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Assessing the growth and physiological performance of juvenile tilapia (*Oreochromis niloticus*) with the inclusion of new banana by-products in starter diets

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ABSTRACT

The high worldwide amount of residues derived from the banana harvest is an opportunity to create synergies between the banana industry and aquaculture, from a circular economy perspective. The present work objective is to evaluate the inclusion of banana by-products, such as banana pseudo-stem and banana flower in tilapia feeds, and to explore the extracts from the banana flower on the development and health in tilapia juveniles. Three consecutive feeding trials were performed to evaluate the test by-products inclusions: Experiment I) with 0%, 2.5%, 5%, 15% and 20% of banana pseudo-stem; Experiment II) with 0%, 0.2%, 0.5%, 1% and 3% of banana flower; and Experiment III) with 3% of banana flower against its correspondent organic extract and residue of the extraction. Salinity stress challenges were conducted after the feeding trials in Experiments II and III. From the obtained results, up to 5% of banana pseudo-stem and 3% of banana flower inclusion were suitably regarded the fish growth and the liver health, also, as the essential fatty acids proportion in the muscle, despite the reduction in total lipid percentage. Regarding the stress challenges, the flower and more specific, the remaining residue from its organic extraction, appears to regulate the levels of plasma cortisol and glucose and reduce the oxidation parameters in fish liver and muscle, which may be due to the polyphenols present in both, the whole banana flower and in its organic extraction residue.

1. Introduction

In the next decade, new challenges are emerging as is shown in the UN Sustainable Development Goals, which address the degradation of freshwater ecosystems and declining water availability and the need for sustainable and secure food production, among others (U.N., 2020). It has been reported that aquaculture, through its sustainable development, contributes to most of the UN's SDO through Blue Growth (Bartley, 2022), representing moreover one of the most resilient productive sectors due to the variety of species cultivated and the diverse farming methods (FAO, 2021b).

Among fish species, and according to FAO (2020), tilapia spp. represents the second most-produced in aquaculture worldwide, due to its high adaptation capacity to different ambient, fast growth and low technology dependence under culture in most cases. The production of tilapia is mostly extended in tropical areas worldwide but becoming interesting as emergent species in many other geographical areas due to the increase in consumption in countries such as the USA, Canada and Central Europe (Prabu et al., 2019; FAO, 2020). The adaptability of the species to increasing salinity water enables the adaptation from the fresh aquaculture to brackish or even salt water (El-Leithy et al., 2019), which gives it versatility in dealing with the problem of lack of fresh water. Furthermore, opportunities for using this species in alternative and recirculated production systems, like aquaponics (Kloas et al., 2015), extend tilapias' sustainable production research worldwide. Apart from that, it is well known the capacity of feeding tilapia with low-cost feeds including a high variety of regular vegetable materials like different products from corn, wheat, rice and cassava (Ng and Wee, 1989; Chiayvareesajja et al., 1990; Liti et al., 2005), but also the demonstrated opportunity of using against the global trade ingredients, other locally

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available by-products such as Aloe vera, *Azadirachta indica*, sacha inchi or sweet orange peel in tropical areas (Obaroh and Nzeh, 2014; Acar et al., 2015; Gabriel et al., 2015; Khieokhajonkhet et al., 2021).

Bananas are the most sought-after fruit worldwide (FAO, 2021a). The European Union is the region with more importation of banana fruit, with 90% of the home production developed in the Canary Islands (Spain) and the French West Indias (FAO, 2021a). The harvesting process generates a high amount of residues, almost 80% of the total plant mass (Padam et al., 2014). The male flowers fall off during the development of the fruits and when the banana is harvested, the rest of the plant has to be cut to let the rest of the plantain grow up again (Lau et al., 2020). There is a long tradition of using banana residues as feed for livestock (Marie-Magdeleine et al., 2010; Wadhwa et al., 2015; Wang et al., 2016; Menezes et al., 2019), with also some studies in rats to evaluate the bioactives present in the pseudo-stem and the flower (Bhaskar et al., 2012). On the aquaculture side, a few studies have reported the utilisation of banana by-products, most of them on green bananas discard during packaging (Giri et al., 2016; Palintorn et al., 2019; Felix e Silva et al., 2021; Karaket et al., 2021; Yossa et al., 2021), but to our knowledge, there is no one focus on the utilisation of banana pseudo-stem and banana flower in fish, nor on tilapia feeds. The interest in these two by-products under aquaculture perspectives is not only due to the high availability throughout the year, but to the interesting nutritional and polyphenol profile described in a previous study (Ramírez-Bolaños et al., 2021), where it was established the potential use of both, the banana pseudo-stem and banana flower as ingredients.

The potential synergy between the two industries can be developed and may have several benefits, on the one hand by reducing the waste generated in banana cultivation, and on the other hand by increasing the list of alternative available raw materials, moreover, reducing the need for imported ingredients in some tilapia production regions, thus decreasing the impact of food and feed production on the environment. The circular economy is one of the paths toward sustainability that must be taken to solve the problems that lie ahead.

The main objective of this work is to evaluate the dietary inclusion of different banana by-products such as banana pseudo-stem and banana flower and to explore the extracts from the banana flower in the development and health of tilapia juveniles.

