids were trans-esterified with 1% sulfuric acid in methanol following the methodology of (Christie 1982). A dilution was made in hexane, and the separation, identification and quantification of the different fatty acids was carried out through gas chromatography, following the protocol described by (Izquierdo 1989).

5.2.5. Histology

Individual samples were fixed in 4% neutral-buffered formalin (4% formalin, 0.08M sodium phosphate, pH 7.0), embedded in paraffin (Histokinette 2000; Leica, Nussloch, Germany), sectioned using a Leica RM 2135 microtome (Leica, Nussloch, Germany), mounted onto coated slides, and stained with hematoxylin and eosin (H&E) (Luna, 1968) for optical examination. The micrographs from each stained slide were made using an Olympus CX41 microscope (Olympus Optical, PA, USA) incorporated with an Olympus DP50 camera (Olympus Optical Co. LTD, Shinjuku-ku, Tokyo, Japan). Blinded evaluation for two independent trained evaluators was performed using a semi-quantitative scoring system ranging from 0 to 3. Score 0 was given to the normal tissue appearance and subsequent scores accounted for increasing alterations in tissue histomorphology.

5.2.5.1. Liver

In the hepatic tissue, the vacuolization grade (steatosis), necrosis foci or pyknotic nuclei presence, nuclear pleomorphism or vascular changes occurrence were evaluated following the semi-quantitative scoring system.

5.2.5.2. Foregut

Two parameters were measured: villus height (the length from the villus bottom to the tip) and villus width (width at the middle part of the villus). These intestinal parameters were measured from 9 villi for each slide and only complete villi were selected and measured under 10× magnification for each section. The sections were photographed and evaluated using an analySIS® (Image Pro Plus®, Media Cybernetics, Silver Spring, MD, USA) software package.

5.2.5.3. Posterior intestine

The posterior intestine samples were stained with specific Alcian Blue/PAS staining (pH = 2.5) (Luna 1968). In hindgut sections, in addition to evaluating all the parameters described for the foregut, the number of cells stained for specific acid mucin staining by unit of fold area were determined.

5.2.6. Statistical analysis

The results were expressed as mean ± standard deviation (SD). Normality and homogeneity of the variance of all the variables were evaluated using the Kolmogorov-Smirnoff test and Levene test respectively. The homogeneity of variances has been determined with the Levene test ($P \leq 0.05$). The analysis of variance was performed using one-way ANOVA, and the means compared by Duncan, Scheffé and Tukey post-hoc tests ($P \leq 0.05$). All statistical analyses were conducted by IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp.).

5.3. Results

5.3.1. Growth

Regarding the different growth parameters and feed utilization of the fish (Table 5.2), no statistically significant differences have been found in none of the indices calculated.

The evolution of the average weight of the fish fed with the different diets throughout the experiment is shown in Figure 5.1. The animals doubled their initial weight after three months from the beginning of the test, resulting in a mean of 31.91 ± 4.02 g of weight and 12.01 ± 0.94 cm of total height (Figure 5.2). There were no significant differences for either of these two parameters throughout the experimental test nor at the end of the test.

Table 5.2 Values for parameters of growth performance and use of diets.

	CONTROL	BP5	BP10	BP20
IBW	**6.87±0.15**	**6.94±0.20**	**6.87±0.09**	**6.82±0.32**
ITL	**7.41±0.09**	**7.41±0.04**	**7.36±0.04**	**7.33±0.12**
FBW	**30.24±2.66**	**31.11±5.98**	**28.53±2.65**	**31.92±4.45**
FTL	**11.71±0.52**	**11.76±0.65**	**11.51±0.35**	**12.01±0.33**
LW	**0.32±0.01**	**0.33±0.12**	**0.32±0.07**	**0.33±0.03**
GW	**2.05±0.37**	**2.28±0.59**	**1.86±0.34**	**2.09±0.24**
FI	**27.18±1.49**	**27.05±1.86**	**26.79±2.45**	**27.71±0.71**
SGR	**0.8±0.06**	**0.81±0.10**	**0.77±0.05**	**0.84±0.06**
BWG	**23.37±2.81**	**24.17±5.84**	**21.66±2.56**	**25.09±4.19**
FCR	**1.17±0.10**	**1.16±0.24**	**1.24±0.08**	**1.12±0.18**

IBW: initial WEIGHT
ITL: initial TOT LENGTH
FBW: final Weight
FTL: final Total length

LW: Liver weight
GW: Gut weight
FI: Feed intake (g) per fish for the experimental-day period
SGR: ((Ln Final weight – Ln Initial weight)/ nº days) x 100
BWG: body Weight gain
FCR: Feed intake (g) / Weight increase (g)

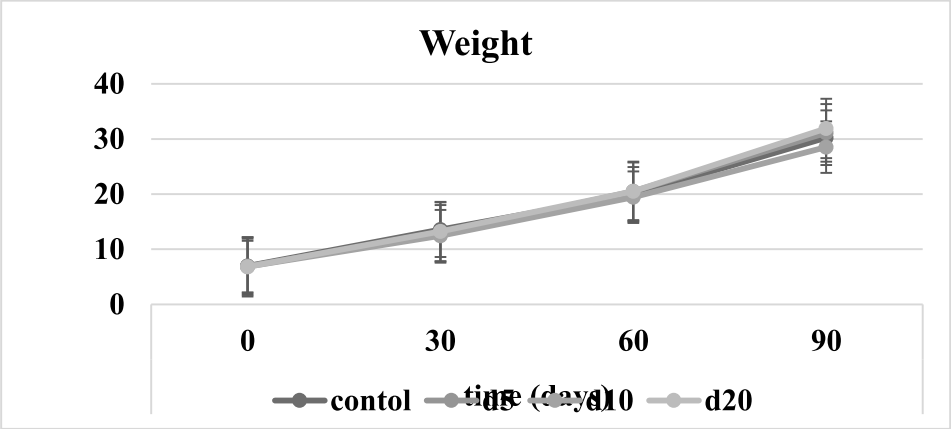

Figure 5.1. Evolution of the average weight of the fish fed with the different diets throughout the experiment.

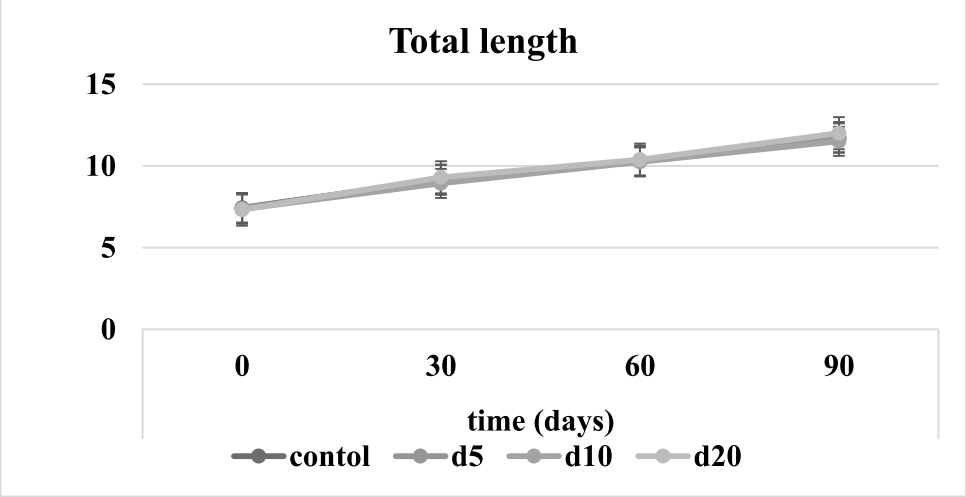

Figure 5.2. Evolution of the average Total length of the fish fed with the different diets throughout the experiment.

5.3.2. Biochemistry

5.3.2.1. Diets

The Tables 5.3 and 5.4 show the results obtained for the proximal composition and fatty acids of the 4 evaluated feeds. As it can be observed, the results for proteins, lipids and ashes are quite similar between diets and in accordance with their formulation with significant differences, for example in proteins we found that BP20 had the lowest value in comparison to the other diets and control which was the highest one, as well it was the lowest in lipids and the highest concentration was in BP5. In contrast, BP20 had the highest value in moisture followed by the other similar values in BP5, BP10 and control. The results were more variable in the case of ash, the order was ascending from control to BP20. While there were no significant differences in fatty acids.

Table 5.3 Experimental diets proximal composition (mean ± SD).

	CONTROL	BP5	BP10	BP20
Protein	**40.61±3.00^{a}**	**38.25±0.09^{a}**	**37.82±0.43^{a}**	**33.09±0.68^{b}**
Lipid	**12.36±0.37^{b}**	**14.47±0.32^{a}**	**10.11±0.26^{c}**	**10.67±0.18^{c}**
Ash	**8.42±0.16^{d}**	**9.21±0.39^{c}**	**10.04±0.01^{b}**	**12.17±0.16^{a}**
Moisture	**6.62±0.10^{b}**	**6.7±0.1^{b}**	**6.76±0.06^{b}**	**8.25±0.05^{a}**

Table 5.4 Experimental diets fatty acid profile expressed in % of the total fatty acids identified.

	CONTROL	BP5	BP10	BP20
14:00	**0.94**	**0.36**	**0.47**	**0.30**
14:1n-7	**0.01**	**0.01**	**0.01**	**0.00**
14:1n-5	**0.02**	**0.01**	**0.02**	**0.01**
15:00	**0.18**	**0.12**	**0.14**	**0.12**
15:1n-5	**0.01**	**0.00**	**0.01**	**0.00**
16:OISO	**0.02**	**0.01**	**0.02**	**0.02**
16:00	**11.63**	**9.72**	**10.06**	**8.07**
16:1n-7	**1.38**	**1.17**	**1.17**	**0.89**
16:1n-5	**0.05**	**0.04**	**0.05**	**0.04**
16:2n-6	**0.00**	**0.00**	**0.00**	**0.00**
16:2n-4	**0.05**	**0.04**	**0.04**	**0.03**
17:00	**0.03**	**0.02**	**0.03**	**0.02**
16:3n-4	**0.16**	**0.16**	**0.15**	**0.15**
16:3n-3	**0.06**	**0.05**	**0.05**	**0.05**

16:3n-1	0.01	0.02	0.01	0.01
16:4n-3	0.03	0.03	0.03	0.02
16:4n-1	0.00	0.01	0.00	0.00
18:00	4.76	4.93	5.03	4.86
18:1n-9	22.02	21.75	21.70	19.11
18:1n-7	1.44	1.57	1.28	1.33
18:1n-5	0.05	0.05	0.04	0.04
18:2n-9	0.01	0.01	0.01	0.01
18.2n-6	20.77	20.47	19.87	18.13
18:2n-4	0.03	0.04	0.03	0.03
18:3n-6	0.07	0.09	0.03	0.10
18:3n-4	0.00	0.04	0.01	0.02
18:3n-3	29.57	31.52	32.03	38.14
18.3n-1	0.00	0.01	0.02	0.01
18:4n-3	0.28	0.31	0.27	0.29
18:4n-1	0.01	0.01	0.01	0.01
20:00	0.29	0.30	0.31	0.29
20:1n-9	0.17	0.19	0.17	0.17
20:1n-7	0.83	0.90	0.90	0.92
20.1n-5	0.06	0.07	0.07	0.07
20:2n-9	0.02	0.01	0.01	0.01
20:2n-6	0.14	0.14	0.15	0.14
20:3n-9	0.02	0.02	0.02	0.02
20:3n-6	0.04	0.05	0.05	0.05
21:00	0.00	0.00	0.00	0.00
20:4n-6	0.31	0.36	0.35	0.37
20:3n-3	0.07	0.08	0.09	0.10
20:4n-3	0.07	0.09	0.11	0.13
20:5n-3	0.76	0.85	0.86	0.95
22:1n-11	0.47	0.52	0.51	0.55
22:1n-9	0.23	0.25	0.23	0.24
22:4n-6	0.06	0.08	0.07	0.08
22:5n-6	0.18	0.23	0.24	0.29
22:5n-3	0.21	0.25	0.25	0.29
22:6n-3	2.49	3.04	3.01	3.49
[1]Total Saturates	17.54	15.15	15.73	13.38
[2]Total Monoenoic	26.64	26.41	26.04	23.26
[3]Total n-3	33.55	36.23	36.70	43.47
[4]Total n-6	21.55	21.42	20.77	19.16

[5]Total n-9	22.46	22.24	22.14	19.56
[6]Total n-3HUFA	3.61	4.31	4.33	4.96
ARA	0.31	0.36	0.35	0.37
EPA	0.76	0.85	0.86	0.95
DHA	2.49	3.04	3.01	3.49
ARA/EPA	0.40	0.42	0.41	0.39
DHA/EPA	3.26	3.57	3.50	3.68
DHA/ARA	8.08	8.44	8.61	9.53
[7]Total PUFA	25.55	26.20	25.47	24.53
n-3/n-6	0.18	1.69	1.77	2.27

[1]14:00,15:00, 16:00, 17:00, 18:00, 20:00
[2]14:1n-7; 14:1n-5; 15:1n-5; 16:1n-7 16:1n-5; 18:1n-9; 18:1n-7; 18:1n-5; 20:1n-9; 20:1n-7; 20:1n-5; 22:1n-11; 22:1n-9.
[3]16:3n-3; 18:3n-3; 18:4n-3; 20:3n-3; 20:4n-3; 20:5n-3; 22:5n-3; 22:6n-3.
[4]18:2n-6; 18:3n-6; 20:2n-6; 20:3n-6; 20:4n-6; 22:4n-6; 22:5n-6
[5]18:1n-9; 18:2n-9; 20:1n-9; 20:2n-9; 20:3n-9
[6]20:3n-3; 20:4n-3; 20:5n-3; 22:5n-3; 22:6n-3
[7]18:2n-9; 18:2n-6; 18:2n-4; 18:3n-6; 18:3n-4; 18:4n-3; 18:4n-1; 20:2n-9; 20:2n-6; 20:3n-9; 20:3n-6; 20:4n-6; 20:3n-3, 20:4n-3; 20:5n-3; 22:4n-6, 22:5n-6; 22:5n-3; 22:6n-3

5.3.2.2. Fish Muscles

5.3.2.2.1 Proximal composition

Regarding to the biochemical analysis, no statistically significant differences were found between the fish fed in the different diets for the fish muscles as shown in Table 5.5, where the muscle composition expressed in dry weight shows existence of protein with an average (19%), fat (1.3%), lipids (1.6%) and moisture (78%).

Table 5.5 Proximal composition, expressed in % of dry weight, for muscle of fish fed with experimental diets.

	CONTROL	BP5	BP10	BP20
Protein	19.81±0.93	19.74±0.74	20.07±0.95	19.93±0.94
Lipid	1.71±0.58	1.52±0.39	1.57±0.33	1.50±0.15
Ash	1.34±0.31	1.28±0.31	1.28±0.29	1.36±0.21
Moisture	78.69±3.32	78.24±1.80	78.88±3.16	79.51±3.70

5.3.2.2.2. Fatty acids

Muscle fatty acid profile expressed as percentage of fatty acids; it was more affected by different diets. Regarding the levels of fatty acids in the fillet, as can be seen in Table 5.6, the levels of

monoenoic fatty acids, DHA, n-3 HUFA and total PUFA, were quite similar in the different inclusion of the banana by-product with no significant differences in fish fed. However, in n-3/n-6 the values were increasing with the increasing of banana by-product inclusion with a significant difference though the highest value was in BP 20% in comparison with the control.

Table 5.6 Fatty acid profile expressed in% of fatty acids identified, from the muscle tilapia of the different experimental groups at the end of the experiment.

	CONTROL	BP5	BP10	BP20
14:00	0.67±0.27	0.62±0.31	0.38±0.18	0.48±0.27
14:1n-7	0.02±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{b}	0.01±0.00^{ab}
14:1n-5	0.02±0.01	0.02±0.01	0.03±0.02	0.03±0.01
15:00	0.12±0.03	0.12±0.04	0.09±0.03	0.12±0.04
15:1n-5	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
16:OISO	0.02±0.01	0.02±0.01	0.01±0.01	0.02±0.01
16:00	13.97±1.55	12.97±1.99	12.33±1.61	12.50±1.41
16:1n-7	1.53±0.45	1.42±0.58	1.12±0.39	1.08±0.44
16:1n-5	0.07±0.019	0.07±0.02	0.07±0.016	0.09±0.02
16:2n-6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
16:2n-4	0.02±0.01	0.02±0.01	0.02±0.02	0.02±0.01
17:00	0.04±0.01^{a}	0.03±0.01^{b}	0.03±0.01^{b}	0.03±0.01^{b}
16:3n-4	0.14±0.02	0.13±0.05	0.13±0.02	0.13±0.02
16:3n-3	0.06±0.015	0.05±0.01	0.06±0.02	0.04±0.02
16:3n-1	0.39±0.09	0.36±0.09	0.42±0.07	0.41±0.06
16:4n-3	0.95±0.21	0.869±0.24	1.02±0.18	0.95±0.09
16:4n-1	0.03±0.03	0.04±0.03	0.06±0.01	0.05±0.03
18:00	8.26±1.11	7.89±0.90	8.97±0.69	8.35±0.76
18:1n-9	17.88±2.61	17.11±3.50	15.77±1.53	16.01±1.64
18:1n-7	2.56±0.16	2.52±0.08	2.70±0.26	2.59±0.10
18:1n-5	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01
18:2n-9	0.10±0.03	0.09±0.03	0.09±0.03	0.07±0.01
18.2n-6	11.90±0.72	11.53±1.40	11.47±0.72	11.38±0.70
18:2n-4	0.09±0.01	0.09±0.02	0.10±0.01	0.10±0.01
18:3n-6	0.34±0.03	0.30±0.09	0.35±0.08	0.27±0.04
18:3n-4	0.05±0.01	0.06±0.01	0.05±0.01	0.05±0.01
18:3n-3	10.88±1.98	10.51±2.61	9.97±1.49	12.06±1.47
18.3n-1	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.00
18:4n-3	0.30±0.05	0.24±0.09	0.26±0.04	0.26±0.05