2. Materials and methods

2.1. Raw materials

Banana pseudo-stem and banana flower were supplied by local Canarian producers of banana cultivars in the framework of the UE project LIFEBAQUA (code: LIFE15 ENV/ES/000157). The banana pseudo-stem was processed at the University of Las Palmas de Gran Canaria, Fabricación Integrada y Avanzada Research Group facilities, to mechanically separate the external long fibre (patent: WO2014/174115) normally used for bioplastic purposes, from the residue that remained in the machine, which represent up to 76% of the dry pulp (LifeBaqua, 2019). This high quantity of secondary by-product was considered pseudo-stem fibre (BP), to be studied as a dietary fish ingredient. The proximate composition of BP and banana flower (BF) was described in previous work (Ramírez-Bolaños et al., 2021) being 7.23% and 13.59% protein, 1% and 8.66% lipid, 15.97% and 18.07% ash and 76% and 60% carbohydrates, respectively. According to its carbohydrates content, as reported to be for banana pseudo-stem 26% cellulose, 19% hemicellulose, 8% lignin and 22% starch (Díaz et al., 2021), a pre-treatment was proposed to make the carbohydrate and fibre fraction present in this waste more accessible and thus favour fermentation in the digestive tract of the juveniles. For this, acid hydrolysis was performed under diluted acid conditions (H_2SO_4 2% w/w, 500 g/L wet weight, 100 °C) for 30 min (Souza et al., 2014). Then, the product obtained was neutralised, dried, and milled, generating the BP meal used in this study (0.93 ± 0.36 lipids (%), 1.91 ± 0.35 proteins (%), 50.32 ± 0.67 ash (%), 4.12 ± 0.06 moisture (%) and 44.51 ± 0.10 carbohydrates (%). The banana flower was freeze-dried (Lyobeta, Spain) to finally ground in a mill (Ultra Centrifugal Mill ZM200, Retsch, Germany) down to 125–250 μm. Interestingly, banana flower has reported high polyphenol content and profile: 1075.02 ± 20.06 mg/100 g of extractable polyphenols, = 735.23 ± 103.91 mg/100 g of hydrolysable polyphenols and 11,200 ± 3357.61 mg/100 g of non extractable proanthocyanidins (Ramírez-Bolaños et al., 2021). Based on that, an organic extract from the banana flower was performed with methanol:water and acetone: water; then the organic solvents were evaporated in a rotary evaporator (Laborota 4000 Efficient, Heidolph, Germany) until only the aqueous fraction remained, and the residues from that extraction were dried in an oven at 37 °C (Digitheat-TFT, J. P. Selecta, Spain), being both stored at − 80 °C until use.

2.2. Experimental conditions

Three consecutive experiments were carried out at the aquaculture facilities of the Ecoaqua Institute belonging to the University of Las Palmas de Gran Canaria. Triplicate groups of fish obtained by natural reproduction on-site were randomly distributed in recirculated systems (80 L/tank, 3 per treatment) for the 3 experiments, being fish manually fed to apparent satiation twice a day, six days a week during the trials. All the experiments followed the bioethics protocol followed by the Bioethics Committee of the University of Las Palmas de Gran Canaria (Real Decreto, 2013).

A previously tested diet in our facilities based on a comprehensive review (Table 1), where used as control for the three trials. In Experiment I, BP highly available worldwide, was used as raw material in tilapia diets by substituting the cornmeal present in the basal diet (0%, 2.5%, 5%, 15% and 20%). In Experiment II, due to the lower amount of BF in the banana culture and also the high quantity of bioactive, this by-product was used as an additive (Ramírez-Bolaños et al., 2021) increasing levels of BF (0.2%, 0.5%, 1% and 3%). In Experiment III the 3% BF diet was assayed against a diet containing the organic extract corresponding to 3% BF and a diet with its solid remaining residue (Table 2).

2.2.1. Experiment I: hydrolysed banana pseudo-stem (BP)

Triplicate groups of 12 fish per tank (3.9 ± 0.64 g) were manually fed with five diets with increasing levels of BP (0%, 2.5%, 5%, 15% and

Table 1
Formulation of basal diet for the tilapia trials.

Formulation (%)	
Fish meal	21.00
Corn meal	20.00
Corn gluten	7.00
Soy meal	26.00
Wheat meal	9.00
Linseed Oil	6.00
Vitamin mix[a]	1.00
Mineral mix[b]	2.00
Bi-Calcium Phosphate	0.50
Bread Meal	5.00
α-Cellulose	1.50

[a] Vitamin mix (g kg^{-1}) = thiamine 0.02, riboflavin 0.025, pyridoxine 0.02, calcium pantothenate 0.0585, nicotinic acid 0.1, biotin 0.0005, cyanocobalamin 0.0003, choline chloride 1.350, myo-inositol 1, ascorbic acid 2.5, α-tocopherol 0.125, menadione 0.01, cholecalciferol 0.0025, retinyl acetate 0.0125, ethoxyquin 0.1

[b] Mineral mix (g kg^{-1}) = $Ca(H_2PO)$ 1.605, $CaCO_3$ 4, $FeSO_4$ x 7 H_2O 1.5, $MgSO_4$ x 7 H_2O 1.605, K_2HPO_4 2.8, Na_2PO_4 x H_2O 1, $Al(SO_4)_3$ x 6 H_2O 0.02, $ZnSO_4$ x 5 H_2O 0.24, $CuSO_4$ x 5 H_2O 0.12, $MnSO_4$ x H_2O 0.08, KI 0.02, $CaSO_4$ x 7 H_2O 0.08

Table 2
Proximate composition of experimental diets including hydrolysed banana pseudo-stem (Experiment I), banana flower (Experiment II) and banana flower extract and residue (Experiment III).

Diet					
	Control	BP2.5	BP5	BP15	BP20
Experiment I (%dw)					
Protein	37.32 ± 1.41	38.12 ± 0.40	38.31 ± 0.60	38.72 ± 0.14	38.69 ± 1.53
Lipids	11.09 ± 0.61	10.70 ± 0.18	10.60 ± 0.09	10.05 ± 0.27	10.33 ± 0.28
Ash	8.71 ± 0.04	9.76 ± 0.07	10.75 ± 0.05	15.67 ± 0.06	18.52 ± 0.90
CHO & fibre	48.04 ± 2.07	46.20 ± 0.58	45.14 ± 0.77	41.11 ± 0.61	38.42 ± 0.87
	Control	BF 0.2	BF 0.5	BF 1	BF 3
Experiment II (%dw)					
Protein	32.93	36.03 ± 1.08	35.96 ± 1.13	34.01 ± 1.10	36.43 ± 1.20
Lipids	13.64	13.70	12.94 ± 0.85	13.29 ± 0.09	12.72 ± 0.16
Ash	8.64 ± 0.04	8.66 ± 0.33	8.54 ± 0.11	8.77 ± 0.13	8.84 ± 0.08
CHO & Fibre	47.45 ± 0.02	44.13 ± 0.64	44.66 ± 0.94	46.17 ± 0.21	43.86 ± 0.33
	Control	BF3	BF3R	BF3E	
Experiment III (% dw)					
Protein	41.46 ± 0.30	40.92 ± 0.09	35.80 ± 0.35	39.79 ± 1.02	
Lipids	13.77 ± 0.77	13.54 ± 0.41	15.17 ± 1.12	13.81 ± 0.27	
Ash	8.75 ± 0.16	9.87 ± 0.15	9.01 ± 0.13	8.65 ± 0.13	
CHO & fibre	40.39 ± 1.86	39.97 ± 1.40	43.91 ± 1.40	40.25 ± 0.16	

20%) for 47 days with 25.56 ± 0.61 °C and 5.04 ± 0.62 mg/L O_2 during the whole trial. At the end of the experiment, 9 samples (3 per replicate) for biochemical composition, histopathology and growth parameters were taken.

2.2.2. Experiment II: banana flower (BF)

Triplicate groups of 12 fish per tank (7.85 ± 0.39 g) were manually fed for 47 days, with a control diet and four diets with BF flour inclusion (0.2%, 0.5%, 1% and 3%) regarded the polyphenols content in banana flower and the results obtained from Gabriel et al. (2015). Measured temperature and oxygen ranged between 22.65 ± 1.03 °C and 5.32 ± 0.52 mg/L O_2, respectively. After the feeding trial, an osmotic challenge was performed in brackish water with salinity of 21 ppt, unfortunately, the fish from the BF1 diet were removed due to a failure in the system that could not be restored in time to perform the challenge. Blood samples (2 fish per tank, 6 fish per treatment) were collected at the beginning of the challenge (t0), at 3 h (t3) and 24 h (t24) according to Kammerer et al. (2010). Individual samples were centrifuged, and each plasma was stored at − 80 °C for cortisol and glucose analysis. Also, survival was observed during the 48 h hours and the time of fish death in the case was recorded.

2.2.3. Experiment III: banana flower extracts (BF3E) and residue (BF3R)

Twenty fish per tank (0.94 ± 0.005 g) were fed in triplicate groups with four diets with 3% banana flower inclusion (BF3), against the organic extraction equivalent to that of 3% of banana flower (BF3E) and the residues remained from that extraction (BF3R). All diets were tested against the control diet during 45 days of feeding, at 25.73 ± 0.80 °C and 5.61 ± 0.50 mg/L O_2 for temperature and oxygen respectively. After the feeding period, blood samples were taken from two fish per tank and cortisol and glucose were determined. To better understand the results obtained in experiment II, the osmotic challenge was performed in two phases being the fish acclimated in brackish water (18 ppt, 50% of salt water) for five days, before increasing the salinity to salt water (35ppt) and mortality was recorded.

2.3. Growth performance

To evaluate the growth performance, fish were individually weighted every fifteen days until the end of each feeding experiment and the subsequent parameters were determined: FW, Final Weight (g); FI, Feed Intake (g/fish/day); FCR, Feed Conversion Ratio = Feed intake (g) / Weight increase (g); PER, Protein Efficiency Ratio= weight gain (g) / protein intake (g); SGR, Specific Growth Rate = ((Ln Final weight – Ln Initial weight)/ nº days)x100; K, Condition Factor= (Final Weight (g) / Total Length $(cm)^3$)x100; VSI, Viscerosomatic index (%) = (Weight of whole fish (g) -Weight of fish without viscera (g)/ Weight of whole fish (g)) x100; HIS, Hepatosomatic index (%) = (liver weight /body weight) x100.

2.4. Proximal composition

In each experiment, the lipid composition was analysed in muscle and liver. Moisture content was determined following Association of Analytical Communities AOAC (2000) protocol; lipids were determined according to Folch et al. (1957) being fatty acids profiles obtained by transmethylation of total lipids according to Christie (1982) protocol and later separated and quantified by liquid chromatography (Izquierdo et al., 1989). In Experiment II and Experiment III, the protein and ash content in the muscle were determined by Kjeldahl and muffle furnace incineration, respectively (Association of Analytical Communities AOAC, 2000).

2.5. Histopathology

Liver for histopathological analysis was fixed with formaldehyde (4% formalin, 0.08 M sodium phosphate, pH 7.0), dehydrated, included in paraffin, and stained with haematoxylin & eosin (Martoja, Martoja-Pierson, 1970). The vacuolisation grade on the hepatocytes was evaluated following a scale from 1.0 (no evident presence of lipid vacuoles) to 2.5 (presence of lipid vacuoles, core displacement and cellular deformity). Micrographs from each stained slide were taken using an Olympus CX41 microscope (Olympus Optical, PA, USA) incorporated with an Olympus DP50 camera (Olympus Optical Co. LTD, Shinjuku-ku, Tokyo, Japan). The total area of 30 hepatocytes per specimen (180 hepatocytes per experimental diet) was measured, as well as maximum and minimum hepatocyte length with the programme ImageJ (Schneider et al., 2012).

2.6. Oxidation (MDA & liver catalase activity)

In the trial with banana flower (Experiment II) the mannonaldehyde concentration (MDA) was determined in the lipids from fish muscle at t0 and t24 and in the liver at t24, as an indicator of oxidative status. The analysis was performed following the protocol from Burk et al. (1980).

The catalase activity analysis was performed with a catalase assay kit (Cayman Chemical, USA) on the livers at the beginning of the osmotic challenge (t0) and 24 h after placing the fish in SW (21 ppt).

2.7. Plasma analysis (cortisol and glucose)

Blood samples were taken from six fish per treatment, at 0 h, 3 h and 24 h from the caudal vein using a sterile syringe, during the challenge of Experiment II and after the feeding trial in Experiment III. Plasma cortisol and glucose were performed in an external certified laboratory.

2.8. Statistics

The results were expressed as mean ± standard deviation. All the data were analysed with GraphPad Prism 8.0.2 (GraphPad Software, San

Diego, California USA, www.graphpad.com), normal distribution was determined with the Shapiro-Wilk test, one-way ANOVA analysis was performed on the normal data and non-parametric Kruskal-Wallis test was performed on the data without normal distribution. The significance was established with a p-value = 0.05.

3. Results

Tilapiaś performance was barely affected by the inclusion of banana residues. In experiment I, banana pseudo-stem decreased tilapia's performance (Table 3), but to a higher extent for BP15 and BP20 concerning BP2.5 and BP5, with about 50% and 30% less final fish weight for the higher and lower substitution levels, respectively. Feed acceptance, as shown by feed intake for the whole trial, and the condition factor (K) was not reduced among the increasing banana pseudo-stem inclusion. The FCR values maintain close to control by up to 5%, being significantly higher in the case of 15% and 20% inclusion (Table 3). The protein efficiency ratio was reduced with the banana pseudo-stem inclusion up to 5%. Regarding body indexes, all BP inclusion levels and banana flower extract and its residue, increased the VSI concerning control fish, while only the 20% of BP in the case of the liver (Table 3).