18:4n-1	**0.01±0.01**	**0.01±0.01**	**0.01±0.01**	**0.04±0.10**
20:00	**0.24±0.02**	**0.25±0.01**	**0.23±0.01**	**0.23±0.02**
20:1n-9	**0.09±0.01**	**0.10±0.02**	**0.08±0.01**	**0.09±0.04**
20:1n-7	**1.11±0.14^{ab}**	**1.19±0.07^{a}**	**1.03±0.06^{b}**	**1.06±0.07^{b}**
20.1n-5	**0.06±0.01^{ab}**	**0.07±0.01^{a}**	**0.06±0.01^{b}**	**0.06±0.01^{b}**
20:2n-9	**0.10±0.04**	**0.10±0.02**	**0.07±0.02**	**0.07±0.01**
20:2n-6	**0.73±0.09**	**0.83±0.20**	**0.82±0.11**	**0.8±0.07**
20:3n-9	**0.01±0.01**	**0.01±0.00**	**0.01±0.01**	**0.01±0**
20:3n-6	**1.00±0.13^{ab}**	**0.97±0.17^{ab}**	**1.08±0.08^{a}**	**0.90±0.10^{b}**
21:00	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**
20:4n-6	**2.71±0.49**	**2.74±0.66**	**3.24±0.39**	**2.78±0.36**
20:3n-3	**1.47±0.11^{b}**	**1.76±0.30^{ab}**	**1.74±0.27^{ab}**	**1.90±0.23^{a}**
20:4n-3	**0.49±0.04**	**0.47±0.036**	**0.51±0.03**	**0.51±0.04**
20:5n-3	**0.92±0.17**	**0.88±0.17**	**1.05±0.13**	**1.02±0.15**
22:1n-11	**0.15±0.03^{ab}**	**0.18±0.03^{a}**	**0.12±0.02^{b}**	**0.16±0.03^{a}**
22:1n-9	**0.18±0.05**	**0.24±0.09**	**0.23±0.06**	**0.23±0.06**
22:4n-6	**0.65±0.08**	**0.76±0.19**	**0.76±0.09**	**0.66±0.09**
22:5n-6	**1.44±0.26**	**1.64±0.55**	**1.60±0.16**	**1.58±0.25**
22:5n-3	**2.37±0.27^{b}**	**2.58±0.57^{ab}**	**2.92±0.39^{a}**	**2.66±0.35^{ab}**
22:6n-3	**15.76±2.70**	**18.04±5.91**	**18.86±2.97**	**18.09±2.95**
[1]Total Saturates	**23.06±2.22**	**21.63±2.08**	**21.79±1.40**	**21.47±1.56**
[2]Total Monoenoic	**23.63±3.12**	**22.89±4.07**	**21.17±1.82**	**21.35±1.95**
[3]Total n-3	**33.19±1.64^{b}**	**35.39±4.59^{ab}**	**36.40±2.38^{ab}**	**37.49±2.36^{a}**
[4]Total n-6	**18.78±0.65^{ab}**	**18.78±0.68^{ab}**	**19.30±0.68^{a}**	**18.37±0.52^{b}**
[5]Total n-9	**18.36±2.68**	**17.65±3.49**	**16.25±1.55**	**16.47±1.64**
[6]Total n-3HUFA	**21.00±3.14**	**23.73±6.89**	**25.09±3.35**	**24.18±3.51**
ARA	**2.71±0.49**	**2.74±0.66**	**3.24±0.39**	**2.78±0.36**
EPA	**0.92±0.17**	**0.88±0.17**	**1.05±0.13**	**1.02±0.15**
DHA	**15.76±2.70**	**18.04±5.91**	**18.86±2.97**	**18.09±2.95**
ARA/EPA	**2.96±0.33^{ab}**	**3.10±0.33^{a}**	**3.08±0.10^{a}**	**2.73±0.23^{b}**
DHA/EPA	**17.22±1.59**	**20.11±3.12**	**17.99±2.42**	**17.72±1.83**
DHA/ARA	**5.82±0.18**	**6.48±0.78**	**5.84±0.75**	**6.49±0.40**
[7]Total PUFA	**40.46±3.52**	**43.13±7.16**	**45.00±3.47**	**43.23±3.42**
n-3/n-6	**1.77±0.05^{b}**	**1.88±0.21^{ab}**	**1.89±0.13^{ab}**	**2.04±0.13^{a}**

Different letter indicates significant differences (p-value<0.05)

[1]14:00,15:00, 16:00, 17:00, 18:00, 20:00

[2]14:1n-7; 14:1n-5; 15:1n-5; 16:1n-7 16:1n-5; 18:1n-9; 18:1n-7; 18:1n-5; 20:1n-9; 20:1n-7; 20:1n-5; 22:1n-11; 22:1n-9.

[3]16:3n-3; 18:3n-3; 18:4n-3; 20:3n-3; 20:4n-3; 20:5n-3; 22:5n-3; 22:6n-3.

[4]18:2n-6; 18:3n-6; 20:2n-6; 20:3n-6; 20:4n-6; 22:4n-6; 22:5n-6

[5]18:1n-9; 18:2n-9; 20:1n-9; 20:2n-9; 20:3n-9

[6]20:3n-3; 20:4n-3; 20:5n-3; 22:5n-3; 22:6n-3
[7]18:2n-9; 18:2n-6; 18:2n-4; 18:3n-6; 18:3n-4; 18:4n-3; 18:4n-1; 20:2n-9; 20:2n-6; 20:3n-9; 20:3n-6; 20:4n-6; 20:3n-3, 20:4n-3; 20:5n-3; 22:4n-6, 22:5n-6; 22:5n-3; 22:6n-3

5.3.3. Histology

5.3.3.1. Liver

After 3 months of feeding with the different experimental diets, histological examination of fish livers fed different experimental revealed liver histomorphology was generally good among study fish, showing a regular morphology of hepatic tissue with hepatocytes of medium size. However, some minor adverse cellular alterations were observed. Livers of tilapia fed BP20 showed a significant lower tendency for accumulation of lipids in the hepatocytes. Control diet was characterized by an increase in the cytoplasmic vacuolation, a marked nuclear displacement and in the severity of lipid infiltration. Also, lesser presence of necrotic foci with broken cytoplasmatic hepatocytes but not statistically significant. Connected with nuclear morphology no differences were found in the occurrence of small, dark, pyknotic nuclei. BP20 was the feeding that less occurrence of hepatocellular nuclear pleomorphism, presenting regular uniform central nucleus. Vascular changes represented by wide, dilated sinusoid with a greater presence of erythrocytes, was related to feeding Control and BP10 diet. Table 5.7 shows the values of different parameters such as the steatosis, necrosis foci, pyknotic nuclei, nuclear pleomorphism and vascular changes found feeding the different experimental diets.

Table 5.7 Histopathological analysis of the liver from tilapia fed experimental diets.

	CONTROL	BP5	BP10	BP20
Steatosis	**2.50±0.50[a]**	**2.56±0.53[a]**	**2.17±0.79[a]**	**1.56±0.53[b]**
Necrosis Foci	**1.67±0.50**	**2.00±0.50**	**1.67±0.50**	**1.44±0.73**
Pyknotic nuclei	**1.44±0.53**	**1.67±0.50**	**1.22±0.44**	**1.22±0.44**
Nuclear pleomorphism	**2.00±0.00[a]**	**2.22±0.67[a]**	**1.89±0.60**	**1.33±0.71[b]**
Vascular changes	**2.44±0.73[a]**	**1.44±0.53[b]**	**2.00±0.50[a]**	**1.33±0.50[b]**

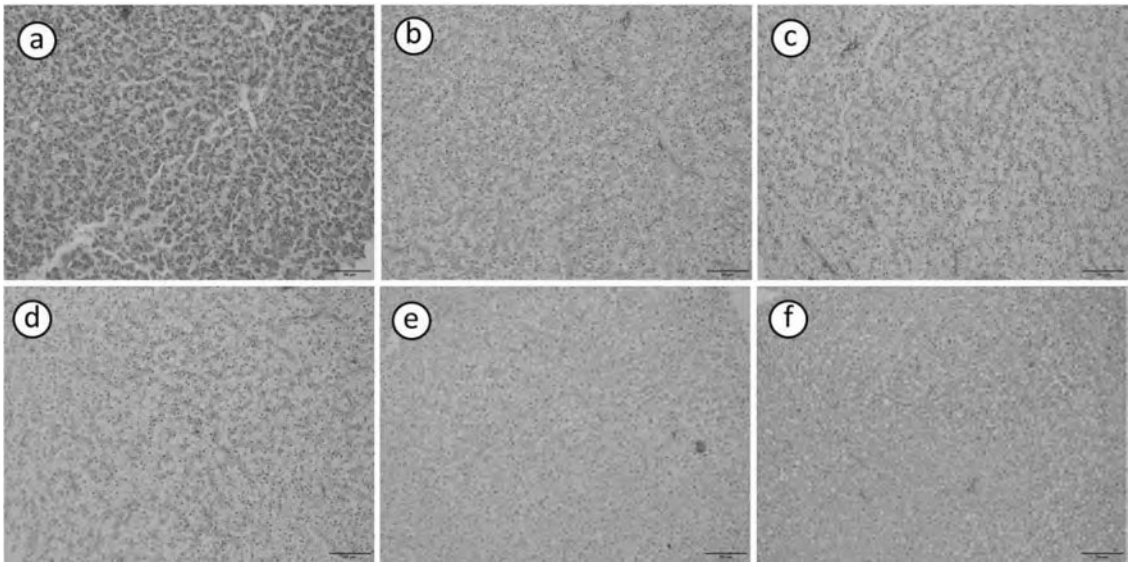

Figure 5.3: Liver histological appearance of tilapia fed different experimental diets. a: BP20 diet shows regular nuclei and eosinophilic aspect connected with relevant reserves of glycogen, no steatosis appearance. b: Control diet steatosis phenomena, cytoplasmic vacuolation and marked nuclear displacement. c: BP5 diet necrosis foci with broken cytoplasm. d: BP10 pyknosis nuclei. e: nuclei pleomorphism. f: slight vascular hyperemia. H&E; scale 50 µm).

5.3.3.2. Intestine

5.3.3.2.1. Foregut

In the anterior intestine, fish fed experimental diets showed normal epithelial layers, mucosa, muscularis and serosa. The tunica mucosa had normal folds (villi), and the intestinal epithelium shown normal enterocyte with basal oval nucleus. The tip of the fold was damaged with different grades of reparation. Villus length was not significantly influenced by the dietary treatments ($P > 0.05$) (Figure 5.4. & 5.5.). However, as shown in Table 8, the intestinal villus of the fish fed diet BP5 showed the highest villus width and BP20 the lowest ($P < 0.05$).

5.3.3.2.2. Hindgut

Table 5.8 illustrate height and width values in the hindgut. The intestinal epithelium shown normal structure with not damages on the folds and normal enterocyte with no evident intraepithelial

lymphocytes' presence (Figure 5.6). The posterior intestine of fish fed BP10 presented a reduction on the intestinal folds both on length and width with the lowest values ($P < 0.05$).

Table 0.8 Histopathological analysis of the intestine from tilapia fed experimental diets.

		CONTROL	BP5	BP10	BP20
Foregut	Height	257.96±70.88	247.37±37.32	245.07±44.31	261.70±90.93
	Width	66.12±18.42^{bc}	75.64±19.64^{a}	73.42±14.99^{ab}	62.27±21.66^{c}
Hindgut	Height	148.04±36.70^{a}	143.68±26.05^{a}	103.55±18.95^{b}	138.36±29.74^{a}
	Width	59.48±15.25^{a}	57.41±16.04^{a}	46.49±16.49^{b}	60.5±20.81^{a}

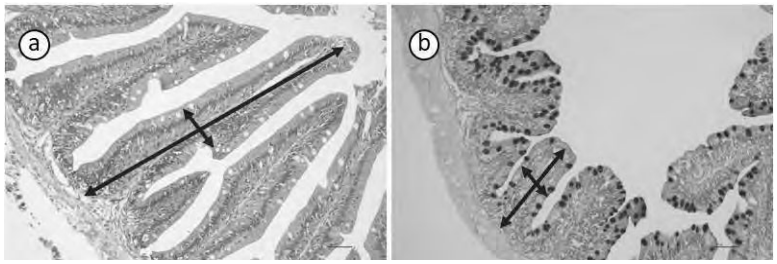

Figure 5.4: Photomicrograph of tilapia foregut intestine stained with a: H&E (×400) and b: Alcian Blue/PAS (pH = 2.5), fed on the control basal diet showing intestinal mucosal folds (villi) length and width. scale 100 µm.

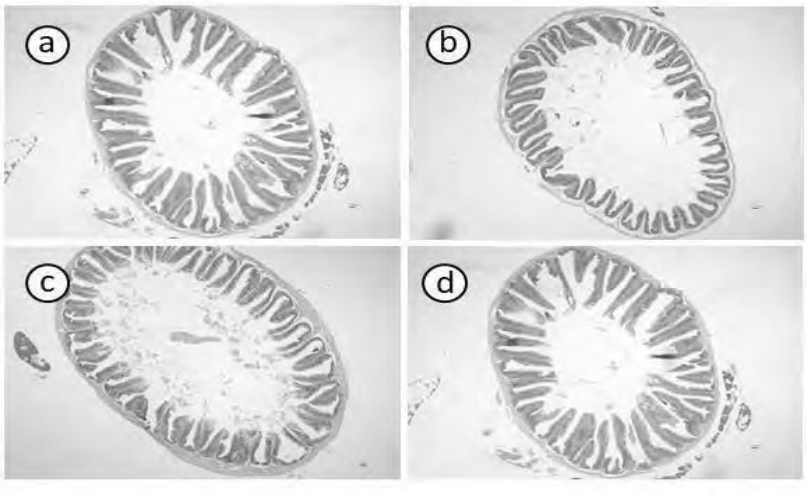

Figure 5.5: Foregut histological morphology of tilapia fed different experimental diets. a: Control diet b: BP5. c: BP10. d: BP20. H&E; scale 100 µm.

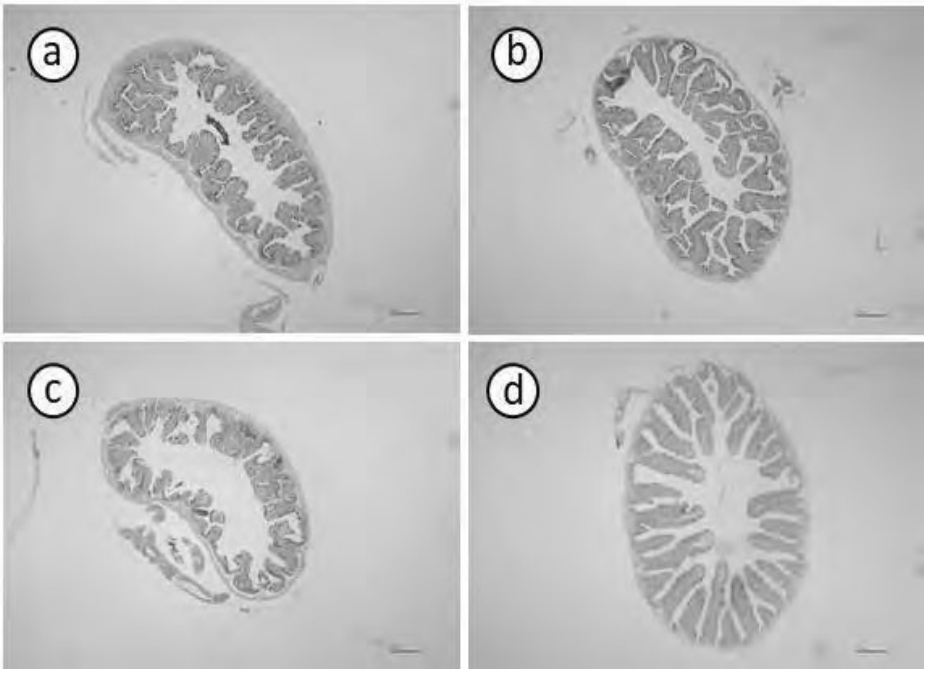

Figure 5.6: Hindgut histological morphology of tilapia fed different experimental diets. a: Control diet b: BP5. c: BP10. d: BP20. Alcian Blue/PAS (pH = 2.5), scale 100 µm.

5.4. Discussion

The main goal of aquaculture is to increase maximal growth rate while preserving lowest feed conversion ratio (Mengistu, Mulder et al. 2020). Extensive studies have been done to achieve this goal, and feed additives are among the most promising elements to fulfill this objective (Dawood and Koshio 2016, Reverter, Tapissier-Bontemps et al. 2017). At the beginning of the present experiment, the Nile tilapia quickly acclimatized to the feed and to the experimental tanks. No variations in appetite were observed feeding with the experimental diets, nor were increases of up to 20% observed in BP levels. Our results confirm that the distinct levels of inclusion of banana by-products had no detrimental effects on the growth rates findings consistent with previous results reported by (Medina-Villacis, Italo et al. 2022) where the biological parameters and meat yields were unaffected by dietary consume of banana by-products. A recent study (Ramírez-Bolaños, Pérez-Jiménez et al. 2021) proved that the presence of banana residues in the diets for Nile tilapia had a minor impact on performance parameters. Similarly, in the case of banana peel, tested with GIFT strain of Nile tilapia an increase in size around to a 30% (Yossa, Ahmad Fatan et al. 2022). Also, (Palintorn, Rujinanont et al. 2019) on Nile tilapia recorded no difference in growth

parameters and survival rate, testing different inclusion levels of flesh ripe banana. In this context, (Suehs and Gatlin 2022) found that for indicators of growth performance and condition, there were no discernible variations in the measured growth performance measures for juvenile Nile tilapia fed by Dried Grain with Soluble (HP-DDGS). On the other hand, Herath et al. (2016) showed that the growth parameters results were affected by the feeding treatment when insert the corn co-products to the Nile tilapia diets. Even though the fish accepted the diet well, (Karaket, Somtua et al. 2021) discovered that just 5% of ripe bananas had no effect on the hybrid tilapia's growth. With another by-product, this time sugarcane bagasse powder, (Lumsangkul, Tapingkae et al. 2021) it was recorded an increased the final weight (FW), weight gain (WG), and specific growth rate (SGR) compared to fish fed a control diet that has been previously studied in peninsula carp (*Labeo fimbriatus*) (Dharmaraj, Manissery et al. 2002).