In experiment II, the banana flower inclusion did not produce any effect on the growth of tilapia juveniles but did affect some of the productive parameters (Table 4). The protein efficiency ratio decreased with the dietary inclusion of banana flower being significantly higher for BF1 and above.

In experiment III, the banana flower extract and residue did not affect fish growth, but the BF3R diet showed a reduction in condition factor (K), also, BF3 in the present trial and similarly BF3R decreased the protein efficiency ratio compared to the control diet (Table 5).

In the three experiments, the survival after the feeding period was no affected by the diets with banana by-products inclusion.

3.1. Proximate composition

The lipids composition of muscle and liver decreased with the inclusion of hydrolysed banana pseudo-stem (Tables 6 and 7) in Experiment I. Otherwise, in Experiment II, none of the experimental diets did affect the composition of the muscle and liver (Tables 8 and 9) and non also on the fish muscle and liver in Experiment III diets (Tables 10 and 11).

Table 3
Fish growth and performance parameters from Experiment I with hydrolysed banana pseudo-stem inclusion.

	DIET				
	Control	BP2.5	BP5	BP15	BP20
FW (g)	30.70 ± 6.60^{a}	20.59 ± 4.11^{b}	21.15 ± 5.99^{b}	14.99 ± 4.27^{c}	18.38 ± 4.04^{b}
[1]FI (g fish^{-1})	84.78 ± 0.54	92.77 ± 2.02	86.93 ± 0.40	92.47 ± 5.91	93.22 ± 4.84
[2]FCR	0.97 ± 0.00^{c}	1.13 ± 0.03^{abc}	1.07 ± 0.01^{bc}	1.28 ± 0.10^{a}	1.18 ± 0.09^{ba}
[3]PER	3.01 ± 0.11^{a}	2.52 ± 0.08^{ab}	2.66 ± 0.07^{bc}	2.22 ± 0.17^{c}	2.39 ± 0.23^{bc}
[4]SGR	6.62 ± 0.14^{a}	5.50 ± 0.03^{b}	5.42 ± 0.24^{b}	4.14 ± 0.12^{c}	5.01 ± 0.31^{b}
[5]K	1.79 ± 0.14	1.75 ± 0.08	1.81 ± 0.27	1.76 ± 0.14	1.71 ± 0.13
[6]HSI (%)	0.79 ± 0.01^{b}	0.96 ± 0.13^{ab}	0.96 ± 0.09^{ab}	0.86 ± 0.14^{b}	1.27 ± 0.95^{a}
[7]VSI	10.71 ± 0.17^{b}	13.44 ± 0.21^{a}	12.38 ± 0.70^{a}	13.73 ± 0.51^{a}	12.69 ± 0.95^{a}

Different letter in same arrow indicates significant differences (p-value<0.05)
[1]FI= Feed intake (g) per fish for the experimental-day period
[2]FCR= Feed intake (g) / Weight increase (g)
[3]PER= weight gain / protein intake (g)
[4]SGR= ((Ln Final weight – Ln Initial weight)/ nº days) x 100
[5]K= (Final Weight (g) / Total Length (cm)^{3}) x 100
[6]HIS (%) = 100 ×wet liver weight /body weight.
[7]VSI (%) = ((Weight of whole fish (g) − Weight of fish without viscera (g))/ Weight of whole fish (g))x 100

Table 4
Fish growth and performance parameters from Experiment II with banana flower inclusion.

	DIET				
	C	BF0.2	BF0.5	BF1	BF3
FW(g)	20.65 ± 7.33	17.58 ± 5.70	18.12 ± 7.60	16.53 ± 2.33	17.28 ± 6.44
[1]FI (g fish-1)	79.14 ± 2.07	78.65 ± 2.75	77.36 ± 1.36	80.02 ± 4.14	82.14 ± 2.03
[2]FCR	1.33 ± 0.11	1.41 ± 0.09	1.35 ± 0.10	1.46 ± 0.13	1.55 ± 0.10
[3]PER	2.42 ± 0.21^{a}	2.04 ± 0.17^{ab}	2.14 ± 0.16^{ab}	1.90 ± 0.22^{b}	1.74 ± 0.18^{b}
[4]SGR	1.96 ± 0.27	1.74 ± 0.09	1.81 ± 0.14	1.69 ± 0.11	1.62 ± 0.21
[5]K	1.68 ± 0.01^{b}	1.67 ± 0.11^{b}	1.67 ± 0.14^{b}	1.81 ± 0.06^{a}	1.78 ± 0.03^{b}
[6]VSI	14.86 ± 3.65	13.38 ± 1.40	13.63 ± 0.13	15.08 ± 1.80	14.29 ± 1.05

Different letter in same arrow indicates significant differences (p-value<0.05)
[1]FI= Feed intake (g) per fish for the experimental-day period
[2]FCR= Feed intake (g) / Weight increase (g)
[3]PER= weight gain / protein intake (g)
[4]SGR= ((Ln Final weight – Ln Initial weight)/ nº days) x 100
[5]K= (Final Weight (g) / Total Length (cm)^{3}) x 100
[6]VSI (%) = ((Weight of whole fish (g) − Weight of fish without viscera (g))/ Weight of whole fish (g))x 100

Table 5
Fish growth and performance parameters from Experiment III with the inclusion of banana flower extract and residue.

DIET				
	C	BF3	BF3R	BF3E
FW (g)	7.09 ± 0.52	6.19 ± 0.28	6.26 ± 0.04	7.09 ± 0.78
[1]FI (g fish-1)	81.07 ± 4.47	89.64 ± 2.76	98.82 ± 26.41	86.98 ± 5.90
[2]FCR	0.94 ± 0.05	1.06 ± 0.02	1.16 ± 0.31	1.00 ± 0.08
[3]PER	1.03 ± 0.09^{a}	0.79 ± 0.04^{b}	0.74 ± 0.01^{b}	0.92 ± 0.12^{ab}
[4]SGR	4.48 ± 0.17	4.18 ± 0.10	4.21 ± 0.00	4.48 ± 0.23
[5]K	1.73 ± 0.04^{a}	1.73 ± 0.06^{a}	1.61 ± 0.02^{b}	1.75 ± 0.02^{a}
[6]HSI (%)	0.99 ± 0.39	1.43 ± 0.66	0.87 ± 0.20	1.07 ± 0.13
[7]VSI	10.73 ± 2.89^{b}	12.56 ± 1.51^{ab}	15.68 ± 2.26^{a}	13.46 ± 4.04^{a}