The proximal composition of banana by-product in our study presented some differences with precedent studies. (Udo, Etokakpan et al. 2021, Hefnawy, Awad et al. 2022) reported lower values in all terms of proximal composition in banana except in moisture that it was higher comparing with our results. The study by (Ramírez-Bolaños, Pérez-Jiménez et al. 2021) showed that the banana flower and pseudo stem presented lower protein, moisture and lipid values while the ash content was higher, possibly related to specific variations in the samples collected. The biochemistry analysis in the Nile tilapia muscle in our study showed that no statistically significant differences found comparing the fish fed different experimental diets. There are scarce studies that discussed the inclusion of banana by-product in feeds which resulted in changes in body composition. Accordingly, we discuss the effect of other by-products on Nile tilapia or the effect of by-product on alternative fish species. (Gabriel, Qiang et al. 2017) reported that the Aloe vera by-product led to a decrease in the protein content and muscle moisture in GIFT tilapia juveniles. According to (Suehs and Gatlin 2022) in the whole-body tissues of juvenile Nile tilapia, fed with different diets of Commercial High-Protein Distiller's dried grain with Soluble (HP-DDGS), the contents of dry matter, crude protein, crude fat, and ash were found not different. However, the values of different classes of fatty acids feeding diverse levels of inclusion of banana by-product produce lower values than that in the control. In this sense, the levels of unsaturated fatty acids were quite similar between diets. It interesting to highlight that banana inflorescence (Sheng, Ma et al. 2010) was found to contain up more than 60% of the total fatty acid unsaturated fatty acids (UFAs), such as linoleic acid, oleic acid, and alpha-linolenic acid. The extract of ripe pulp of

banana fruit presented a lower rate of unsaturated fatty acids (Vilela, Santos et al. 2014). In banana peels has been shown that linoleic acid (Omega-6) and -linolenic acid (Omega-3), make up more than 40% of the total fatty acids (Emaga, Andrianaivo et al. 2007, Khawas and Deka 2016). Linoleic acid reduces blood cholesterol levels and protects against atherosclerosis (Ramu, Shirahatti et al. 2017), consequently, the bananas by-products are a valuable source of beneficial UFAs that may reduce the risk of cardiovascular disease (Lau, Kong et al. 2020). Also, the advantages of MUFA and PUFA are mentioned in several studies including their role in the innate immune system, their ability to decrease the risk of cardiovascular disease, and their ability to rearrange adipose tissue (Tvrzicka, Kremmyda et al. 2011, Lau, Kong et al. 2020). Essential fatty acids (EFAs) have been regarded as functional foods and nutraceuticals. EFAs are essential for synthesis of several biologically active chemicals and the structural elements of cells, tissues, and organs. Several research studies have demonstrated the significance of EFAs in a variety of meta-folic processes (Gupta and Houston 2017). (Tadesse 2010) and (Nemova, Nefedova et al. 2020) discussing the effect of the diet on fatty acids composition from fish tissues focusing on the impact of temperature and diet.

The level of unsaturated fatty acids (PUFA) is higher than the Nile tilapia muscle in all diets comparing with the amount of PUFA in the banana by-product which describe that utilization and absorption in these types of fatty acids was high. Our studies proved that BP10 produced the highest accumulation of the PUFA in the Nile tilapia muscle when the fish was fed diets treated with crude papain enzyme but it was less preferred (Kirimi, Musalia et al. 2022).

In the same context, (Opiyo, Muendo et al. 2022) reported that the Nile tilapia fed with dietary duckweed increased the proportions of omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) in muscle. Also, (Chepkirui, Orina et al. 2021) recorded an increase in the PUFA including water spinach (*Ipomoea aquatica*) in Nile tilapia diets.

The histological studies of the liver and intestine (foregut and hindgut) shown that the different concentration of banana by-product included in Nile tilapia diets had scarce effects on the studied organs. In the liver, studying lipids content, cytoplasmic vacuolation and broken cytoplasmatic hepatocytes or epithelial structure in the intestine, BP20 diet, which was the highest inclusion of banana by-product, presented the lowest effects. Also, the gut samples in both the experimental and control groups had normal histology, which suggested that plant by-products can be included as an ingredient in Nile tilapia diets. This result is in concordance with (Palintorn, Rujinanont et

al. 2019) which observed normal characteristics and regular features in the liver and intestine in Nile tilapia fed with Thai local cultivated banana (CV *Kluai Namwa*). Similar results were found by (Ramírez-Bolaños, Pérez-Jiménez et al. 2021) who reported that the inclusion of banana by-product in Nile tilapia diets had no effect on liver histology.

Some minor variations were reported as in (Yossa, Ahmad Fatan et al. 2022) that noticed a reduction in the degree of vacuolization with the increase of inclusion the banana peel, associated with a lower content of lipid in the liver. Also, (Mohammadi, Imani et al. 2020) observed that adding canola meal in diets significantly impacted the histoarchitecture of the gut and the liver in immature Nile tilapia. Another study reported that when compared to the control, the sodium butyrate and *Lippia origanoides* supplementation encouraged the development of more intestinal villi and fish fed a diet that only contained Lippia showed an increase in the red blood cell count and the villus perimeter in the gut's posterior area (Jesus, Owatari et al. 2021). Our histology analysis of the liver and intestine of Nile tilapia shows a normal appearance without lesions or damage after the addition of banana by-products at all experimentally tested levels of 5, 10 and 20%, implying that it can be used without showing unwanted effects.

5.5. Conclusion

The present study, one of the few studies discussing the use of banana by-product in Nile tilapia diets, demonstrated that diet supplemented with 5, 10, and 20% banana ensured positive effects on growth and efficiency of tilapia. feeding parameters without negative effects on growth or proximal composition and without abnormal effects on either the liver or the intestine during histological analysis.

Taking into account the above, and from a circular economy perspective, the results showed an effective and environmentally friendly use of a banana by-product as a diet for Nile tilapia that contributes to the reduction of food loss and waste.

Chapter 6. Conclusions and recommendations

1- Reduction the demand-production cost gap and promote circular economy strategies, replacing typically expensive fish diets with plant-derived wastes remains an achievable option. Obviously, our results make a good impression of using Aloe vera and banana wastes as an alternative ingredient in fish diets (*liza aurata* and *Oreochromis niloticus*) under the name of circular economy as a way to reduce the cost of the fish diets and for its safe effects on fish and then humans.

2- All heavy metal concentrations were below the established acceptable limits for intake in both golden mullet tissue and Aloe vera diets. Through the food chain, Aloe vera products and by-products were safe for fish as well as for humans. National and international agencies devised a number of risk and benefit assessment methods, and they concluded that using *Liza aurata* was relatively safe according to the results of THQ, HI, As-CR and Se-HBV.

3- Evaluation and research of (EDI) and (MSC) showed that if a 70 kg person consumes more than 58 g of golden mullet per day, health hazards are not totally eliminated.

4- The nutritional value for most of the essential metals is sufficiently covered at both low and high intake levels in golden mullet.

5- The metal content in banana by-products diets for Nile tilapia showed that the level of inclusion 10% has consistently the greatest concentration of all the heavy and essential elements in diets, however the contents of metals in raw fillet the accumulation of metals was non-significant variable.

6- Tracking metal levels in edible tissues is essential for assessing the potential risk to human health using the EDI, THQ, and HI indices because the heavy metal concentration of fish edible sections is greatly dependent on diet composition, in our study *O. niloticus* fed on varying concentrations of banana by-products had heavy metal and trace element toxicity levels were far lower than the level allowed for human consumption.

7- The values of As (CR) and Se-HBV in the Nile tilapia raw fillet which was within the acceptable limits and give us a good influence on human health showed an evidence of safety banana by-products

8- The nutritional analysis of the farmed Nile tilapia revealed that this species can be regarded as a rich source of necessary metals, so it is clear that the presence of banana by-products

in the tilapia diet had a minor impact on the fillet's metal content but not enough to reduce its nutritional value.

9- On the other hand, it was concluded that the inclusion of banana by-product in *O. niloticus* diet affect positively the growth rate which represented in weight and length, both during the experimental test and afterwards, neither of these two parameters showed any significant differences.

10- Concerning the biochemical composition and fatty acids of the banana by-product, even though their respective fatty acid profiles are not directly related, the addition of banana products dramatically decreases the lipid content of tilapia fillets. The lowest banana levels (5%) considerably raised most of the values relating to the control whereas the greatest banana levels (10% and 20%) reduced the lipid content to levels comparable to the control fish. This proves the species' biological ability to biosynthesize necessary fatty acids when fed large amounts of banana by-products.

11- Nutritionally, the higher concentration (10% and 20%) showed the higher accumulation of protein and humidity values in the tilapia raw fillet and lower in the lipids content, so the nutritional values increase with the increasing of banana by-product concentration in tilapia diet.

Chapter 7. Resumen en español

El concepto de economía circular hace referencia a una forma de economía que preserva el medio ambiente, reduciendo la huella de carbono y haciendo un uso más sostenible de los recursos. Para ello, se debe mejorar la gestión de las prácticas agrícolas a través de la reducción de los residuos además de introducirlos en la cadena de valor. En este sentido, los nuevos modelos económicos deben adaptarse a las exigencias de sostenibilidad que trae consigo el cambio climático. El reciclaje de productos que pueden aportar valor en otros ciclos productivos es una forma de utilizar los recursos de manera más eficiente y así minimizar el impacto en el medio ambiente. Esta nueva forma de pensar impulsará las bioindustrias para la recuperación, transformación y revalorización de subproductos de los sectores primario y secundario.

En la formulación de dietas para los peces, debe evaluarse la potencialidad de los ingredientes que la componen, no sólo a partir de los rendimientos productivos obtenidos, sino también de sus posibles efectos sobre el bienestar y la salud de los animales. Además, deben analizarse los posibles contaminantes ligados al proceso de desarrollo de las plantas así como los derivados del procesado de las mismas y que están presentes en sus subproductos. El objetivo del presente trabajo ha sido desarrollar dietas con diferentes niveles de inclusión de subproductos provenientes del cultivo de *Aloe vera* y del de platanera como alternativas sostenibles a los ingredientes habituales de las dietas acuícolas bajo la aproximación del concepto de economía circular, determinando el contenido en metales y valorando el riesgo y valor nutricional de los peces así alimentados. En el caso de los subproductos de platanera se han estudiado también los efectos sobre el crecimiento, composición bioquímica y morfología del hígado e intestino en juveniles de tilapia.

Para cada uno de los dos subproductos utilizados, *Aloe vera* en mújil *(Liza aurata*) y platanera en tilapia (*Oreochromis niloticus*), se testaron cuatro dietas isocalóricas e isoproteicas, una de ellas comercial adaptada a la especie en estudio, y las otras tres con cantidades crecientes del subproducto: 5, 10 y 20% de inclusión de subproducto de platanera, y 2, 4 y 6% de subproductos de *Aloe vera*. Todas las dietas fueron analizadas tanto en su composición bioquímica como en su contenido en metales. Los riesgos derivados del contenido de metales en el pescado se han determinado atendiendo a la ingesta diaria estimada (EDI), el máximo consumo seguro (MSCA), la tasa de peligro objetivo (THQ), el índice de riesgo (HI), el riesgo carcinogénico de As (As-CR),

el valor del beneficio para la salud según contenido en selenio (Se HBV) y por último el valor nutricional.

En los trabajos con las dos especies y las diferentes proporciones de inclusión de los subproductos, se ha constatado que el contenido de todos los elementos estudiados fue menor a los límites máximos permitidos. De acuerdo con los análisis de riesgo-beneficio propuestos por las autoridades nacionales e internacionales, el consumo de *Liza aurata* y de *Oreochromis niloticus* es seguro. Aunque se detectaron niveles de As inorgánico potencialmente peligrosos, estuvieron por debajo de límites marcados como seguros. Por otra parte, los valores de Se-HBV se pueden considerar como beneficios para la salud de los potenciales consumidores.

El tercer estudio estuvo enfocado a evaluar la tasa de crecimiento, la composición proximal del filete y el efecto sobre la histología del hígado y del intestino en la tilapia del Nilo alimentada con las dietas formuladas incluyendo subproductos de la platanera. En el hígado se estudió el nivel de vacuolización (esteatosis), la presencia de focos de necrosis o núcleos picnóticos, pleomorfismo nuclear y la aparición de alteraciones vasculares. A nivel intestinal se midió la altura y el ancho de las vellosidades, tanto en su tramo anterior como en el posterior.

Los resultados obtenidos avalaron un adecuado crecimiento de las tilapias alimentadas con dietas que incluyeron subproductos de la platanera. Tampoco se detectaron efectos adversos en la morfología del hígado y del intestino, aunque si se apreció un ligero cambio en los pliegues del intestino anterior y posterior.

En definitiva, los hallazgos obtenidos en el marco de la economía circular, respaldan las expectativas de poder incluir subproductos de *Aloe vera* y de platanera como ingredientes en dietas para *Liza aurata* y *Oreochromis niloticus*, reduciendo su coste, manteniendo la salud de los peces y garantizando la seguridad alimentaria para los consumidores.

References

Abdullah, K. M., A. Abdullah, M. L. Johnson, J. J. Bilski, K. Petry, D. A. Redmer, L. P. Reynolds and A. T. Grazul-Bilska (2003). "Effects of Aloe vera on gap junctional intercellular communication and proliferation of human diabetic and nondiabetic skin fibroblasts." The Journal of Alternative Complementary Medicine 9(5): 711-718.

Abdy, E., M. Alishahi, M. Tollabi, M. Ghorbanpour and T. Mohammadian (2017). "Comparative effects of Aloe vera gel and Freund's adjuvant in vaccination of common carp (*Cyprinus carpio* L.) against Aeromonas hydrophila." Aquaculture international 25: 727-742.

Abowei, J., A. Ekubo and Toxicology (2011). "A review of conventional and unconventional feeds in fish nutrition." British Journal of Pharmacology 2(4): 179-191.

Adesuyi, A., O. Awosanya, F. Adaramola and A. Omeonu (2012). "Nutritional and phytochemical screening of Aloe barbadensis." Current Research of Journal Biological Science 4(1): 4-9.

Afonso, C., H. M. Lourenco, C. Cardoso, N. M. Bandarra, M. L. Carvalho, M. Castro and M. L. Nunes (2013). "From fish chemical characterisation to the benefit-risk assessment–Part A." Food Chemistry 137(1-4): 99-107.

Ahmed, I., K. Jan, S. Fatma and M. A. O. Dawood (2022). "Muscle proximate composition of various food fish species and their nutritional significance: A review." Journal of Animal Physiology and Animal Nutrition 106(3): 690-719.

Akhtar, M., A. Hai, M. M. Awais, Z. Iqbal, F. Muhammad, A. ul Haq and M. I. Anwar (2012). "Immunostimulatory and protective effects of Aloe vera against coccidiosis in industrial broiler chickens." Veterinary parasitology 186(3-4): 170-177.

Al-Ghanem, K. A. J. T. (2011). "Effect of cobalt-supplemented diets on bioaccumulation, digestive enzyme activities and growth of *Cyprinus carpio*." Toxicological Environmental Chemistry 93(5): 985-995.

Al-Kahtani, M. A. (2009). "Accumulation of heavy metals in tilapia fish (*Oreochromis niloticus*) from Al-Khadoud Spring, Al-Hassa, Saudi Arabia." American Journal of Applied Sciences 6(12): 2024-2029.

Alaa, G. (2012). "Biomarkers in Nile tilapia *Oreochromis niloticus* niloticus (Linnaeus, 1758) to assess the impacts of river Nile pollution: bioaccumulation, biochemical and tissues biomarkers." Journal of Environmental Protection 2012.

Alasalvar, C., K. Taylor, E. Zubcov, F. Shahidi and M. Alexis (2002). "Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition." Food chemistry 79(2): 145-150.

Alishahi, M., Z. T. Dezfuly, M. Mesbah and T. Mohammadian (2017). "Effects of Aloe vera crude extract on growth performance and some hemato-immunological indices of *Oncorhynchus mykiss* in farm scale." Iranian Journal of Veterinary Medicine 11(4).

Alonso, D. M., J. Q. Bond and J. A. Dumesic (2010). "Catalytic conversion of biomass to biofuels." Green chemistry 12(9): 1493-1513.

ALOVERIA, 2023. https://www.aloveria.com

Altug, N., N. Yuksek and Z. T. Agaoglu (2010). "Immunostimulatory effects of Aloe vera and β-Glucan on cellular and humoral immune responses following vaccination with polyvalent vaccines in dogs." accines in Dogs Kafkas Univ Vet Fak Derg 16(3): 405-412.

Alves, L., C. Glover and C. J. A. o. e. c. Wood (2006). "Dietary Pb accumulation in juvenile freshwater rainbow trout (*Oncorhynchus mykiss*)." Archives of environmental contamination toxicology 51: 615-625.

Alzate Acevedo, S., J. Díaz Carrillo Á, E. Flórez-López and C. D. Grande-Tovar (2021). "Recovery of Banana Waste-Loss from Production and Processing: A Contribution to a Circular Economy." Molecules 26(17).

Amlund, H., K. A. Francesconi, C. Bethune, A. K. Lundebye and M. H. J. E. T. Berntssen (2006). "Accumulation and elimination of dietary arsenobetaine in two species of fish, Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.)." nvironmental Toxicology Chemistry: An International Journal 25(7): 1787-1794.

Anal, A. K., S. Jaisanti, A. J. J. o. F. S. Noomhorm and Technology (2014). "Enhanced yield of phenolic extracts from banana peels (*Musa acuminat* a Colla AAA) and cinnamon barks (*Cinnamomum varum*) and their antioxidative potentials in fish oil." Journal of Food Science Technology 51: 2632-2639.

Andreini, C., I. Bertini, G. Cavallaro, G. L. Holliday and J. M. Thornton (2008). "Metal ions in biological catalysis: from enzyme databases to general principles." JBIC Journal of Biological Inorganic Chemistry 13: 1205-1218.

APROMAR, 2021. Aquaculture production in Spain 2021. the Spanish Aquaculture Business Association.

Armstrong, G. R., C. E. Dewey and A. J. Summerlee (2017). "Iron release from the Lucky Iron Fish®: safety considerations." Asia Pac J Clin Nutr 26(1): 148-155.

Arunkumar, S. and M. Muthuselvam (2009). "Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens." World journal of agricultural sciences 5(5): 572-576.

Arvanitoyannis, I. and A. Mavromatis (2009). "Banana cultivars, cultivation practices, and physicochemical properties." Critical Reviews in Food Science Nutrition 49(2): 113-135.

Arvanitoyannis, I. S. and A. Kassaveti (2008). "Fish industry waste: treatments, environmental impacts, current and potential uses." International journal of food science technology 43(4): 726-745.

Aschner, J. L. and M. Aschner (2005). "Nutritional aspects of manganese homeostasis." Mol Aspects Med 26(4-5): 353-362.

Ashraf, M., M. Maah and I. Yusoff (2012). "Bioaccumulation of heavy metals in fish species collected from former tin mining catchment."