Different letter indicates significant differences (p-value<0.05)
[1]FI= Feed intake (g) per fish for the experimental-day period
[2]FCR= Feed intake (g) / Weight increase (g)
[3]PER= weight gain / protein intake (g)
[4]SGR= ((Ln Final weight – Ln Initial weight)/ nº days) x 100
[5]K= (Final Weight (g) / Total Length (cm)^{3}) x 100
[6]HIS (%) = 100 ×wet liver weight /body weight.
[7]VSI (%) = ((Weight of whole fish (g) − Weight of fish without viscera (g))/ Weight of whole fish (g))x 100

3.2. Fatty acids composition

BP inclusion affects the fatty acid profile of both liver and muscle. Up to 5% of inclusion of hydrolysed banana pseudo-stem the levels of EPA, ARA, DHA, n-3, n-6, n-3 HUFA and total PUFA were increased, which contribute to maintaining the same amount of these fatty acids as the control diet in the whole muscle, in compensation to the decreasing amount of the total lipids. Stearic acid, oleic acid and 20:3n-3 increase with BP inclusion in diets (Table 6). In the liver, the inclusion of BP decreases some saturated acids such as myristic and palmitic (14:00 and

Table 6
Muscle lipids, moisture and fatty acids composition from tilapia fed with hydrolysed banana pseudo-stem inclusion in Experiment I.

	Diet				
	Control	BP2.5	BP5	BP15	BP20
Lipid (% dw)	9.01 ± 1.20[a]	4.92 ± 0.40[b]	5.02 ± 0.41[b]	5.04 ± 0.75[b]	6.10 ± 0.13[b]
Moisture (% dw)	80.13 ± 0.67	80.73 ± 0.39	80.93 ± 0.45	79.39 ± 1.44	79.16 ± 1.05
Fatty acid (%)					
14:00	1.56 ± 0.36	1.14 ± 0.34	1.12 ± 0.01	1.50	1.36 ± 0.22
14:1n-7	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.03	0.03 ± 0
14:1n-5	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.09	0.12 ± 0.01
15:00	0.19 ± 0.03	0.22 ± 0.03	0.24 ± 0.01	0.27	0.30 ± 0.01
15:1n-5	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.02	0.02 ± 0.00
16:OISO	0.06 ± 0.01[b]	0.07 ± 0.01[ab]	0.09 ± 0.01[ab]	0.10	0.13 ± 0.01[a]
16:00	15.97 ± 3.13	14.83 ± 1.25	15.64 ± 2.17	19.57	19.11 ± 1.21
16:1n-7	2.25 ± 1.65	2.00 ± 0.73	1.61 ± 0.07	2.22	2.45 ± 0.23
16:1n-5	0.22 ± 0.01[b]	0.30 ± 0.02[ab]	0.37 ± 0.05[ab]	0.47	0.54 ± 0.06[a]
16:2n-4	0.06 ± 0.01	0.05 ± 0.02	0.05 ± 0.00	0.06	0.06 ± 0.00
17:00	0.02 ± 0.02	0.05 ± 0.01	0.06 ± 0.00	0.05	0.06 ± 0.01
16:3n-4	0.25 ± 0.01[a]	0.18 ± 0.05[b]	0.17 ± 0.00[b]	0.22	0.30 ± 0.00[a]
16:3n-3	0.08 ± 0.00[c]	0.13 ± 0.01[b]	0.15 ± 0.03[ab]	0.15	0.18 ± 0.01[a]
16:3n-1	0.01 ± 0.01[b]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.03	0.03 ± 0.00[a]
16:4n-3	0.03 ± 0.01[ab]	0.04 ± 0.01[ab]	0.04 ± 0.01[a]	0.03	0.02 ± 0.00[b]
16:4n-1	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01	0.01 ± 0.00
18:00	5.02 ± 5.02[b]	13.83 ± 1.37[ab]	19.26 ± 4.30[a]	16.86	16.69 ± 1.70[a]
18:1n-9	18.96 ± 10.97[b]	17.82 ± 3.91[ab]	19.09 ± 0.44[a]	23.14	23.43 ± 0.22[a]
18:1n-7	0.90 ± 0.90	1.98 ± 0.19	2.41 ± 0.49	2.64	3.19 ± 0.12
18:1n-5	0.86 ± 0.76	0.10 ± 0.01	0.11 ± 0.02	0.10	0.13 ± 0.01
18:2n-9	0.12 ± 0.02	0.10 ± 0.03	0.08 ± 0.00	0.08	0.08 ± 0.01
18:2n-6	6.11 ± 6.11	11.70 ± 1.55	11.6 ± 0.61	10.67	11.45 ± 0.60
18:2n-4	0.08 ± 0.00[b]	0.10 ± 0.02[ab]	0.10 ± 0.00[ab]	0.11	0.13 ± 0.01[a]
18:3n-6	0.27 ± 0.00	0.30 ± 0.04	0.25 ± 0.07	0.16	0.20 ± 0.00
18:3n-4	0.04 ± 0.00[b]	0.05 ± 0.00[ab]	0.06 ± 0.01[a]	0.07	0.06 ± 0.00[ab]
18:3n-3	8.79 ± 0.07	7.78 ± 2.10	6.86 ± 1.40	6.70	7.45 ± 1.33
18:4n-3	0.17 ± 0.02	0.17 ± 0.03	0.15 ± 0.05	0.08	0.09 ± 0.00
18:4n-1	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.02	0.04	0.03 ± 0.01
20:00	0.18 ± 0.01[b]	0.25 ± 0.05[ab]	0.38 ± 0.09[a]	0.46	0.39 ± 0.05[a]
20:1n-9	0.03 ± 0.03[b]	0.05 ± 0.01[ab]	0.05 ± 0.00[ab]	0.12	0.08 ± 0.01[a]
20:1n-7	0.63 ± 0.01[b]	0.56 ± 0.00[b]	0.66 ± 0.16[b]	1.28	0.94 ± 0.03[a]
20:1n-5	0.07 ± 0.01[b]	0.08 ± 0.00[b]	0.10 ± 0.02[ab]	0.14	0.12 ± 0.01[a]
20:2n-9	0.05 ± 0.01[a]	0.04 ± 0.00[ab]	0.04 ± 0.01[b]	0.06	0.04 ± 0.00[b]
20:2n-6	0.37 ± 0.01[b]	0.71 ± 0.17[a]	0.76 ± 0.11[a]	0.98	0.89 ± 0.10[a]
20:3n-9	0.01 ± 0.01[b]	0.05 ± 0.02[a]	0.05 ± 0.00[a]	0.07	0.03 ± 0.00[ab]
20:3n-6	0.22 ± 0.01[a]	0.53 ± 0.15[b]	0.44 ± 0.02[b]	0.41	0.36 ± 0.05[ab]
20:4n-6	0.92 ± 0.00[b]	2.44 ± 0.64[a]	2.16 ± 0.45[a]	1.47	0.96 ± 0.11[b]
20:3n-3	1.00 ± 0.12[c]	1.84 ± 0.39[ab]	1.62 ± 0.12[ac]	2.35	2.51 ± 0.41[a]
20:4n-3	0.18 ± 0.03	0.32 ± 0.07	0.25 ± 0.05	0.20	0.22 ± 0.05
20:5n-3	0.59 ± 0.08[b]	1.26 ± 0.24[a]	0.87 ± 0.26[ab]	0.39	0.50 ± 0.00[b]
22:1n-11	0.04 ± 0.00[b]	0.04 ± 0.00[b]	0.06 ± 0.02[b]	0.37	0.11 ± 0.02[a]
22:1n-9	0.09 ± 0.00[c]	0.10 ± 0.03[bc]	0.21 ± 0.03[ab]	0.81	0.32 ± 0.08[a]
22:4n-6	0.11 ± 0.00[b]	0.30 ± 0.10[a]	0.22 ± 0.01[ab]	0.16	0.12 ± 0.03[b]
22:5n-6	0.14 ± 0.00[b]	0.38 ± 0.13[a]	0.30 ± 0.07[ab]	0.17	0.15 ± 0.03[b]
22:5n-3	0.64 ± 0.11[b]	1.70 ± 0.54[a]	1.03 ± 0.30[ab]	0.67	0.77 ± 0.20[b]
22:6n-3	5.49 ± 0.25[b]	16.15 ± 6.16[a]	11.01 ± 4.69[ab]	4.41	3.79 ± 0.90[b]
[1]Saturades	22.94 ± 8.58[b]	30.32 ± 0.23[ab]	36.70 ± 6.57[ab]	38.70	37.91 ± 3.19[a]
[2]Monoenoics	24.10 ± 12.84	23.08 ± 4.51	24.63 ± 1.14	30.62	31.14 ± 0.48
[3]∑n-3	16.97 ± 0.55[b]	29.38 ± 5.24[a]	21.97 ± 6.61[ab]	14.98	15.54 ± 2.88[b]
[4]∑n-6	8.15 ± 6.08[b]	16.38 ± 0.40[a]	15.74 ± 1.11[ab]	14.02	14.15 ± 0.69[ab]
[5]∑n-9	19.26 ± 11.03	18.16 ± 3.90	19.53 ± 0.47	24.29	23.98 ± 0.14
[6]∑n-3 HUFA	7.90 ± 0.59[b]	21.26 ± 7.39[a]	14.77 ± 5.19[ab]	8.02	7.79 ± 1.56[b]
ARA/EPA	1.54 ± 0.20[b]	1.94 ± 0.15[b]	2.50 ± 0.27[a]	3.78	1.93 ± 0.20[b]
DHA/EPA	9.26 ± 0.77[ab]	12.86 ± 2.58[a]	12.70 ± 1.76[ab]	11.38	7.65 ± 1.87[b]
DHA/ARA	6.00 ± 0.27	6.62 ± 0.86	5.09 ± 1.18	3.01	3.95 ± 1.41
[7]Total PUFA	25.79 ± 5.61[b]	46.43 ± 4.72[a]	38.37 ± 7.75[ab]	29.76	30.49 ± 3.58[b]
n-3/n-6	1.55 ± 1.69	1.79 ± 0.36	1.40 ± 0.32	1.07	1.10 ± 0.15