Atamanalp, M., E. M. Kocaman, A. Ucar and G. Alak (2010). "The alterations in the hematological parameters of brown trout Salmo trutta fario, exposed to cobalt chloride." Journal of Animal Veterinary Advances 9(16): 2167-2170.

Authority, E. F. S. (2017). Dietary reference values for nutrients summary report, Wiley Online Library.

Ayanda, I. O., U. I. Ekhator and O. A. Bello (2019). "Determination of selected heavy metal and analysis of proximate composition in some fish species from Ogun River, Southwestern Nigeria." Heliyon 5(10): e02512.

Aydın, D. and Ş. Tokalıoğlu (2015). "Trace metals in tissues of the six most common fish species in the Black Sea, Turkey." Food Additives Contaminants: Part B 8(1): 25-31.

Aznar-Sánchez, J. A., J. F. Velasco-Muñoz, L. J. Belmonte-Ureña and F. Manzano-Agugliaro (2019). "The worldwide research trends on water ecosystem services." Ecological indicators 99: 310-323.

Aznar-Sánchez, J. A., J. F. Velasco-Muñoz, D. García-Arca and B. López-Felices (2020). "Identification of opportunities for applying the circular economy to intensive agriculture in Almería (South-East Spain)." Agronomy 10(10): 1499.

Baiano, A. (2014). "Recovery of biomolecules from food wastes—A review." Molecules 19(9): 14821-14842.

Balali-Mood, M., K. Naseri, Z. Tahergorabi, M. R. Khazdair and M. Sadeghi (2021). "Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic." 12.

Bandara, T. (2018). "Alternative feed ingredients in aquaculture: Opportunities and challenges." Entomol. Zool. Stud 6(2): 3087-3094.

Banerjee, R. and S. W. Ragsdale (2003). "The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes." Annual review of biochemistry 72(1): 209-247.

Basim, Y., Z. Khoshnood and i. health (2016). "Target hazard quotient evaluation of cadmium and lead in fish from Caspian Sea." Toxicology 32(2): 215-220.

Begum, A., M. N. Amin, S. Kaneco and K. Ohta (2005). "Selected elemental composition of the muscle tissue of three species of fish, Tilapia nilotica, Cirrhina mrigala and Clarius batrachus, from the fresh water Dhanmondi Lake in Bangladesh." Food chemistry 93(3): 439-443.

Bennett, R. N., T. M. Shiga, N. M. Hassimotto, E. A. Rosa, F. M. Lajolo, B. R. Cordenunsi and f. chemistry (2010). "Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (*Musa acuminata* Juss.) cultivars." Journal of agricultural 58(13): 7991-8003.

Beveridge, M., D. Baird and exploitation (2000). "Diet, feeding and digestive physiology." Tilapias: Biology: 59-87.

Bosch, A. C., B. O'Neill, G. O. Sigge, S. E. Kerwath and L. C. Hoffman (2016). "Heavy metals in marine fish meat and consumer health: a review." Journal of the Science of Food Agriculture 96(1): 32-48.

Boscolo, W. R., C. Hayashi, C. M. Soares, W. M. Furuya and F. Meurer (2001). "Desempenho e características de carcaça de machos revertidos de tilápias do Nilo (*Oreochromis niloticus*), linhagens tailandesa e comum, nas fases inicial e de crescimento." Revista Brasileira de Zootecnia 30.

Boudreau, M. D. and F. A. Beland (2006). "An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera." Journal of Environmental Science Health Part C 24(1): 103-154.

Boyd, C. E. (2004). "Farm-level issues in aquaculture certification: Tilapia." Report commissioned by WWF-US in: 1-29.

Boyd, C. E., L. R. D'Abramo, B. D. Glencross, D. C. Huyben, L. M. Juarez, G. S. Lockwood, A. A. McNevin, A. G. Tacon, F. Teletchea and J. R. Tomasso Jr (2020). "Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges." Journal of the World Aquaculture Society 51(3): 578-633.

Boyd, D. M. and N. B. Ellison (2007). "Social network sites: Definition, history, and scholarship." Journal of computer-mediated Communication 13(1): 210-230.

Bury, N. R., P. A. Walker and C. N. Glover (2003). "Nutritive metal uptake in teleost fish." Journal of experimental biology 206(1): 11-23.

Caballero, B., P. Finglas and F. Toldrá (2015). Encyclopedia of food and health, Academic Press.

Cai, G., J. Zhu, C. Shen, Y. Cui, J. Du and X. Chen (2012). "The effects of cobalt on the development, oxidative stress, and apoptosis in zebrafish embryos." Biological trace element research 150: 200-207.

Campos, D. A., R. Gómez-García, A. A. Vilas-Boas, A. R. Madureira and M. M. Pintado (2020). "Management of fruit industrial by-products—a case study on circular economy approach." Molecules 25(2): 320.

Canli, M. and G. Atli (2003). "The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species." Environmental pollution 121(1): 129-136.

Canonico, G. C., A. Arthington, J. K. McCrary and M. L. Thieme (2005). "The effects of introduced tilapias on native biodiversity." 15(5): 463-483.

Carline, R. F., P. M. Barry and H. G. Ketola (2004). "Dietary uptake of polychlorinated biphenyls (PCBs) by rainbow trout." North American Journal of Aquaculture 66(2): 91-99.

Castro-González, M. and M. Méndez-Armenta (2008). "Heavy metals: Implications associated to fish consumption." Environmental toxicology pharmacology 26(3): 263-271.

Censi, P., S. Spoto, F. Saiano, M. Sprovieri, S. Mazzola, G. Nardone, S. Di Geronimo, R. Punturo and D. Ottonello (2006). "Heavy metals in coastal water systems. A case study from the northwestern Gulf of Thailand." Chemosphere 64(7): 1167-1176.

Chakraborty, T. K., G. Chandra Ghosh, M. R. Hossain, M. S. Islam, A. Habib, S. Zaman, H. Bosu, M. S. Nice, M. Haldar and A. S. Khan (2022). "Human health risk and receptor model-

oriented sources of heavy metal pollution in commonly consume vegetable and fish species of high Ganges river floodplain agro-ecological area, Bangladesh." Heliyon 8(10): e11172.

Chan, S., B. Gerson and S. Subramaniam (1998). "The role of copper, molybdenum, selenium, and zinc in nutrition and health." Clinics in laboratory medicine 18(4): 673-685.

Chen, L., Z. Xu, M. Liu, Y. Huang, R. Fan, Y. Su, G. Hu, X. Peng and X. Peng (2012). "Lead exposure assessment from study near a lead-acid battery factory in China." Science of the total environment 429: 191-198.

Chen, Y.-J., Y.-J. Liu, L.-X. Tian, J. Niu, G.-Y. Liang, H.-J. Yang, Y. Yuan and Y.-Q. Zhang (2013). "Effect of dietary vitamin E and selenium supplementation on growth, body composition, and antioxidant defense mechanism in juvenile largemouth bass (*Micropterus salmoide*) fed oxidized fish oil." Fish physiology biochemistry 39: 593-604.

Chepkirui, M., P. Orina, M. Opiyo, P. Muendo, K. Mbogo and R. Omondi (2021). "Fatty acids composition of Nile tilapia (*Oreochromis niloticus*) fingerlings fed diets containing different levels of water spinach (*Ipomoea aquatica*)." Journal of Agriculture Food Research 5: 100156.

Chien, L.-C., T.-C. Hung, K.-Y. Choang, C.-Y. Yeh, P.-J. Meng, M.-J. Shieh and B.-C. Han (2002). "Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan." Science of the total environment 285(1-3): 177-185.

Chovanec, A., R. Hofer, F. Schiemer, A. Markert and H. Zechmeister (2003). "Chapter 18 Fish as bioindicators, Trace Metals and other Contaminants in the Environment." Elsevier, pp 6: 639-676.

Christie, P. M. J., I.-W. G. Kwon, P. A. Stoeberl and R. Baumhart (2003). "A cross-cultural comparison of ethical attitudes of business managers: India Korea and the United States." Journal of Business Ethics 46: 263-287.

Christie, W. W. (1982). Lipid analysis, Pergamon press Oxford.

Ciardullo, S., F. Aureli, E. Coni, E. Guandalini, F. Iosi, A. Raggi, G. Rufo and F. Cubadda (2008). "Bioaccumulation potential of dietary arsenic, cadmium, lead, mercury, and selenium in organs and tissues of rainbow trout (*Oncorhyncus mykiss*) as a function of fish growth." Journal of agricultural food chemistry 56(7): 2442-2451.

Clarke, W. P., P. Radnidge, T. Lai, P. Jensen and M. Hardin (2008). "Digestion of waste bananas to generate energy in Australia." Waste Management 28(3): 527-533.

Colombo, S. M. and G. M. Turchini (2021). "'Aquafeed 3.0': creating a more resilient aquaculture industry with a circular bioeconomy framework." Reviews in Aquaculture 13(3): 1156-1158.

Combs Jr, G. and S. B. Combs (1986). The role of selenium in nutrition, Academic Press, Inc.

Copat, C., G. Arena, M. Fiore, C. Ledda, R. Fallico, S. Sciacca and M. Ferrante (2013). "Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories." Food chemical toxicology 53: 33-37.

Copat, C., A. Grasso, M. Fiore, A. Cristaldi, P. Zuccarello, S. Santo Signorelli, G. O. Conti and M. Ferrante (2018). "Trace elements in seafood from the Mediterranean sea: an exposure risk assessment." Food chemical toxicology 115: 13-19.

Copat, C., M. Vinceti, M. G. D'Agati, G. Arena, V. Mauceri, A. Grasso, R. Fallico, S. Sciacca and M. Ferrante (2014). "Mercury and selenium intake by seafood from the Ionian Sea: A risk evaluation." Ecotoxicology environmental safety 100: 87-92.

Cretì, P., F. Trinchella and R. Scudiero (2010). "Heavy metal bioaccumulation and metallothionein content in tissues of the sea bream *Sparus aurata* from three different fish farming systems." Environmental monitoring assessment 165: 321-329.

Czarnek, K., S. Terpiłowska and A. K. Siwicki (2015). "Selected aspects of the action of cobalt ions in the human body." Cent Eur J Immunol 40(2): 236-242.

D'hont, A., F. Denoeud, J.-M. Aury, F.-C. Baurens, F. Carreel, O. Garsmeur, B. Noel, S. Bocs, G. Droc and M. Rouard (2012). "The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants." Nature 488(7410): 213-217.

Da, C. T., T. Lundh and J. E. Lindberg (2012). "Evaluation of local feed resources as alternatives to fish meal in terms of growth performance, feed utilisation and biological indices of striped catfish (*Pangasianodon hypophthalmus*) fingerlings." Aquaculture 364: 150-156.

Dan, N. C. and D. C. Little (2000). "Overwintering performance of Nile tilapia *Oreochromis niloticus* (L.) broodfish and seed at ambient temperatures in northern Vietnam." Aquaculture Research 31(6): 485-493.

Dang, F. and W.-X. Wang (2011). "Antagonistic interaction of mercury and selenium in a marine fish is dependent on their chemical species." Environmental science technology 45(7): 3116-3122.

Dave, G. and R. Xiu (1991). "Toxicity of mercury, copper, nickel, lead, and cobalt to embryos and larvae of zebrafish, Brachydanio rerio." Archives of Environmental Contamination Toxicology 21: 126-134.

Davis Jr, R., A. Welty, J. Borrego, J. Morales, J. Pendon and J. G. Ryan (2000). "Rio Tinto estuary (Spain): 5000 years of pollution." Environmental Geology 39: 1107-1116.

Dawood, M. A. and S. Koshio (2016). "Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review." Aquaculture 454: 243-251.

De Corato, U. (2020). "Agricultural waste recycling in horticultural intensive farming systems by on-farm composting and compost-based tea application improves soil quality and plant health: A review under the perspective of a circular economy." Science of the Total Environment 738: 139840.

De Langhe, E., L. Vrydaghs, P. De Maret, X. Perrier and T. Denham (2009). "Why bananas matter: an introduction to the history of banana domestication." Ethnobotany Research Applications 7: 165-177.

De Silva, S. S. and R. M. Gunasekera (1989). "Effect of dietary protein level and amount of plant ingredient (*Phaseolus aureus*) incorporated into the diets on consumption, growth performance and carcass composition in *Oreochromis niloticus* (L.) fry." Aquaculture 80(1-2): 121-133.

DeSA, U. (2015). "World population projected to reach 9.7 billion by 2050." United Nations Department of Economic Social Affairs.

Devatkal, S. K., R. Kumboj and D. Paul (2014). "Comparative antioxidant effect of BHT and water extracts of banana and sapodilla peels in raw poultry meat." Journal of food science technology 51: 387-391.

Dharmaraj, M., J. K. Manissery and P. Keshavanath (2002). "Effects of a biodegradable substrate, sugarcane bagasse and supplemental feed on growth and production of fringe-lipped peninsula carp, *Labeo fimbriatus* (Bloch)." Acta Ichthyologica et Piscatoria 32(2): 137-144.

Di Giulio, R. T. and D. E. Hinton (2008). The toxicology of fishes, Crc Press.

Dickie, A. (2007). "Biofuels sustainability: a UK perspective." Renewable Energy Focus 8(6): 59-61.

Dissanayake, C., R. Chandrajith, C. Dissanayake and R. Chandrajith (2009). "Bioavailability of Trace Elements and Risk Assessment." Introduction to Medical Geology: 47-58.

Djikanović, V., S. Skorić, I. Jarić and M. t. Lenhardt (2016). "Age-specific metal and accumulation patterns in different tissues of nase (*Chodrostoma nasus*) from the Medjuvršje Reservoir." Science of the Total Environmen 566: 185-190.

Doran, I., B. Sen and Z. Kaya (2005). "The effects of compost prepared from waste material of banana on the growth, yield and quality properties of banana plants." Journal of environmental biology 26(1): 7-12.

Dotta, G., J. I. A. de Andrade, E. L. T. Gonçalves, A. Brum, J. J. Mattos, M. Maraschin and M. L. Martins (2014). "Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed supplemented diet with natural extracts of propolis and Aloe barbadensis." Fish Shellfish Immunology 39(2): 280-284.

Dotta, G., J. Ledic-Neto, E. Gonçalves, A. Brum, M. Maraschin and M. Martins (2015). "Acute inflammation and hematological response in Nile tilapia fed supplemented diet with natural extracts of propolis and Aloe barbadensis." Brazilian Journal of Biology 75: 491-496.

Dunz, A. R. and U. K. Schliewen (2013). "Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as "Tilapia"." Mol Phylogenet Evol 68(1): 64-80.

Dural, M., M. Lugal Göksu, A. A. Özak and B. Derici (2006). "Bioaccumulation of some heavy metals in different tissues of *Dicentrarchus labrax* L, 1758, *Sparus aurata* L, 1758 and *Mugil cephalus* L, 1758 from the Camlik lagoon of the eastern cost of mediterranean (turkey)." Environmental Monitoring Assessment 118: 65-74.

Durigon, E., D. Kunz, N. Peixoto, J. Uczay and R. Lazzari (2019). "Selênio na dieta melhora o sistema de defesa antioxidante de juvenis de Tilápia do Nilo (*Oreochromis niloticus* L.)." Brazilian Journal of Biology 79(3): 527-532.

Edwards, P. (1997). "Sustainable food production through aquaculture." Aquaculture Asia 2(1): 4-7.

Eid, R., N. T. Arab and M. T. Greenwood (2017). "Iron mediated toxicity and programmed cell death: A review and a re-examination of existing paradigms." Biochimica et Biophysica Acta -Molecular Cell Research 1864(2): 399-430.

Eisler, R. (2000). "Handbook of chemical risk assessment: health hazards to humans, plants, and animals, three volume set."

El-Batrawy, O. A., M. I. El-Gammal, L. I. Mohamadein, D. H. Darwish and K. M. El-Moselhy (2018). "Impact assessment of some heavy metals on tilapia fish, Oreochromis niloticus, in Burullus Lake, Egypt." The Journal of Basic and Applied Zoology 79(1): 13.

El-Naggar, H. A., H. M. M. Khalaf Allah, M. F. Masood, W. M. Shaban and M. A. E. Bashar (2019). "Food and feeding habits of some Nile River fish and their relationship to the availability of natural food resources." The Egyptian Journal of Aquatic Research 45(3): 273-280.

El-Sayed, A.-F. M. (1999). "Alternative dietary protein sources for farmed tilapia, Oreochromis spp." Aquaculture 179(1-4): 149-168.

El-Sayed, A.-F. M. (2006). Tilapia culture, CABI publishing.

El-Sayed, A.-F. M. and S.-i. Teshima (1992). "Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry." Aquaculture 103(1): 55-63.

El-Sayed, A.-F. M. and S.-i. J. A. Teshima (1992). "Protein and energy requirements of Nile tilapia, *Oreochromis niloticus*, fry." 103(1): 55-63.

El Hendy, H. A., M. I. Yousef and N. I. A. El-Naga (2001). "Effect of dietary zinc deficiency on hematological and biochemical parameters and concentrations of zinc, copper, and iron in growing rats." Toxicology 167(2): 163-170.

Emadi, H., Z. Samavat, A. Saeedi and H. Hosseinzadeh Sahafi (2015). Short Communication: A comparative study on manganese accumulation in liver, kidney, fatty tissues including muscle of rainbow trout (*Oncorhynchus mykiss*) fed with different levels of manganese in the diet.

Emaga, T. H., R. H. Andrianaivo, B. Wathelet, J. T. Tchango and M. Paquot (2007). "Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels." Food chemistry 103(2): 590-600.

Emaga, T. H., C. Robert, S. N. Ronkart, B. Wathelet and M. Paquot (2008). "Dietary fibre components and pectin chemical features of peels during ripening in banana and plantain varieties." Bioresource Technology 99(10): 4346-4354.

FAO (2010a) FAOSTAT: Banana Production by Countries 2010 http://faostat.fao.org/site/339/default.aspx [Assessed 27 May 2012]

FAO (2010b) FAOSTAT: Total World Banana Production http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID0567#ancor

Fao and Who, (2002). Evaluation of certain food additives. Fifty-Ninth report of the joint FAO/WHO expert committee on food additives. World Health Org. Tech. Rep. Series 913 i–viii, 1–153, back cover. http://www.ncbi.nlm.nih.gov/pubmed/12677645.

FAO, F. (2012). "The state of world fisheries and aquaculture." Opportunities challenges. Food Agriculture Organization of the United Nations.

FAO. (2009). How to feed the world in 2050. [Online]. Available: http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf. [Accessed 23 Apil 2017]

FAO. (2010). National Aquaculture Sector Overview. National Aquaculture Sector Overview Fact Sheets, Greece Text by Christofilogiannis. http://www.fao.org/fishery/countrysector/naso_%0Agreece/en%0A.

FAO. (2018). Transforming Food and Agriculture to Achieve the SDGs: 20 interconnected actions to guide decision-makers. Technical Reference Document. Rome, 132 pp.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. FAO, Rome. https://doi.org/10.4060/ca9229en

FAO. (2022). Blue Transformation - Roadmap 2022–2030: A vision for FAO's work on aquatic food systems. FAO, Rome. https://doi.org/10.4060/cc0459en

Felix e Silva, A., C. E. Copatti, E. P. de Oliveira, H. C. Bonfá, F. V. S. T. d. Melo, A. C. d. S. Camargo and J. F. B. Melo (2020). "Effects of whole banana meal inclusion as replacement for corn meal on digestibility, growth performance, haematological and biochemical variables in practical diets for tambaqui juveniles (*Colossoma macropomum*)." Aquaculture Reports 17: 100307.

Fernandes, C., A. Fontaínhas-Fernandes, D. Cabral and M. A. Salgado (2008). "Heavy metals in water, sediment and tissues of Liza saliens from Esmoriz–Paramos lagoon, Portugal." Environmental monitoring assessment 136: 267-275.

Ferreira, M., M. Caetano, J. Costa, P. Pousão-Ferreira, C. Vale and M. A. Reis-Henriques (2008). "Metal accumulation and oxidative stress responses in, cultured and wild, white seabream from Northwest Atlantic." Science of the total environment 407(1): 638-646.

Figgitt, M., R. Newson, I. J. Leslie, J. Fisher, E. Ingham and C. P. Case (2010). "The genotoxicity of physiological concentrations of chromium (Cr (III) and Cr (VI)) and cobalt (Co (II)): an in vitro study." Mutation Research/Fundamental Molecular Mechanisms of Mutagenesis 688(1-2): 53-61.

Folch, J., M. Lees and G. H. Sloane Stanley (1957). "A simple method for the isolation and purification of total lipids from animal tissues." biol Chem 226(1): 497-509.

Fowler, B. A., G. F. Nordberg, M. Nordberg and L. Friberg (2011). Handbook on the Toxicology of Metals, Academic Press.

Freyhof, J. and M. Kottelat (2008). "Hucho hucho." IUCN Red List of Threatened Species.

Furuya, W. M., K. M. Fujii, L. D. d. Santos, T. S. d. C. Silva, L. C. R. d. Silva and P. J. P. Sales (2008). "Exigência de fósforo disponível para juvenis de tilápia-do-nilo." Revista Brasileira de Zootecnia 37: 1517-1522.

Gaber, H. (2007). "Impact of certain heavy metals on the gill and liver of the Nile tilapia (*Oreochromis niloticus*)." Egyptian Journal of Aquatic Biology Fisheries 11(2): 79-100.

Gabriel, N., J. Qiang, X. Ma, P. Xu and D. Nakwaya (2017). "Effects of dietary Aloe vera crude extracts on digestive enzyme activities and muscle proximate composition of GIFT tilapia juveniles." South African Journal of Animal Science 47(6): 904-913.

Gabriel, N. N., J. Qiang, J. He, X. Y. Ma, M. D. Kpundeh and P. Xu (2015). "Dietary Aloe vera supplementation on growth performance, some haemato-biochemical parameters and disease resistance against Streptococcus iniae in tilapia (GIFT)." Fish Shellfish Immunology 44(2): 504-514.

Gaithersburg, M. (1984). "Association of Analytical Communities (AOAC)." Off. Methods Anal. Int 24: 974-981.

Galali, Y., Z. A. Omar and S. M. Sajadi (2020). "Biologically active components in by-products of food processing." Food Science & Nutrition 8(7): 3004-3022.

Galappaththi, E. K., S. T. Ichien, A. A. Hyman, C. J. Aubrac and J. D. Ford (2020). "Climate change adaptation in aquaculture." Reviews in Aquaculture 12(4): 2160-2176.

García-Meilán, I., B. Ordóñez-Grande and M. A. Gallardo (2014). "Meal timing affects protein-sparing effect by carbohydrates in sea bream: Effects on digestive and absorptive processes." Aquaculture 434: 121-128.

Gatlin III, D. M., F. T. Barrows, P. Brown, K. Dabrowski, T. G. Gaylord, R. W. Hardy, E. Herman, G. Hu, Å. Krogdahl and R. Nelson (2007). "Expanding the utilization of sustainable plant products in aquafeeds: a review." Aquaculture research 38(6): 551-579.

Geoffrey, W. (2008). "Whey and whey proteins-From 'gutter-to-gold'." International Dairy Journal 18(7): 695-704.

Ghisellini, P., C. Cialani and S. Ulgiati (2016). "A review on circular economy: the expected transition to a balanced interplay of environmental and economic systems." Journal of Cleaner Production 114: 11-32.

Giannakoudakis, D. A., A. Hosseini-Bandegharaei, P. Tsafrakidou, K. S. Triantafyllidis, M. Kornaros and I. Anastopoulos (2018). "Aloe vera waste biomass-based adsorbents for the removal of aquatic pollutants: A review." Journal of environmental management 227: 354-364.

Giri, S. S., J. W. Jun, V. Sukumaran and S. C. Park (2016). "Dietary administration of banana (*Musa acuminata*) peel flour affects the growth, antioxidant status, cytokine responses, and disease susceptibility of rohu, *Labeo rohita*." Journal of immunology research 2016.

Gobas, F. A., J. B. Wilcockson, R. W. Russell and G. D. Haffner (1999). "Mechanism of biomagnification in fish under laboratory and field conditions." Environmental science technology 33(1): 133-141.

Gobi, N., B. Vaseeharan, R. Rekha, S. Vijayakumar and C. Faggio (2018). "Bioaccumulation, cytotoxicity and oxidative stress of the acute exposure selenium in *Oreochromis mossambicus*." Ecotoxicology environmental safety 162: 147-159.

Gordon, J. and J. Quastel (1948). "Effects of organic arsenicals on enzyme systems." Biochemical Journal 42(3): 337.

Goswami, B. and A. Borthakur (1996). "Chemical and biochemical aspects of developing culinary banana (Musa ABB)'Kachkal'." Food chemistry 55(2): 169-172.

Guallar, E., M. I. Sanz-Gallardo, P. v. t. Veer, P. Bode, A. Aro, J. Gómez-Aracena, J. D. Kark, R. A. Riemersma, J. M. Martín-Moreno and F. J. Kok (2002). "Mercury, fish oils, and the risk of myocardial infarction." New England Journal of Medicine 347(22): 1747-1754.

Gupta, A. and B. Houston (2017). "A comprehensive review of the bioenergetics of fatty acid and glucose metabolism in the healthy and failing heart in nondiabetic condition." Heart failure reviews 22: 825-842.

Gupta, G., M. Baranwal, S. Saxena and M. S. Reddy (2022). "Utilization of banana waste as a resource material for biofuels and other value-added products." Biomass Conversion and Biorefinery.

Halwart, M. and J. F. Moehl (2006). FAO Regional Technical Expert Workshop on Cage Culture in Africa, 20-23 October 2004, Entebbe, Uganda, Food & Agriculture Org.

Hamman, J. H. (2008). "Composition and applications of Aloe vera leaf gel." Molecules 13(8): 1599-1616.

Hardy, R. W. (2010). "Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal." Aquaculture research 41(5): 770-776.

Hardy, R. W. and F. T. Barrows (2003). Diet formulation and manufacture. Fish nutrition, Elsevier: 505-600.

Harris, C., K. Pierce, G. King, K. Yates, J. Hall and I. Tizard (1991). "Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms." Molecular biotherapy 3(4): 207-213.

Harter, T., F. Buhrke, V. Kumar, U. Focken, H. Makkar and K. Becker (2011). "Substitution of fish meal by Jatropha curcas kernel meal: effects on growth performance and body composition of white leg shrimp (*Litopenaeus vannamei*)." Aquaculture Nutrition 17(5): 542-548.

Hashim, R., T. H. Song, N. Z. Muslim and T. P. Yen (2014). "Determination of Heavy Metal Levels in Fishes from the Lower Reach of the Kelantan River, Kelantan, Malaysia." 25(2): 21-39.

Hattink, J., G. De Boeck and R. Blust (2006). "Toxicity, accumulation, and retention of zinc by carp under normoxic and hypoxic conditions." Environmental Toxicology Chemistry: An International Journal 25(1): 87-96.

Hefnawy, A. H., A. Awad, K. Wahdan and M. Doheim (2022). "Chemical and functional properties of the native banana (musa) pseudo-stem." Zagazig Journal of Agricultural Research 49(6): 841-852.

Hem, J. D. (1985). Study and interpretation of the chemical characteristics of natural water, Department of the Interior, US Geological Survey.

Herath, S. S., Y. Haga and S. Satoh (2016). "Effects of long-term feeding of corn co-product-based diets on growth, fillet color, and fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*." Aquaculture 464: 205-212.

Herreros, M., S. Iñigo-Nuñez, E. Sanchez-Perez, T. Encinas and A. Gonzalez-Bulnes (2008). "Contribution of fish consumption to heavy metals exposure in women of childbearing age from a Mediterranean country (Spain)." Food chemical toxicology 46(5): 1591-1595.

Higgs, D., B. Dosanjh, A. Prendergast, R. Beames, R. Hardy, W. Riley and G. Deacon (1995). "Use of rapeseed/canola protein products in finfish diets." Nutrition utilization technology in aquaculture.: 130-156.

Himeno, S. and N. Imura (2000). "New aspects of physiological and pharmacological roles of selenium." Journal of health science 46(6): 393-398.

Ho, E. (2004). "Zinc deficiency, DNA damage and cancer risk." The Journal of nutritional biochemistry 15(10): 572-578.

Hogstrand, C., S. D. Reid and C. M. Wood (1995). "Ca2+ versus Zn2+ transport in the gills of freshwater rainbow trout and the cost of adaptation to waterborne Zn2+." Journal of Experimental Biology 198(2): 337-348.

Hogstrand, C., R. Wilson, D. Polgar and C. M. Wood (1994). "Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc." Journal of Experimental Biology 186(1): 55-73.

Holley, A. K., V. Bakthavatchalu, J. M. Velez-Roman and D. K. St. Clair (2011). "Manganese superoxide dismutase: guardian of the powerhouse." International journal of molecular sciences 12(10): 7114-7162.

Housagul, S., U. Sirisukpoka, S. Boonyawanich and N. Pisutpaisal (2014). "Biomethane production from co-digestion of banana peel and waste glycerol." Energy Procedia 61: 2219-2223.

Howard, H. (2002). "Human health and heavy metals exposure." The environment human health. Michael McCally: 1-14.

HUGHES, J. P., L. POLISSAR and G. Van Belle (1988). "Evaluation and synthesis of health effects studies of communities surrounding arsenic producing industries." International journal of epidemiology 17(2): 407-413.

Hussain, M. (2004). "Farming of tilapia: Breeding plans, mass seed production and aquaculture techniques." Habiba Akter Hussain 55: 149.

Igic, P. G., E. Lee, W. Harper and K. W. Roach (2002). "Toxic Effects Associated With Consumption of Zinc." Mayo Clinic Proceedings 77(7): 713-716.

Ikem, A. and N. O. Egiebor (2005). "Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America)." Journal of food composition analysis 18(8): 771-787.

Iqbal, S., U. Atique, S. Mahboob, M. S. Haider, H. S. Iqbal, K. A. Al-Ghanim, F. Al-Misned, Z. Ahmed and M. S. Mughal (2020). "Effect of supplemental selenium in fish feed boosts growth and gut enzyme activity in juvenile tilapia (*Oreochromis niloticus*)." Journal of King Saud University-Science 32(5): 2610-2616.

Iqbal, S., U. Atique, M. S. Mughal, N. Khan, M. S. Haider, K. Iqbal and M. Akmal (2017). "Effect of selenium incorporated in feed on the hematological profile of tilapia (*Oreochromis niloticus*)." Journal of Aquaculture Research Development 8(10): 1000513.

Irfan, M., S. Hayat, A. Ahmad and M. N. Alyemeni (2013). "Soil cadmium enrichment: Allocation and plant physiological manifestations." Saudi journal of biological sciences 20(1): 1-10.

Izquierdo, M. S. (1989). "Requirement of larval red seabream Pagrus major for essential fatty acids." 日本水産学会誌 55(5): 859-867.

Järup, L. (2003). "Hazards of heavy metal contamination." British medical bulletin 68(1): 167-182.

Jauncey, K. (1998). "Tilapia feeds and feeding."

Javed, M. and N. Usmani (2011). "Accumulation of heavy metals in fishes: a human health concern." International journal of environmental sciences 2(2): 659-670.

Jayathilakan, K., K. Sultana, K. Radhakrishna and A. Bawa (2012). "Utilization of by-products and waste materials from meat, poultry and fish processing industries: a review." Journal of food science technology 49: 278-293.

Jesus, G. F. A., M. S. Owatari, S. A. Pereira, B. C. Silva, N. M. Syracuse, G. R. Lopes, K. Addam, L. Cardoso, J. L. P. Mouriño and M. L. Martins (2021). "Effects of sodium butyrate and Lippia origanoides essential oil blend on growth, intestinal microbiota, histology, and haemato-immunological response of Nile tilapia." Fish Shellfish Immunology 117: 62-69.

Jezierska, B. and M. Witeska (2006). The metal uptake and accumulation in fish living in polluted waters. Soil and water pollution monitoring, protection and remediation, Springer.

Jim, F., P. Garamumhango and C. Musara (2017). "Comparative analysis of nutritional value of *Oreochromis niloticus* (Linnaeus), Nile tilapia, meat from three different ecosystems." Journal of Food Quality 2017.

Jobling, M. (2012). National Research Council (NRC): Nutrient requirements of fish and shrimp: The National Academies Press, Washington, DC, 2011, 376+ XVI pp,£ 128 (Hardback), ISBN: 978-0-309-16338-5, Springer.

Josupeit, H. (2005). World market of tilapia, FAO.

Ju, Y.-R., C.-W. Chen, C.-F. Chen, X.-Y. Chuang and C.-D. Dong (2017). "Assessment of heavy metals in aquaculture fishes collected from southwest coast of Taiwan and human consumption risk." International Biodeterioration & Biodegradation 124: 314-325.

Kaithwas, G., K. Dubey and K. Pillai (2011). "Effect of aloe vera (Aloe barbadensis Miller) gel on doxorubicin-induced myocardial oxidative stress and calcium overload in albino rats."

Kalantzi, I., K. D. Black, S. A. Pergantis, T. M. Shimmield, N. Papageorgiou, K. Sevastou and I. Karakassis (2013). "Metals and other elements in tissues of wild fish from fish farms and comparison with farmed species in sites with oxic and anoxic sediments." Food chemistry 141(2): 680-694.

Kalantzi, I., S. A. Pergantis, K. D. Black, T. M. Shimmield, N. Papageorgiou, M. Tsapakis and I. Karakassis (2016). "Metals in tissues of seabass and seabream reared in sites with oxic and anoxic substrata and risk assessment for consumers." Food Chemistry 194: 659-670.

Kalogeropoulos, N., S. Karavoltsos, A. Sakellari, S. Avramidou, M. Dassenakis and M. Scoullos (2012). "Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish." Food Chemical Toxicology 50(10): 3702-3708.

Kandasamy, S., C. Baggu, M. R. Javagal, J. R. Lingamallu, V. Yenamandra and S. M. Aradhya (2014). "Antioxidant properties of isolated compounds from banana rhizome." Journal of food science 79(5): H988-H1001.

Kaneko, J. J. and N. V. Ralston (2007). "Selenium and mercury in pelagic fish in the central north Pacific near Hawaii." Biological trace element research 119: 242-254.

Kang, H.-Y. (2007). Yeast from papaya processing wastes as aquaculture feed supplement.

Kang, M.-C., S. Y. Kim, Y. T. Kim, E.-A. Kim, S.-H. Lee, S.-C. Ko, W. Wijesinghe, K. W. Samarakoon, Y.-S. Kim and J. H. Cho (2014). "In vitro and in vivo antioxidant activities of polysaccharide purified from aloe vera (*Aloe barbadensis*) gel." Carbohydrate polymers 99: 365-371.

Karaket, T., C. Somtua, P. Ponza and N. Areechon (2021). "Potential Benefits of Ripe Cultivated Banana (*Musa sapientum* Linn.) in Practical Diet on Growth Performance, Feed Utilization and Disease Resistance of Hybrid Tilapia (*Oreochromis niloticus* x *O. mossambicus*)." Turkish Journal of Fisheries Aquatic Sciences 21(10): 501-508.

Karapanagiotidis, I. T., V. Karalazos, N. Kougioumtzis, V. Tsiamis, V. Tsiara, C. Neofitou, I. Karacostas and I. Nengas (2014). "Growth and feed utilization by golden grey mullet (*Liza aurata*) in a coastal lagoon ecosystem, fed compound feeds with varying protein levels." Israeli Journal of Aquaculture-Bamidgeh 66.

Karuppiah, P. and M. Mustaffa (2013). "Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection." Asian Pacific journal of tropical biomedicine 3(9): 737-742.

Kasapidou, E., E. Sossidou and P. Mitlianga (2015). "Fruit and vegetable co-products as functional feed ingredients in farm animal nutrition for improved product quality." Agriculture 5(4): 1020-1034.

Katselis, G., K. Koukou, E. Dimitriou and C. Koutsikopoulos (2007). "Short-term seaward fish migration in the Messolonghi–Etoliko lagoons (Western Greek coast) in relation to climatic variables and the lunar cycle." Estuarine, Coastal Shelf Science 73(3-4): 571-582.

Kaur, R. and T. K. Shah (2017). "A review on role of plant waste products on fish growth, health and production." Entomol Zool Stud 5(3): 583-589.

Kaushik, S. (2001). "Carbohydrate nutrition: importance and limits of carbohydrate supplies."

Kaushik, S. J., S. Panserat and J. W. Schrama (2022). Chapter 7 - Carbohydrates. Fish Nutrition (Fourth Edition). R. W. Hardy and S. J. Kaushik, Academic Press: 555-591.

Keen, C. L., J. L. Ensunsa, B. Lönnerdal and S. Zidenberg-Cherr (2013). Manganese. Encyclopedia of Human Nutrition (Third Edition). B. Caballero. Waltham, Academic Press: 148-154.