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

16:00), monoenoic and n-9, with a reduction of oleic acid (18:2n-6) but increases total PUFA and n-3/n-6 ratio. In addition, BP2.5 and BP5 decrease the amount of linolenic acid (18:3n-3 HUFA) but increase stearic acid (18:00) (Table 7).

Regarded dietary banana flower (Experiment II), there was no effect observed in the case of the muscle being the closer results observed among fish from BF3 and the control diets (Table 8). Similarly, no effect on the livers by BF inclusion apart from a slight reduction in the EPA content among other HUFAs which affect the FAA ratios although not significantly (Table 9).

In experiment III, the fatty acid profile of the muscle was affected by

Table 7
Liver lipids, moisture and fatty acids composition from tilapias fed with hydrolysed banana pseudo-stem inclusion in Experiment I.

	Diet				
	Control	BP2.5	BP5	BP15	BP20
Lipid (% dw)	34.42 ± 6.52[a]	22.64 ± 2.16[b]	17.80 ± 1.36[b]	20.47 ± 3.44[b]	18.71 ± 3.03[b]
Moisture (% dw)	72.54 ± 0.89	76.59 ± 1	75.91 ± 1.34	76.37 ± 0.49	72.65 ± 1.13
Fatty acid					
14:00	1.27 ± 0.21[a]	0.39 ± 0.06[b]	0.38 ± 0.09[b]	0.45 ± 0.05[b]	0.69
14:1n-7	0.08 ± 0.00[ab]	0.12 ± 0.01[a]	0.12 ± 0.04[a]	0.06 ± 0.01[b]	0.02
14:1n-5	0.06 ± 0.01[a]	0.02 ± 0.00[b]	0.04 ± 0.01[ab]	0.03 ± 0.02[ab]	0.09
15:00	0.18 ± 0.03	0.13 ± 0.02	0.16 ± 0.01	0.16 ± 0.03	0.19
15:1n-5	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02
16:OISO	0.05 ± 0.01[a]	0.03 ± 0.00[b]	0.04 ± 0.00[ab]	0.03 ± 0.01[ab]	0.09
16:00	17.21 ± 2.43[a]	16.60 ± 1.44[b]	15.18 ± 0.41[b]	15.15 ± 0.49[b]	11.54
16:1n-7	2.78 ± 0.21[a]	0.66 ± 0.11[b]	0.85 ± 0.12[b]	0.91 ± 0.11[b]	1.65
16:1n-5	0.21 ± 0.04	0.14 ± 0.02	0.20 ± 0.04	0.17 ± 0.05	0.41
16:2n-6	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.00	0.00
16:2n-4	0.08 ± 0.02	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.06
17:00	0.05 ± 0.02	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05
16:3n-4	0.21 ± 0.04[a]	0.08 ± 0.02[b]	0.11 ± 0.01[b]	0.10 ± 0.02[b]	0.24
16:3n-3	0.09 ± 0.03	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.12
16:3n-1	0.19 ± 0.02[b]	0.56 ± 0.00[a]	0.56 ± 0.17[a]	0.45 ± 0.08[a]	0.03
16:4n-3	0.23 ± 0.07[b]	0.74 ± 0.04[a]	0.63 ± 0.21[a]	0.59 ± 0.13[a]	0.04
16:4n-1	0.03 ± 0.02	0.05 ± 0.01	0.07 ± 0.02	0.04 ± 0.01	0.01
18:00	11.10 ± 0.38[b]	14.98 ± 1.32[a]	13.74 ± 1.41[ab]	11.55 ± 0.48[b]	10.79
18:1n-9	28.06 ± 1.56[a]	15.08 ± 2.26[b]	17.14 ± 1.56[b]	14.22 ± 1.25[b]	17.67
18:1n-7	2.62 ± 0.33	2.90 ± 0.13	2.97 ± 0.17	2.93 ± 0.33	2.35
18:1n-5	0.10 ± 0.02[a]	0.06 ± 0.01[b]	0.07 ± 0.01[ab]	0.06 ± 0.01[b]	0.11
18:2n-9	0.12 ± 0.02[a]	0.07 ± 0.01[b]	0.09 ± 0.01[ab]	0.05 ± 0.00[b]	0.06
18:2n-6	12.58 ± 0.53[a]	9.80 ± 0.54[b]	10.84 ± 1.35[ab]	11.16 ± 1.21[ab]	11.64
18:2n-4	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.14
18:3n-6	0.22 ± 0.04	0.17 ± 0.08	0.14 ± 0.02	0.15 ± 0.01	0.26
18:3n-4	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.07
18:3n-3	7.83 ± 0.89[ab]	4.03 ± 0.51[c]	5.44 ± 1.08[bc]	8.77 ± 1.17[a]	11.46
18:3n-1	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.00	0.02
18:4n-3	0.11 ± 0.04	0.08 ± 0.01	0.13 ± 0.02	0.14 ± 0.02	0.17
18:4n-1	0.03 ± 0.01	0.02 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.05
20:00	0.30 ± 0.05[b]	0.40 ± 0.06[ab]	0.49 ± 0.07[a]	0.36 ± 0.01[ab]	0.24
20:1n-9	0.08 ± 0.01	0.07 ± 0.03	0.10 ± 0.02	0.07 ± 0.01	0.07
20:1n-7	0.91 ± 0.11	0.83 ± 0.10	0.97 ± 0.15	0.72 ± 0.10	0.76
20:1n-5	0.10 ± 0.01	0.11 ± 0.03	0.12 ± 0.03	0.10 ± 0.00	0.10
20:2n-9	0.07 ± 0.01	0.05 ± 0.01	0.08 ± 0.04	0.04 ± 0.00	0.05
20:2n-6	0.66 ± 0.12[b]	1.07 ± 0.06[a]	1.09 ± 0.07[a]	0.99 ± 0.06[a]	0.95
20:3n-9	0.03 ± 0.01[c]	0.05 ± 0.01[a]	0.04 ± 0.00[bc]	0.04 ± 0.00[ab]	0.05
20:3n-6	0.41 ± 0.06[b]	0.81 ± 0.11[a]	0.73 ± 0.03[a]	0.73 ± 0.06[a]	0.55
20:4n-6	1.20 ± 0.19[b]	2.60 ± 0.23[a]	2.37 ± 0.23[a]	2.03 ± 0.24[a]	2.03
20:3n-3	1.42 ± 0.35[b]	1.98 ± 0.30[ab]	2.21 ± 0.20[a]	2.55 ± 0.22[a]	3.57
20:4n-3	0.22 ± 0.05[c]	0.30 ± 0.04[bc]	0.38 ± 0.02[ab]	0.42 ± 0.05[a]	0.52
20:5n-3	0.67 ± 0.21[b]	1.50 ± 0.22[a]	1.74 ± 0.32[a]	1.72 ± 0.17[a]	1.13
22:1n-11	0.09 ± 0.03[b]	0.13 ± 0.02[b]	0.24 ± 0.05[a]	0.12 ± 0.03[b]	0.09
22:1n-9	0.23 ± 0.02	0.33 ± 0.06	0.40 ± 0.04	0.32 ± 0.09	0.23
22:4n-6	0.18 ± 0.04[c]	0.44 ± 0.05[a]	0.33 ± 0.04[b]	0.36 ± 0.04[ab]	0.29
22:5n-6	0.25 ± 0.07[b]	0.73 ± 0.07[a]	0.73 ± 0.10[a]	0.58 ± 0.05[a]	0.37
22:5n-3	1.10 ± 0.30[b]	3.20 ± 0.54[a]	3.38 ± 0.31[a]	4.01 ± 0.57[a]	2.87
22:6n-3	6.37 ± 2.18[b]	18.31 ± 2.57[a]	15.23 ± 1.61[a]	17.27 ± 3.73[a]	16.09
[1]Saturades	30.11 ± 3.06	32.54 ± 2.81	30.01 ± 1.60	27.72 ± 0.89	23.50
[2]Monoenoics	35.12 ± 1.59[a]	20.15 ± 2.36[b]	22.83 ± 1.93[b]	19.41 ± 1.42[b]	23.33
[3]∑n-3	18.05 ± 3.78[b]	30.22 ± 4.12[a]	29.22 ± 2.76[a]	35.52 ± 2.85[a]	35.97
[4]∑n-6	15.52 ± 0.87	15.64 ± 0.90	16.25 ± 1.28	16.00 ± 0.97	16.10
[5]∑n-9	28.59 ± 1.56[a]	15.65 ± 2.30[b]	17.84 ± 1.63[b]	14.75 ± 1.17[b]	18.13
[6]∑n-3 HUFA	9.78 ± 3.01[b]	25.29 ± 3.63[a]	22.94 ± 2.06[a]	25.96 ± 4.03[a]	24.18
ARA/EPA	1.80 ± 0.25[a]	1.74 ± 0.12[a]	1.36 ± 0.21[ab]	1.18 ± 0.05[b]	1.80
DHA/EPA	9.56 ± 2.18	12.21 ± 0.65	8.74 ± 1.99	10.06 ± 1.29	14.27
DHA/ARA	5.33 ± 1.15[b]	7.03 ± 0.67[ab]	6.42 ± 0.42[ab]	8.52 ± 1.26[a]	7.94
[7]Total PUFA	34.49 ± 4.56[b]	46.95 ± 4.98[a]	46.72 ± 3.57[a]	52.52 ± 2.01[a]	52.84
n-3/n-6	1.16 ± 0.19[b]	1.93 ± 0.17[a]	1.80 ± 0.17[a]	2.22 ± 0.30[a]	2.23