Kelly, B. C., M. G. Ikonomou, D. A. Higgs, J. Oakes and C. Dubetz (2008). "Mercury and other trace elements in farmed and wild salmon from British Columbia, Canada." Environmental Toxicology Chemistry: An International Journal 27(6): 1361-1370.

Kern, C. H., G. D. Stanwood and D. R. Smith (2010). "Preweaning manganese exposure causes hyperactivity, disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels." Synapse 64(5): 363-378.

Khalil, M. E., E. K. Moustafa and H. O. A. Osman (1980). "Composition of bolti (Tilapia nilotica) muscle proteins." Food Chemistry 5(2): 175-184.

Khan, K. U., A. Zuberi, J. B. K. Fernandes, I. Ullah and H. Sarwar (2017). "An overview of the ongoing insights in selenium research and its role in fish nutrition and fish health." Fish physiology biochemistry 43: 1689-1705.

Khawas, P. and S. C. Deka (2016). "Comparative nutritional, functional, morphological, and diffractogram study on culinary banana (Musa ABB) peel at various stages of development." International Journal of Food Properties 19(12): 2832-2853.

Kim, K. H., Y. J. Hwang and S. C. Bai (1999). "Resistance to Vibrio alginolyticus in juvenile rockfish (*Sebastes schlegeli*) fed diets containing different doses of aloe." Aquaculture 180(1-2): 13-21.

Kimball, B. A., K. Walton-Day and R. L. Runkel (2007). "Quantification of metal loading by tracer injection and synoptic sampling." Integrated investigations of environmental effects of historical mining in the Animas River Watershed, San Juan County, Colorado 1: 417.

Kirimi, J. G., L. M. Musalia, J. M. Munguti and A. Magana (2022). "Carcass fatty acid composition and sensory properties of Nile tilapia (*Oreochromis niloticus*) fed on oilseed meals with crude papain enzyme." East African Journal of Science, Technology Innovation 3(4).

Koca, Y. B., S. Koca, Ş. Yıldız, B. Gürcü, E. Osanç, O. Tunçbaş and G. Aksoy (2005). "Investigation of histopathological and cytogenetic effects on Lepomis gibbosus (Pisces: Perciformes) in the Çine stream (Aydın/Turkey) with determination of water pollution." Environmental Toxicology: An International Journal 20(6): 560-571.

Kontas, A., H. Alyuruk, M. Bilgin, E. Uluturhan, A. Ünlüoğlu, E. Darilmaz and O. Altay (2022). "Metal Bioaccumulation and Potential Health Risk Assessment in Different Tissues of Three Commercial Fish Species (*Merluccius merluccius, Mullus barbatus,* and *Pagellus*

erythrinus) from Edremit Bay (Aegean Sea), Turkey." Biological Trace Element Research: 1-13.

Korhonen, J., A. Honkasalo and J. Seppälä (2018). "Circular economy: the concept and its limitations." Ecological economics 143: 37-46.

Krishnan, P. (2006). "The scientific study of herbal wound healing therapies: Current state of play." Current Anaesthesia Critical Care 17(1-2): 21-27.

Kubiriza, G., A. Akol, J. Arnason, Ó. Sigurgeirsson, S. Snorrason, T. Tómasson and H. Thorarensen (2018). "Practical feeds for juvenile Nile tilapia (*Oreochromis niloticus*) prepared by replacing Rastrineobola argentea fishmeal with freshwater shrimp (*Caridina nilotica*) and mung bean (*Vigna radiata*) meals." Aquaculture Nutrition 24(1): 94-101.

Kubrak, O. I., V. V. Husak, B. M. Rovenko, J. M. Storey, K. B. Storey and V. I. Lushchak (2011). "Cobalt-induced oxidative stress in brain, liver and kidney of goldfish *Carassius auratus*." Chemosphere 85(6): 983-989.

Kubrak, O. I., B. M. Rovenko, V. V. Husak, O. Y. Vasylkiv, K. B. Storey, J. M. Storey and V. I. Lushchak (2012). "Goldfish exposure to cobalt enhances hemoglobin level and triggers tissue-specific elevation of antioxidant defenses in gills, heart and spleen." Comparative Biochemistry Physiology Part C: Toxicology Pharmacology 155(2): 325-332.

Küçükbay, F., H. Yazlak, I. Karaca, N. Sahin, M. Tuzcu, M. Cakmak and K. Sahin (2009). "The effects of dietary organic or inorganic selenium in rainbow trout (*Oncorhynchus mykiss*) under crowding conditions." Aquaculture Nutrition 15(6): 569-576.

Kumar, N., K. Krishnani, M. Brahmane, S. Gupta, P. Kumar and N. Singh (2018). "Temperature induces lead toxicity in Pangasius hypophthalmus: an acute test, antioxidative status and cellular metabolic stress." International journal of environmental science technology 15: 57-68.

Kumar, N., K. Krishnani, S. K. Gupta and N. P. Singh (2017). "Selenium nanoparticles enhanced thermal tolerance and maintain cellular stress protection of Pangasius hypophthalmus reared under lead and high temperature." Respiratory physiology neurobiology 246: 107-116.

Kumar, N., K. Krishnani, P. Kumar, R. Sharma, R. Baitha, D. K. Singh and N. P. Singh (2018). "Dietary nano-silver: Does support or discourage thermal tolerance and biochemical status

in air-breathing fish reared under multiple stressors?" Journal of thermal biology 77: 111-121.

Kumar, N., K. Krishnani, K. Meena, S. K. Gupta and N. Singh (2017). "Oxidative and cellular metabolic stress of *Oreochromis mossambicus* as biomarkers indicators of trace element contaminants." Chemosphere 171: 265-274.

Kuo, J.-M., A. Hwang, D.-B. Yeh, M.-H. Pan, M.-L. Tsai and B. S. Pan (2006). "Lipoxygenase from banana leaf: purification and characterization of an enzyme that catalyzes linoleic acid oxygenation at the 9-position." Journal of agricultural food chemistry 54(8): 3151-3156.

Kuyu, C. G. and Y. B. Tola (2018). "Assessment of banana fruit handling practices and associated fungal pathogens in Jimma town market, southwest Ethiopia." Food science nutrition utilization technology in aquaculture. 6(3): 609-616.

Lall, S. (2002). "The minerals. Fish nutrition." Elsevier Academic Press, San Diego, CA, USA: 259-308.

Lamas, S., J. Fernández, J. Aboal and A. Carballeira (2007). "Testing the use of juvenile *Salmo trutta* L. as biomonitors of heavy metal pollution in freshwater." Chemosphere 67(2): 221-228.

Langmead, L., R. M. Feakins, S. Goldthorpe, H. Holt, E. Tsironi, A. De Silva, D. P. Jewell and D. S. Rampton (2004). "Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active *ulcerative colitis*." Alimentary pharmacology therapeutics 19(7): 739-747.

Lau, B. F., K. W. Kong, K. H. Leong, J. Sun, X. He, Z. Wang, M. R. Mustafa, T. C. Ling and A. Ismail (2020). "Banana inflorescence: Its bio-prospects as an ingredient for functional foods." Trends in Food Science Technology 97: 14-28.

Lee, J., J. R. Prohaska and D. J. Thiele (2001). "Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development." Proceedings of the National Academy of Sciences 98(12): 6842-6847.

Lee, K. Y., S. T. Weintraub and B. P. Yu (2000). "Isolation and identification of a phenolic antioxidant from Aloe barbadensis." Free radical biology medicine 28(2): 261-265.

Lee, S., R. W. Nambi, S. Won, K. Katya and S. C. Bai (2016). "Dietary selenium requirement and toxicity levels in juvenile Nile tilapia, *Oreochromis niloticus*." Aquaculture 464: 153-158.

Lemly, A. D. (1993). "Metabolic stress during winter increases the toxicity of selenium to fish." Aquatic Toxicology 27(1-2): 133-158.

Li, J., Z. Y. Huang, Y. Hu and H. Yang (2013). "Potential risk assessment of heavy metals by consuming shellfish collected from Xiamen, China." Environmental Science Pollution Research 20: 2937-2947.

Li, J., Z. Y. Huang, Y. Hu and H. Yang (2013). "Potential risk assessment of heavy metals by consuming shellfish collected from Xiamen, China." Environmental Science Pollution Research 20: 2937-2947.

Li, J., Z. Y. Huang, Y. Hu, H. J. E. S. Yang and P. Research (2013). "Potential risk assessment of heavy metals by consuming shellfish collected from Xiamen, China." 20: 2937-2947.

Li, M., X. Wang, C. Qi, E. Li, Z. Du, J. G. Qin and L. Chen (2018). "Metabolic response of Nile tilapia (*Oreochromis niloticus*) to acute and chronic hypoxia stress." Aquaculture 495: 187-195.

Li, M. R. and C. H. Huang (2016). "Effect of dietary zinc level on growth, enzyme activity and body trace elements of hybrid tilapia, *Oreochromis niloticus*× *O. aureus*, fed soya bean meal-based diets." Aquaculture Nutrition 22(6): 1320-1327.

Liang, J. J., S. Wang, B. Han, L. X. Tian, H. J. Yang and Y. J. Liu (2015). "Dietary manganese requirement of juvenile grass carp (C *tenopharyngodon idella* V al.) based on growth and tissue manganese concentration." Aquaculture Research 46(12): 2991-2998.

Lin, Y.-H. and S.-Y. Shiau (2007). "The effects of dietary selenium on the oxidative stress of grouper, Epinephelus malabaricus, fed high copper." Aquaculture 267(1-4): 38-43.

Linnik, P. M. and I. B. Zubenko (2000). "Role of bottom sediments in the secondary pollution of aquatic environments by heavy-metal compounds." Lake Reservoirs: Research Management 5(1): 11-21.

Liu, M., Y. Xu, J. Nawab, Z. Rahman, S. Khan, M. Idress, Z. Ud din, A. Ali, R. Ahmad, S. A. Khan, A. Khan, M. Q. Khan, Y.-T. Tang and G. Li (2020). "Contamination features, geo-accumulation, enrichments and human health risks of toxic heavy metal(loids) from fish consumption collected along Swat river, Pakistan." Environmental Technology & Innovation 17: 100554.

Lopes, M. S., I. El-Basyoni, P. S. Baenziger, S. Singh, C. Royo, K. Ozbek, H. Aktas, E. Ozer, F. Ozdemir and A. Manickavelu (2015). "Exploiting genetic diversity from landraces in

wheat breeding for adaptation to climate change." Journal of experimental botany 66(12): 3477-3486.

López-Cervantes, J., D. I. Sánchez-Machado, P. Cruz-Flores, M. F. Mariscal-Domínguez, G. S. de la Mora-López and O. N. Campas-Baypoli (2018). "Antioxidant capacity, proximate composition, and lipid constituents of Aloe vera flowers." Journal of Applied Research on Medicinal Aromatic Plants 10: 93-98.

Lu, C., X. Luo, R. Luo, X. Chen, L. Xing, Z. Tang and H. Li (2013). "Assessment of antibacterial properties and the active ingredient of plant extracts and its effect on the performance of crucian carp (*Carassius auratus* gibelio var. E'erqisi, Bloch)." Journal of the Science of Food Agriculture 93(4): 902-909.

Lucas, J. S., P. C. Southgate and C. S. Tucker (2019). Aquaculture: Farming aquatic animals and plants, John Wiley & Sons.

Lumsangkul, C., W. Tapingkae, K. Sringarm, S. Jaturasitha, C. Le Xuan, S. Wannavijit, P. Outama and H. Van Doan (2021). "Effect of dietary sugarcane bagasse supplementation on growth performance, immune response, and immune and antioxidant-related gene expressions of Nile tilapia (*Oreochromis niloticus*) cultured under Biofloc system." Animals 11(7): 2035.

Luna, L. G. (1968). "Manual of histologic staining methods of the Armed Forces Institute of Pathology."

Lund, I., J. Dalsgaard, H. T. Rasmussen, J. Holm and A. Jokumsen (2011). "Replacement of fish meal with a matrix of organic plant proteins in organic trout (*Oncorhynchus mykiss*) feed, and the effects on nutrient utilization and fish performance." Aquaculture 321(3-4): 259-266.

Lundstedt, L., J. F. B. Melo and G. Moraes (2004). "Digestive enzymes and metabolic profile of Pseudoplatystoma corruscans (Teleostei: Siluriformes) in response to diet composition." Comparative Biochemistry Physiology Part B: Biochemistry Molecular Biology 137(3): 331-339.

Luoma, S. N. and P. S. Rainbow (2005). "Why is metal bioaccumulation so variable? Biodynamics as a unifying concept." Environmental Science Technology 39(7): 1921-1931.

MacArthur, E. (2013). "Towards the circular economy." Journal of Industrial Ecology 2(1): 23-44.

MacFarlane, G. and M. Burchett (2000). "Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh." Aquatic botany 68(1): 45-59.

Mahdavi, M., A. Hajimoradloo and R. Ghorbani (2013). "Effect of Aloe vera extract on growth parameters of common carp (*Cyprinus carpio*)." World Journal of Medical Sciences 9(1): 55-60.

Maity, S., S. Roy, S. Chaudhury and S. Bhattacharya (2008). "Antioxidant responses of the earthworm *Lampito mauritii* exposed to Pb and Zn contaminated soil." Environmental Pollution 151(1): 1-7.

Makkar, H. (1993). "Antinutritional factors in foods for livestock." BSAP Occasional Publication 16: 69-85.

Makwinja, R. and A. Geremew (2020). "Roles and requirements of trace elements in tilapia nutrition: Review." The Egyptian Journal of Aquatic Research 46(3): 281-287.

Manaf, S. R., H. Daud, A. Alimon, N. Mustapha, R. Hamdan, K. Muniand, N. Mohamed, R. Razak and N. Hamid (2016). "The effects of Vitex trifolia, Strobilanthes crispus and Aloe vera herbal-mixed dietary supplementation on growth performance and disease resistance in red hybrid Tilapia (*Oreochromis* sp.)." Journal of Aquaculture Research Development 7(4): 2.

Mapanao, R., T. Rangabpai, Y. R. Lee, H. W. Kuo and W. Cheng (2022). "The effect of banana blossom on growth performance, immune response, disease resistance, and anti-hypothermal stress of Macrobrachium rosenbergii." Fish Shellfish Immunol 124: 82-91.

Marcos, S. V., M. J. Rubio, F. R. Sanchidrián and D. de Robledo (2016). "Spanish National dietary survey in adults, elderly and pregnant women." EFSA Supporting Publications 13(6): 1053E.

Marengo, M., E. D. Durieux, S. Ternengo, P. Lejeune, E. Degrange, V. Pasqualini and S. Gobert (2018). "Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health." Ecotoxicology environmental safety 158: 204-212.

Marshall, F., J. Holden, C. Ghose, B. Chisala, E. Kapungwe, J. Volk, M. Agrawal, R. Agrawal, R. Sharma and R. r. Singh (2007). "Contaminated irrigation water and food safety for the urban and peri-urban poor: appropriate measures for monitoring and control from field research in India and Zambia." Incpetion Report DFID Enka 8160(3).

Martin, R. E., E. P. Carter and L. M. Davis (2000). Marine and freshwater products handbook, CRC Press.

Martoja, R. and M. Martoja-Pierson (1970). "Histoquímica." Técnicas de histología animal: 156-184.

MASKE, N. S. and S. SATYANARAYAN (2012). "Effect of special fish feed prepared using potato peels on fresh water fish labeo-rohita." I Control Pollution 29(1).

Masoudi, A., M. Bojarpour, M. Chaji, M. Eslami and K. Mirzadeh (2010). "Economic value of date Pits replaced with maize in broiler chicken diet." Journal of Animal Veterinary Advances 9(11): 1578-1581.

Mathiesen, Á. M. (2015). The state of world fisheries and aquaculture 2012, Food and Agriculture Organization of the United Nations.

Mechora, Š., V. Stibilj and M. Germ (2013). "The uptake and distribution of selenium in three aquatic plants grown in Se (IV) solution." Aquatic toxicology 128: 53-59.

Medina-Villacis, M., E.-G. Italo, R. M. García-Gimeno and G. Posada-Izquierdo (2022). "Inclusion Of Banana (*Musa Paradisiaca*) Meal In The Diet On The Productive Parameters Of (*Oreochromis* Spp)." Journal of Pharmaceutical Negative Results: 1062-1071.

Mendez, R. (2003). "Banana meal tested as food ingredient for Nile Tilapia fingerlings Globab." Aquaculture Advocate 4: 70-71.

Mengistu, S. B., H. A. Mulder, J. A. Benzie and H. Komen (2020). "A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*)." Reviews in Aquaculture 12(2): 524-541.

Metian, M., M. Warnau, T. Chouvelon, F. Pedraza, A. M. R. y Baena and P. Bustamante (2013). "Trace element bioaccumulation in reef fish from New Caledonia: influence of trophic groups and risk assessment for consumers." Marine Environmental Research 87: 26-36.

Meyers, L. D., J. P. Hellwig and J. J. Otten (2006). Dietary reference intakes: the essential guide to nutrient requirements, National Academies Press.

Milošković, A., B. Dojčinović, S. Kovačević, N. Radojković, M. Radenković, D. Milošević and V. Simić (2016). "Spatial monitoring of heavy metals in the inland waters of Serbia: a multispecies approach based on commercial fish." Environmental Science Pollution Research 23: 9918-9933.

Mmanda, F. P., D. P. Mulokozi, J. E. Lindberg, A. Norman Haldén, M. Mtolera, R. Kitula and T. Lundh (2020). "Fish farming in Tanzania: the availability and nutritive value of local feed ingredients." Journal of Applied Aquaculture 32(4): 341-360.

Mohammadi, M., A. Imani, M. Farhangi, A. Gharaei and M. Hafezieh (2020). "Replacement of fishmeal with processed canola meal in diets for juvenile Nile tilapia (*Oreochromis niloticus*): Growth performance, mucosal innate immunity, hepatic oxidative status, liver and intestine histology." Aquaculture 518: 734824.

Mokbel, M. S. and F. Hashinaga (2005). "Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel." American journal of Biochemistry Biotechnology 1(3): 125-131.

Murray, A., K. Skene and K. Haynes (2017). "The circular economy: an interdisciplinary exploration of the concept and application in a global context." Journal of business ethics 140: 369-380.

Muscio, A. and R. Sisto (2020). "Are agri-food systems really switching to a circular economy model? Implications for European research and innovation policy." Sustainability 12(14): 5554.

Mustafa, S. (2022). Sustainable feed for aquaculture. Frontiers in Sustainable Food Systems, Frontiers Media SA. 6: 1006824.

Mzengereza, K., O. Msiska, F. Kapute, J. Kang'ombe, W. Singini and A. Kamangira (2014). "Nutritional value of locally available plants with potential for diets of Tilapia Rendalli in pond aquaculture in NkhataBay, Malawi."

Nadal, M., N. Ferré-Huguet, R. Martí-Cid, M. Schuhmacher and J. L. Domingo (2008). "Exposure to metals through the consumption of fish and seafood by the population living near the Ebro River in Catalonia, Spain: health risks." Human ecological risk assessment 14(4): 780-795.

Naylor, R., R. Goldburg, J. Primavera, N. Kautsky, M. Beveridge, J. Clay, C. Folke, J. Lubchenco, H. Mooney and M. Troell (2001). "Effects of Aquaculture on World Fish Supplies, Issues in Ecology No. 8." The Ecological Society of America, Washington, DC, 12pp.

Naylor, R. L., R. W. Hardy, D. P. Bureau, A. Chiu, M. Elliott, A. P. Farrell, I. Forster, D. M. Gatlin, R. J. Goldburg and K. Hua (2009). "Feeding aquaculture in an era of finite resources." Proceedings of the National Academy of Sciences 106(36): 15103-15110.

Nelson, D. L., A. L. Lehninger and M. M. Cox (2008). Lehninger principles of biochemistry, Macmillan.

Nelson, G. C., M. W. Rosegrant, J. Koo, R. Robertson, T. Sulser, T. Zhu, C. Ringler, S. Msangi, A. Palazzo and M. Batka (2009). Climate change: Impact on agriculture and costs of adaptation, Intl Food Policy Res Inst.

Nemova, N. N., Z. A. Nefedova, S. N. Pekkoeva, V. P. Voronin, N. S. Shulgina, M. V. Churova and S. A. Murzina (2020). "The Effect of the Photoperiod on the Fatty Acid Profile and Weight in Hatchery-Reared Underyearlings and Yearlings of Atlantic Salmon Salmo salar L." Biomolecules 10(6): 845.

Newton, B. W., T. D. Prowse and B. R. Bonsal (2014). "Evaluating the distribution of water resources in western Canada using synoptic climatology and selected teleconnections. Part 2: Summer season." Hydrological Processes 28(14): 4235-4249.

Ngugi, C. C., E. Oyoo-Okoth, J. O. Manyala, K. Fitzsimmons and A. Kimotho (2017). "Characterization of the nutritional quality of amaranth leaf protein concentrates and suitability of fish meal replacement in Nile tilapia feeds." Aquaculture Reports 5: 62-69.

Nguyen, H. T., G. S. Hilmarsdóttir, T. Tómasson, S. Arason and M. Gudjónsdóttir (2022) "Changes in Protein and Non-Protein Nitrogen Compounds during Fishmeal Processing—Identification of Unoptimized Processing Steps." Processes 10 DOI: 10.3390/pr10040621.

Noël, L., R. Chekri, S. Millour, M. Merlo, J.-C. Leblanc and T. Guérin (2013). "Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas." Chemosphere 90(6): 1900-1910.

Nriagu, J. O. (1979). "Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere." Nature 279(5712): 409-411.

Obirikorang, K. A., S. Amisah, S. C. Fialor and P. V. Skov (2015). "Local agro-industrial by-products with potential use in Ghanaian aquaculture: a review." Aquaculture International 23: 403-425.

Ogunji, J. (2004). Alternative protein sources in diets for farmed tilapia. Nutrition Abstracts and Reviews: Series B: Livestock Feeds and Feeding, CABI Wallingford UK.

Okolie, J. A., O. E. Henry and E. I. Epelle (2016). "Determination of the antioxidant potentials of two different varieties of banana peels in two different solvents." Food Nutrition Sciences 7(13): 1253.

Oliveira, L., N. Cordeiro, D. Evtuguin, I. Torres and A. Silvestre (2007). "Chemical composition of different morphological parts from 'Dwarf Cavendish'banana plant and their potential as a non-wood renewable source of natural products." Industrial Crops Products 26(2): 163-172.

Olivera-Castillo, L., M. Pino-Aguilar, M. Lara-Flores, S. Granados-Puerto, J. Montero-Muñoz, M. Olvera-Novoa and G. Grant (2011). "Substitution of fish meal with raw or treated cowpea (Vigna unguiculata L Walp, IT86-D719) meal in diets for Nile tilapia (*Oreochromis niloticus* L.) fry." Aquaculture Nutrition 17(2): e101-e111.

Olmedo, P., A. Hernández, A. Pla, P. Femia, A. Navas-Acien and F. Gil (2013). "Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury–selenium balance." Food chemical toxicology 62: 299-307.

Olmedo, P., A. Hernández, A. Pla, P. Femia, A. Navas-Acien, F. J. F. Gil and c. toxicology (2013). "Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury–selenium balance." 62: 299-307.

Omoregie, E., L. Igoche, T. Ojobe, K. Absalom, B. J. A. J. o. F. Onusiriuka, Agriculture, Nutrition and Development (2009). "Effect of varying levels of sweet potato (I*pomea Batatas*) peels on growth, feed utilization and some biochemical responses of the cichlid (*Oreochromis Niloticus*)." 9(2): 700-712.

Onsanit, S., C. Ke, X. Wang, K.-J. Wang and W.-X. Wang (2010). "Trace elements in two marine fish cultured in fish cages in Fujian province, China." Environmental Pollution 158(5): 1334-1342.

Opiyo, M. A., P. Muendo, K. Mbogo, C. C. Ngugi, H. Charo-Karisa, P. Orina, W. Leschen, B. D. Glencross and D. R. Tocher (2022). "Inclusion of duckweed (*Lemna minor*) in the diet improves flesh omega-3 long-chain polyunsaturated fatty acid profiles but not the growth of farmed Nile tilapia (*Oreochromis niloticus*)." Animal Feed Science Technology 292: 115442.

Oronsaye, J., O. Wangboje and F. Oguzie (2010). "Trace metals in some benthic fishes of the Ikpoba river dam, Benin City, Nigeria." African Journal of Biotechnology 9(51): 8860-8864.

Osman, A. (2012). "Biomarkers in Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758) to Assess the Impacts of River Nile Pollution: Bioaccumulation, Biochemical and Tissues Biomarkers." Journal of Environmental Protection 03.

Ounsaneha, W., C. Rattanapan, T. T. Suksaroj, D. Kantachote, W. Klawech and T. Rakkamon (2021). "Biogas production by co-digestion of municipal wastewater and food waste: Performance in semi-continuous and continuous operation." Water Environment Research 93(2): 306-315.

Ovando-Martinez, M., S. Sáyago-Ayerdi, E. Agama-Acevedo, I. Goñi and L. A. Bello-Pérez (2009). "Unripe banana flour as an ingredient to increase the undigestible carbohydrates of pasta." Food Chemistry 113(1): 121-126.

Pacitti, D., M. Lawan, J. Feldmann, J. Sweetman, T. Wang, S. Martin and C. Secombes (2016). "Impact of selenium supplementation on fish antiviral responses: a whole transcriptomic analysis in rainbow trout (*Oncorhynchus mykiss*) fed supranutritional levels of Sel-Plex®." BMC genomics 17(1): 1-26.

Padam, B., H. Tin, F. Chye and M. Abdullah (2012). "Antibacterial and Antioxidative Activities of the Various Solvent Extracts of Banana(*Musa paradisiaca* cv. Mysore) Inflorescences." Journal of Biological sciences 12(2): 62-73.

Padam, B. S., H. S. Tin, F. Y. Chye and M. I. Abdullah (2014). "Banana by-products: an under-utilized renewable food biomass with great potential." J Food Sci Technol 51(12): 3527-3545.

Padam, B. S., H. S. Tin, F. Y. Chye, M. I. J. J. o. f. s. Abdullah and technology (2014). "Banana by-products: an under-utilized renewable food biomass with great potential." 51: 3527-3545.

Palermo, F. A., P. Cocci, M. Angeletti, A. Felici, A. M. Polzonetti-Magni and G. Mosconi (2013). "Dietary Aloe vera components' effects on cholesterol lowering and estrogenic responses in juvenile goldfish, *Carassius auratus*." Fish physiology biochemistry 39: 851-861.

Palintorn, N., N. Rujinanont, A. Srisathaporn, S. Gawborisut and P. Wongkaew (2019). "Effects of dietary supplemented with flesh ripe fruit of local cultivated banana CV. Kluai Namwa on growth performance and meat quality of Nile tilapia." Aquaculture, Aquarium, Conservation Legislation 12(5): 1578-1591.

Patterson, M. M., G. B. Paige and K. J. Reddy (2010). "Selenium in surface and irrigation water in the Kendrick irrigation district, Wyoming." Environmental monitoring assessment 171: 267-280.

Paul, M., A. Barman, M. Siddque, A. Bhadra and M. Rahman (2013). "Effect of use of duckweed powder as a fish feed on polyculture of carps." Journal of Environmental Science Natural Resources 6(2): 213-216.

Pauly, D., V. Christensen, J. Dalsgaard, R. Froese and F. Torres Jr (1998). "Fishing down marine food webs." Science 279(5352): 860-863.

Paz, R., M. Monzón, G. Vega, N. Díaz and D. Pestana (2020). Use of Banana Crop Wastes to Develop Products in the Aquaculture and Plastic Sectors. Agricultural, Forestry and Bioindustry Biotechnology and Biodiscovery. P. A. Chong, D. J. Newman and D. A. Steinmacher. Cham, Springer International Publishing: 259-270.

Penglase, S., K. Hamre and S. Ellingsen (2014). "Selenium prevents downregulation of antioxidant selenoprotein genes by methylmercury." Free Radical Biology Medicine 75: 95-104.

Pereira, A. and M. Maraschin (2015). "Banana (Musa spp) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health." Journal of ethnopharmacology 160: 149-163.

Peterson, S. A., N. V. Ralston, D. V. Peck, J. V. Sickle, J. D. Robertson, V. L. Spate and J. S. Morris (2009). "How might selenium moderate the toxic effects of mercury in stream fish of the western US?" Environmental science technology 43(10): 3919-3925.

Prato, E., F. Biandolino, I. Parlapiano, S. Giandomenico, G. Denti, M. Calò, L. Spada and A. Di Leo (2019). "Proximate, fatty acids and metals in edible marine bivalves from Italian market: Beneficial and risk for consumers health." Science of the total environment 648: 153-163.

Preston, F. (2012). "A global redesign? Shaping the circular economy."

Psoma, A., I. Pasias, N. Rousis, K. Barkonikos and N. Thomaidis (2014). "Development, validation and accreditation of a method for the determination of Pb, Cd, Cu and As in seafood and fish feed samples." Food chemistry 151: 72-78.

Pullin, R. (1984). "Tilapia-potentially an international food commodity." Infofish Marketing Digest 3: 35-36.

Pyle, G. G., J. W. Rajotte and P. Couture (2005). "Effects of industrial metals on wild fish populations along a metal contamination gradient." Ecotoxicology Environmental Safety 61(3): 287-312.

Quirós-Pozo, R., A. Ventura-Castellano, S. Ramírez-Bolaños, J. Roo-Filgueira and L. Robaina (2021). "Evaluation of Aloe vera by-product against cereals in feeds for golden mullet (*Liza aurata*)." Aquaculture Reports 20: 100659.

Rabia, S. H., O. P. Luzardo, R. Pozo, M. Abbassy, M. Zumbado, I. Elalfy, L. Robaina and R. Ginés (2022). "Determination of heavy metals from Aloe vera by-product in golden mullet (*Liza aurata*); A consumer health risk assessment." Food Chemical Toxicology 169: 113418.

Rai, A. N., A. Ullah and J. Haider (2015). "Determination of acute toxicity of copper and cobalt for Tilapia nilotica." Journal of Bioresource Management 2(1): 3.

Ralston, N. V., C. R. Ralston, J. L. Blackwell III and L. J. Raymond (2008). "Dietary and tissue selenium in relation to methylmercury toxicity." Neurotoxicology 29(5): 802-811.

Ralston, N. V., C. R. Ralston and L. J. Raymond (2016). "Selenium health benefit values: updated criteria for mercury risk assessments." Biological Trace Element Research 171: 262-269.

Ramesh, M., M. Sankaran, V. Veera-Gowtham and R. K. Poopal (2014). "Hematological, biochemical and enzymological responses in an Indian major carp *Labeo rohita* induced by sublethal concentration of waterborne selenite exposure." Chemico-biological interactions 207: 67-73.

Ramírez-Bolaños, S., J. Pérez-Jiménez, S. Díaz and L. Robaina (2021). "A potential of banana flower and pseudo-stem as novel ingredients rich in phenolic compounds." International Journal of Food Science Technology 56(11): 5601-5608.

Ramos-Miras, J., M. Sanchez-Muros, E. Morote, M. Torrijos, C. Gil, R. Zamani-Ahmadmahmoodi and J. R. Martin (2019). "Potentially toxic elements in commonly consumed fish species from the western Mediterranean Sea (Almería Bay): bioaccumulation in liver and muscle tissues in relation to biometric parameters." Science of the Total Environment 671: 280-287.

Ramu, R., P. S. Shirahatti, K. Anilakumar, S. Nayakavadi, F. Zameer, B. Dhananjaya and M. N. Prasad (2017). "Assessment of nutritional quality and global antioxidant response of banana (Musa sp. CV. Nanjangud Rasa Bale) pseudostem and flower." Pharmacognosy research 9(Suppl 1): S74.

Rankins, D. L. and D. G. Pugh (2012). Chapter 2 - Feeding and Nutrition. Sheep and Goat Medicine (Second Edition). D. G. Pugh and A. N. Baird. Saint Louis, W.B. Saunders: 18-49.

Rattanapan, C. and W. Ounsaneha (2020). "Environmental impact assessment of Thai banana supply chain."

Reinardy, H. C., J. R. Syrett, R. A. Jeffree, T. B. Henry and A. N. Jha (2013). "Cobalt-induced genotoxicity in male zebrafish (*Danio rerio*), with implications for reproduction and expression of DNA repair genes." Aquatic toxicology 126: 224-230.

Rekleitis, G., K.-J. Haralambous, M. Loizidou and K. Aravossis (2020). "Utilization of agricultural and livestock waste in anaerobic digestion (AD): Applying the biorefinery concept in a circular economy." Energies 13(17): 4428.

Renieri, E. A., A. K. Alegakis, M. Kiriakakis, M. Vinceti, E. Ozcagli, M. F. Wilks and A. M. Tsatsakis (2014). "Cd, Pb and Hg biomonitoring in fish of the Mediterranean region and risk estimations on fish consumption." Toxics 2(3): 417-442.

Resma, N. S., A. K. M. M. H. Meaze, S. Hossain, M. U. Khandaker, M. Kamal and N. Deb (2020). "The presence of toxic metals in popular farmed fish species and estimation of health risks through their consumption." Physics Open 5: 100052.

Reverter, M., N. Tapissier-Bontemps, P. Sasal and D. Saulnier (2017). "Use of medicinal plants in aquaculture." Diagnosis control of diseases of fish shellfish: 223-261.

Rider, S. A., S. J. Davies, A. N. Jha, A. A. Fisher, J. Knight and J. W. Sweetman (2009). "Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (*Oncorhynchus mykiss*): implications on selenium status and health responses." Aquaculture 295(3-4): 282-291.

Rodríguez-Hernández, Á., M. Camacho, L. A. Henríquez-Hernández, L. D. Boada, N. Ruiz-Suárez, P. F. Valerón, M. A. González, A. Zaccaroni, M. Zumbado and O. P. Luzardo (2016). "Assessment of human health hazards associated with the dietary exposure to organic and inorganic contaminants through the consumption of fishery products in Spain." Science of the Total Environment 557: 808-818.

Roohani, N., R. Hurrell, R. Kelishadi and R. Schulin (2013). "Zinc and its importance for human health: An integrative review." J Res Med Sci 18(2): 144-157.

Rosegrant, M. W., M. S. Paisner, S. Meijer and J. Witcover (2001). 2020 Global food outlook: Trends, alternatives, and choices, Intl Food Policy Res Inst.

Roselló-Soto, E., C. M. Galanakis, M. Brnčić, V. Orlien, F. J. Trujillo, R. Mawson, K. Knoerzer, B. K. Tiwari and F. J. Barba (2015). "Clean recovery of antioxidant compounds from plant foods, by-products and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions." Trends in Food Science Technology 42(2): 134-149.

Sadiku, S. and A. Orire (2020). "Protein sparing of Carbohydrate in Catfish diet."

Saeedi Saravi, S., S. Karami, B. Karami and M. Shokrzadeh (2009). "Toxic effects of cobalt chloride on hematological factors of common carp (*Cyprinus carpio*)." Biological trace element research 132: 144-152.

Sagar, N. A., S. Pareek, S. Sharma, E. M. Yahia and M. G. Lobo (2018). "Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization." Comprehensive reviews in food science food safety 17(3): 512-531.

Saljooghianpour, M. and T. A. Javaran (2013). "Identification of phytochemical components of aloe plantlets by gas chromatography-mass spectrometry." African Journal of Biotechnology 12(49): 6876-6880.

Saravanan, K. and S. M. Aradhya (2011). "Potential nutraceutical food beverage with antioxidant properties from banana plant bio-waste (pseudostem and rhizome)." Food Function 2(10): 603-610.

Sargent, C. and M. Harris (1992). "Gender ideology, childrearing, and child health in Jamaica." American Ethnologist 19(3): 523-537.

Sarkar, B., S. Bhattacharjee, A. Daware, P. Tribedi, K. Krishnani and P. Minhas (2015). "Selenium nanoparticles for stress-resilient fish and livestock." Nanoscale research letters 10: 1-14.

Satarug, S., S. H. Garrett, M. A. Sens and D. A. Sens (2010). "Cadmium, environmental exposure, and health outcomes." Environmental health perspectives 118(2): 182-190.

Schmidt, M. M., R. C. Prestes, E. H. Kubota, G. Scapin and M. A. Mazutti (2015). "Evaluation of antioxidant activity of extracts of banana inflorescences (*Musa cavendishii*)." CyTA-Journal of Food 13(4): 498-505.

Schneider, C. A., W. S. Rasband and K. W. Eliceiri (2012). "NIH Image to ImageJ: 25 years of image analysis." Nature methods 9(7): 671-675.

Sen, I., A. Shandil and V. Shrivastava (2011). "Study for determination of heavy metals in fish species of the River Yamuna (Delhi) by inductively coupled plasma-optical emission spectroscopy (ICP-OES)." Advances in Applied Science Research 2(2): 161-166.

Seymore, T. (2014). Bioaccumulation of Metals in *Barbus Marequensis* from the Olifants River, Kruger National Park and Lethal Levels of Manganese to Juvenile *Oreochromis Mossambicus*, University of Johannesburg (South Africa).

ShaAto, R., I. S. Eneji and P. Annune (2011). "Bioaccumulation of Heavy Metals in Fish (*Tilapia Zilli* and *Clarias Gariepinus*) Organs from River Benue, North Central Nigeria." Pakistan Journal of Analytical Environmental Chemistry 12(1 & 2): 7.

Sharma, S., A. Kaushik, C. Kaushik and A. Sharma (2007). Impacts of Water Resources Development Project with Special Reference to Dams and Reservoirs. International Conference on Small Hydropower-Hydro Sri Lanka, Citeseer.

Shay, N. F. and H. F. Mangian (2000). "Neurobiology of zinc-influenced eating behavior." The Journal of nutrition 130(5): 1493S-1499S.

Shelton, R. M. (1991). "Aloe vera: its chemical and therapeutic properties." International journal of dermatology 30(10): 679-683.

Sheng, Z.-W., W.-H. Ma, Z.-Q. Jin, Y. Bi, Z.-G. Sun, H.-T. Dou, J. Li and L. Han (2010). "Investigation of dietary fiber, protein, vitamin E and other nutritional compounds of banana flower of two cultivars grown in China." African Journal of Biotechnology 9(25): 3888-3895.

Shiau, S.-Y. and L.-W. Su (2003). "Ferric citrate is half as effective as ferrous sulfate in meeting the iron requirement of juvenile tilapia, *Oreochromis niloticus*× O. *aureus*." The Journal of nutrition 133(2): 483-488.

Shukla, V., P. Rathi and K. Sastry (2002). "Effect of cadmium individually and in combination with other metals on the nutritive value of fresh water fish, *Channa punctatus*." Journal of environmental biology 23(2): 105-110.

Singh, B., J. P. Singh, A. Kaur and N. Singh (2016). "Bioactive compounds in banana and their associated health benefits–A review." Food chemistry 206: 1-11.

Singh, K. P., D. Mohan, S. Sinha and R. Dalwani (2004). "Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area." Chemosphere 55(2): 227-255.

Singh, N., D. Kumar and A. P. Sahu (2007). "Arsenic in the environment: effects on human health and possible prevention." Journal of environmental biology 28(2): 359.

Smárason, B. Ö., B. Alriksson and R. Jóhannsson (2019). "Safe and sustainable protein sources from the forest industry–The case of fish feed." Trends in Food Science Technology 84: 12-14.

Sofoulaki, K., I. Kalantzi, A. Machias, S. A. Pergantis and M. Tsapakis (2019). "Metals in sardine and anchovy from Greek coastal areas: Public health risk and nutritional benefits assessment." Food chemical toxicology 123: 113-124.

Souza, E., G. Liebl and C. Maragoni (2014). "Bioethanol from fresh and dried banana plant pseudostem." Chemical engineering transactions 38.

Souza, S. A. d., A. M. d. Souza, R. C. d. Souza, J. F. B. Melo and L. V. O. Vidal (2020). "Mango pulp meal in diets for Nile tilapia (*Oreochromis niloticus*): Digestibility, Growth performance, Biochemical analysis, Digestive enzyme activity, hematological variables." Revista Brasileira de Saúde e Produção Animal 21.

SPC. 2011. SPC Aquaculture Portal ‑ Countries: Fiji Islands. Secretariat of the Pacific Community. [http://www.spc.int/aquaculture/index.php?option=com_countries&view=country&id=5&Itemid=17]

Srivastava, N. and N. Kaushik (2001). Use of fish as bioindicator of aquatic pollution. Proceedings of the International Congress of Chemistry and Environment.

Srivastava, N. and R. Sharma (1996). "Toxicity of Zinc in Fish (*Channa Punctatus* Block) As Influenced by Temperature and pH of Water." Indian Journal of Animal Nutrition 13(2): 87-90.

Stern, B. R. (2010). "Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations." Journal of Toxicology Environmental Health, Part A 73(2-3): 114-127.

Stevens, J. R., R. W. Newton, M. Tlusty and D. C. Little (2018). "The rise of aquaculture by-products: Increasing food production, value, and sustainability through strategic utilisation." Marine Policy 90: 115-124.

Stone, D. A. J. (2003). "Dietary Carbohydrate Utilization by Fish." Reviews in Fisheries Science 11(4): 337-369.

Storelli, M. (2008). "Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs)." Food Chemical Toxicology 46(8): 2782-2788.

Storelli, M., A. Storelli, R. D'addabbo, C. Marano, R. Bruno and G. Marcotrigiano (2005). "Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation." Environmental pollution 135(1): 163-170.

Subotić, S., Ž. Višnjić Jeftić, S. Spasić, A. Hegediš, J. Krpo-Ćetković and M. Lenhardt (2013). "Distribution and accumulation of elements (As, Cu, Fe, Hg, Mn, and Zn) in tissues of fish species from different trophic levels in the Danube River at the confluence with the Sava River (Serbia)." Environmental Science Pollution Research 20: 5309-5317.

Suehs, B. A. and D. M. Gatlin (2022). "Evaluation of a Commercial High-Protein Distiller's Dried Grain with Solubles (HP-DDGS) Product in the Diet of Juvenile Nile Tilapia (*Oreochromis niloticus*)." Aquaculture Nutrition 2022.

Sundaram, S., S. Anjum, P. Dwivedi and G. K. Rai (2011). "Antioxidant activity and protective effect of banana peel against oxidative hemolysis of human erythrocyte at different stages of ripening." Applied biochemistry biotechnology 164: 1192-1206.

Surai, P. F. (2006). Selenium in nutrition and health, Nottingham university press Nottingham.

Surjushe, A., R. Vasani and D. Saple (2008). "Aloe vera: a short review." Indian journal of dermatology 53(4): 163.

Tabrett, S., D. Blyth, N. Bourne and B. Glencross (2012). "Digestibility of Lupinus albus lupin meals in barramundi (*Lates calcarifer*)." Aquaculture 364: 1-5.

Tacon, A. G. and M. Metian (2008). "Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects." Aquaculture 285(1-4): 146-158.

Tacon, A. G. and M. Metian (2013). "Fish matters: importance of aquatic foods in human nutrition and global food supply." Reviews in fisheries Science 21(1): 22-38.

Tadesse, Z. (2010). "Diet composition impacts the fatty acid contents of Nile tilapia, *Oreochromis niloticus* L., in Ethiopian highland lakes." Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen 30(9): 1363-1368.

Takayanagi, K. (2001). "Acute toxicity of waterborne Se (IV), Se (VI), Sb (III), and Sb (V) on red seabream (*Pargus major*)." Bulletin of environmental contamination toxicology 66: 808-813.

Tan, B. and K. Mai (2001). "Zinc methionine and zinc sulfate as sources of dietary zinc for juvenile abalone, *Haliotis discus hannai* Ino." Aquaculture 192(1): 67-84.

Tan, B. K. and J. Vanitha (2004). "Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review." Current medicinal chemistry 11(11): 1423-1430.

Tesfahun, A., M. J. I. J. o. F. Temesgen and A. Studies (2018). "Food and feeding habits of Nile tilapia *Oreochromis niloticus* (L.) in Ethiopian water bodies: A review." 6: 43-47.

Thompson, K. R., L. A. Muzinic, L. S. Engler and C. D. Webster (2005). "Evaluation of practical diets containing different protein levels, with or without fish meal, for juvenile Australian red claw crayfish (*Cherax quadricarinatus*)." Aquaculture 244(1-4): 241-249.

Thu, K., Y. Y. Mon, T. A. Khaing and O. M. Tun (2013). "Study on Phytochemical Properties, Antibacterial Activity and Cytotoxicity of Aloe vera L." International Journal of Biotechnology Bioengineering 7(5): 285-289.

Tiamiyu, L., V. Okomoda and A. Agbo (2015). "Nutritional suitability of Leucaena leaf meal in the diet of Clarias gariepinus." Journal of Fisheries Sciences 9(2): 1-5.

Tibbetts, J. (2001). "Aquaculture: satisfying the global appetite." Environmental health perspectives 109(7): A318-A323.

Tofighy, M. A. and T. Mohammadi (2011). "Adsorption of divalent heavy metal ions from water using carbon nanotube sheets." Journal of hazardous materials 185(1): 140-147.

Toop, T. A., S. Ward, T. Oldfield, M. Hull, M. E. Kirby and M. K. Theodorou (2017). "AgroCycle–developing a circular economy in agriculture." Energy Procedia 123: 76-80.

Tvrzicka, E., L.-S. Kremmyda, B. Stankova and A. Zak (2011). "Fatty acids as biocompounds: their role in human metabolism, health and disease-a review. part 1: classification, dietary sources and biological functions." Biomedical Papers of the Medical Faculty of Palacky University in Olomouc 155(2).

Udo, I. I., O. Etokakpan, S. Ukwo and E. O. Ukpong (2021). "Evaluation of the proximate compositions, dietary fibre and resistant starch contents of selected varieties of banana and plantain." Journal of Advances in Food Science Technology 8(1): 1-9.

Uneyama, C., M. Toda, M. Yamamoto and K. s. Morikawa (2007). "Arsenic in various foods: cumulative data." Food additives contaminant 24(5): 447-534.

USEPA (2012). "Technology Transfer Network - Air Toxics Web Site Arsenic Compounds Fact Sheet." US Environmental Protection Agency(https://www3.epa.gov/airtoxics/hlthef/%0Aarsenic%0A).

USEPA, I. (2011). "Integrated risk information system." Environmental protection agency region I, Washington DC 20460.

USEPA, M. (2005). Guidelines for carcinogen risk assessment. Risk Assessment Forum US Environmental Protection Agency, Washington, DC EPA/630/P-03 F.

USEPA. (2012). Technology Transfer Network - Air Toxics Web Site Arsenic Compounds Fact Sheet. US Environmental Protection Agency. https://www3.epa.gov/airtoxics/hlthef/%0Aarsenic%0A

USEPA. (2014). Regional Screening Level (RSL) Fish Ingestion. May 2014.

USEPA. (2017). Regional screening levels (RSLs) - generic Tables. https://www.epa.gov/risk/regionalscreening-%0Alevels-rsls-generic-tables-november-2017%0A

USEPA/USFDA. (2017). EPA-FDA advice about eating fish and shellfish.

Usero, J., C. Izquierdo, J. Morillo and I. Gracia (2004). "Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain." Environment international 29(7): 949-956.

Uysal, K., Y. Emre and E. Köse (2008). "The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey)." Microchemical journal 90(1): 67-70.

Valko, M., D. Leibfritz, J. Moncol, M. T. Cronin, M. Mazur and J. Telser (2007). "Free radicals and antioxidants in normal physiological functions and human disease." The international journal of biochemistry cell biology 39(1): 44-84.

Van Raaij, M., G. Van den Thillart, G. Vianen, D. Pit, P. Balm and A. Steffens (1996). "Substrate mobilization and hormonal changes in rainbow trout (*Oncorhynchus mykiss*, L.) and common carp (*Cyprinus carpio*, L.) during deep hypoxia and subsequent recovery." Journal of Comparative Physiology B 166: 443-452.

Varol, M., G. K. Kaya and A. Alp (2017). "Heavy metal and arsenic concentrations in rainbow trout (*Oncorhynchus mykiss*) farmed in a dam reservoir on the Firat (Euphrates) River: Risk-based consumption advisories." Science of the Total Environment 599: 1288-1296.

Velasco-Muñoz, J. F., J. A. Aznar-Sánchez, A. Batlles-delaFuente and M. D. Fidelibus (2019). "Rainwater harvesting for agricultural irrigation: An analysis of global research." Water 11(7): 1320.

Viana, L. F., F. Kummrow, C. A. L. Cardoso, N. A. de Lima, B. do Amaral Crispim, A. Barufatti and A. C. Florentino (2023). "Metal bioaccumulation in fish from the Araguari River (*Amazon biome*) and human health risks from fish consumption." Environ Sci Pollut Res Int 30(2): 4111-4122.

Vieira, C., S. Morais, S. Ramos, C. Delerue-Matos and M. B. P. P. Oliveira (2011). "Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: Intra- and inter-specific variability and human health risks for consumption." Food and Chemical Toxicology 49(4): 923-932.

Vilela, C., S. A. Santos, J. J. Villaverde, L. Oliveira, A. Nunes, N. Cordeiro, C. S. Freire and A. J. Silvestre (2014). "Lipophilic phytochemicals from banana fruits of several Musa species." Food chemistry 162: 247-252.

Vuori, K.-M. (1995). Direct and indirect effects of iron on river ecosystems. Annales Zoologici Fennici, JSTOR.

Waldron, K. W. (2009). Handbook of waste management and co-product recovery in food processing, Elsevier.

Wangkahart, E., B. Bruneel, A. Chantiratikul, M. de Jong, N. Pakdeenarong and P. A. Subramani (2022). "Optimum dietary sources and levels of selenium improve growth, antioxidant status, and disease resistance: re-evaluation in a farmed fish species, Nile tilapia (*Oreochromis niloticus*)." Fish & Shellfish Immunology 121: 172-182.

Watanabe, T., V. Kiron and S. Satoh (1997). "Trace minerals in fish nutrition." Aquaculture 151(1-4): 185-207.

Wee, K. L. and S.-S. Wang (1987). "Nutritive value of Leucaena leaf meal in pelleted feed for Nile tilapia." Aquaculture 62(2): 97-108.

Wei, Y., J. Zhang, D. Zhang, T. Tu and L. Luo (2014). "Metal concentrations in various fish organs of different fish species from Poyang Lake, China." Ecotoxicology and Environmental Safety 104: 182-188.

Wheal, M. S., E. DeCourcy-Ireland, J. R. Bogard, S. H. Thilsted and J. C. R. Stangoulis (2016). "Measurement of haem and total iron in fish, shrimp and prawn using ICP-MS: Implications for dietary iron intake calculations." Food Chemistry 201: 222-229.

Wilson, R. (2002). "Protein and amino acids." Fish nutrition: 144-179.

World Health Organization, (2000) Hazardous Chemical in Human and Environmental Health. W.H.O. Geneva. Switzerland.

World Health Organization, (2017). Mercury and health. W.H.O. Geneva. Switzerland. https://www.who.int/news-room/fact-sheets/detail/mercury-and-health

World Health Organization, (2019). Exposure to cadmium: a major public health concern. W.H.O. Geneva. Switzerland.

World Health Organization, (2022). Arsenic. W.H.O. Geneva. Switzerland. https://www.who.int/news-room/fact-sheets/detail/arsenic

World Health Organization, (2022). Lead poisoning. W.H.O. Geneva. Switzerland. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

Wu, Z., F. You, A. Wen, D. Ma and P. Zhang (2016). "Physiological and morphological effects of severe hypoxia, hypoxia and hyperoxia in juvenile turbot (S *cophthalmus maximus* L.)." Aquaculture Research 47(1): 219-227.

Xie, Q., L. Qian, S. Liu, Y. Wang, Y. Zhang and D. Wang (2020). "Assessment of long-term effects from cage culture practices on heavy metal accumulation in sediment and fish." Ecotoxicology and Environmental Safety 194: 110433.

Yildirim, Y., Z. Gonulalan, I. Narin and M. Soylak (2009). "Evaluation of trace heavy metal levels of some fish species sold at retail in Kayseri, Turkey." Environmental monitoring assessment 149: 223-228.

Yildiz, M. (2008). "Mineral composition in fillets of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*): a comparison of cultured and wild fish." Journal of Applied Ichthyology 24(5): 589-594.

Yossa, R., N. Ahmad Fatan, J. Kumari and J. W. Schrama (2022). "Apparent digestibility coefficients of banana peel, cassava peel, cocoa husk, copra waste, and sugarcane bagasse

in the GIFT strain of Nile tilapia (*Oreochromis niloticus*)." Journal of Applied Aquaculture 34(3): 734-754.

Younus, M., S. Iqbal, M. S. Mughal, A. Javid, M. K. Rafique, A. U. Khan, N. Khan and U. Atique (2015). "Effect of selenium incorporated in feed on the hematological profile of *Oreochromis niloticus*." Abstract of Applied Sciences Engineering 4(4): 1-22.

Zanuzzo, F. S., R. E. Sabioni, L. N. F. Montoya, G. Favero and E. C. Urbinati (2017). "Aloe vera enhances the innate immune response of pacu (*Piaractus mesopotamicus*) after transport stress and combined heat killed Aeromonas hydrophila infection." Fish shellfish immunology 65: 198-205.

Zargaran Hoseini, M. and M. Chelemal Dezfulnejad (2018). "The effect of utilization of banana peel powder in dieton the growth, survival and body composition of common carp (*Cyprinus carpio*)." Applied Biology 31(1): 109-120.

Zein, G. N., A. E. el-Bedawey, A. M. el-Sherbiney and F. M. Dawoud (1985). "Studies on fish protein concentrate and fish meal from river Nile bolti fish (*Tilapia nilotica*)." Nahrung 29(5): 523-532.

Zenebe, T., G. Ahlgren, I. B. Gustafsson and M. Boberg (1998). "Fatty acid and lipid content of *Oreochromis niloticus* L. in Ethiopian lakes — dietary effects of phytoplankton." Ecology of Freshwater Fish 7(3): 146-158.

Zhao, D., Z. Mao, Q. Lin, C. Qin and Y. Hong (2017). "Effect of supplementary manganese on growth performance, tissue manganese content, antioxidant activities, and cytosolic manganese superoxide dismutase gene mRNA expression in juvenile Chinese mitten crabs (*Eriocheir sinensis*)." Aquaculture international 25: 893-903.

Zheng, N., Q. Wang, X. Zhang, D. Zheng, Z. Zhang and S. Zhang (2007). "Population health risk due to dietary intake of heavy metals in the industrial area of Huludao city, China." Science of the Total Environment 387(1-3): 96-104.

Zohra, B. S. and A. Habib (2016). "Assessment of heavy metal contamination levels and toxicity in sediments and fishes from the Mediterranean Sea (southern coast of Sfax, Tunisia)." Environmental Science and Pollution Research 23(14): 13954-13963.

Zou, F., C. Tan, B. Zhang, W. Wu and N. Shang (2022) "The Valorization of Banana By-Products: Nutritional Composition, Bioactivities, Applications, and Future Development." Foods 11 DOI: 10.3390/foods11203170.