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

the experimental diets: all diets with flower and derivatives decreased the fatty acid 20:1n-7 compared to the control diet. In addition, the BF3 diet decreased oleic acid, monoenoic acids and n-9 and increased 22:5n-3, DHA, total n-3 and the n-3/n-6 ratio. On the other hand, the BF3E diet decreased ARA and DHA levels and increased linoleic acid, linolenic acid (18:2n-6 and 18:3n-3), 20:3n-3 and total n-3. Fatty acids n-3 HUFA were reduced by the BF3R diet compared to the BF3 diet (Table 10).

Table 8
Muscle proximate composition and fatty acids content from tilapias fed with banana flower inclusion in Experiment II.

	Diet				
	Control	BF0.2	BF0.5	BF1	BF3
Lipid (% dw)	6.34 ± 0.87	5.65 ± 0.37	6.06 ± 0.88	6.37 ± 0.82	5.41 ± 0.45
Ash (%dw)	7.22 ± 0.69	8.12 ± 0.94	7.29 ± 0.16	7.42 ± 0.57	8.64 ± 0.26
Protein (%dw)	91.71 ± 6.34	91.55 ± 0.77	90.21 ± 4.3	93.16 ± 2.28	93.34 ± 0.48
Moisture (% dw)	78.93 ± 0.63	78.91 ± 0.94	79.95 ± 0.16	79.63 ± 0.26	79.32 ± 0.67
Fatty acid (%)					
14:00	0.59 ± 0.38	0.67 ± 0.29	1.01 ± 0.47	0.52 ± 0.35	0.46 ± 0.36
14:1n-7	0.10 ± 0.09	0.54 ± 0.58	0.18 ± 0.09	0.20 ± 0.25	0.11 ± 0.14
14:1n-5	0.11 ± 0.12	0.41 ± 0.39	0.05 ± 0.03	0.06 ± 0.03	0.07 ± 0.06
15:00	0.23 ± 0.14	0.47 ± 0.40	0.20 ± 0.05	0.14 ± 0.08	0.18 ± 0.12
15:1n-5	0.13 ± 0.16	0.36 ± 0.31	0.06 ± 0.03	0.05 ± 0.03	0.07 ± 0.06
16:OISO	0.14 ± 0.17	0.34 ± 0.24	0.06 ± 0.04	0.06 ± 0.04	0.07 ± 0.06
16:00	16.03 ± 7.12	13.14 ± 1.09	16.96 ± 5.51	12.45 ± 6.07	16.61 ± 4.81
16:1n-7	0.82 ± 0.49	1.20 ± 0.20	1.42 ± 0.80	1.10 ± 0.63	0.97 ± 0.37
16:1n-5	0.18 ± 0.10	0.56 ± 0.53	0.16 ± 0.05	0.16 ± 0.11	0.19 ± 0.14
16:2n-4	0.28 ± 0.20	0.78 ± 0.47	0.16 ± 0.04	0.15 ± 0.03	0.17 ± 0.04
17:00	0.13 ± 0.14	0.88 ± 1.01	0.05 ± 0.02	0.08 ± 0.04	0.07 ± 0.03
16:3n-4	0.17 ± 0.11	1.18 ± 1.34	0.17 ± 0.09	0.19 ± 0.06	0.14 ± 0.04
16:3n-3	0.14 ± 0.11	0.49 ± 0.35	0.08 ± 0.03	0.07 ± 0.03	0.08 ± 0.03
16:3n-1	0.89 ± 0.27	0.86 ± 0.22	0.39 ± 0.08	0.47 ± 0.25	0.68 ± 0.17
16:4n-3	1.21 ± 0.27	1.35 ± 0.17	0.65 ± 0.11	0.70 ± 0.35	1.08 ± 0.24
16:4n-1	0.12 ± 0.12	0.55 ± 0.34	0.08 ± 0.03	0.09 ± 0.04	0.12 ± 0.02
18:00	12.07 ± 4.96	8.48 ± 0.42	9.85 ± 1.79	10.82 ± 2.10	11.65 ± 2.06
18:1n-9	12.55 ± 3.79	11.27 ± 3.44	15.96 ± 4.16	15.42 ± 2.14	12.74 ± 2.56
18:1n-7	2.67 ± 0.90	2.61 ± 1.01	2.44 ± 0.50	2.82 ± 0.39	2.77 ± 0.26
18.1n-5	0.16 ± 0.12	0.52 ± 0.30	0.08 ± 0.01	0.10 ± 0.04	0.09 ± 0.02
18:2n-9	0.18 ± 0.18	0.81 ± 0.61	0.14 ± 0.03	0.13 ± 0.00	0.10 ± 0.03
18.2n-6	9.36 ± 1.66	8.74 ± 1.80	11.75 ± 1.97	12.38 ± 0.93	10.67 ± 1.04
18:2n-4	0.23 ± 0.16	0.75 ± 0.66	0.12 ± 0.04	0.14 ± 0.07	0.19 ± 0.04
18:3n-6	0.67 ± 0.06	1.88 ± 0.14	1.16 ± 0.58	0.82 ± 0.17	0.68 ± 0.08
18:3n-4	0.16 ± 0.18	0.53 ± 0.41	0.07 ± 0.03	0.09 ± 0.04	0.08 ± 0.03
18:3n-3	5.24 ± 0.29	4.98 ± 1.79	8.00 ± 1.78	8.98 ± 1.88	7.13 ± 1.70
18:4n-3	0.25 ± 0.19	0.86 ± 0.63	0.25 ± 0.05	0.30 ± 0.09	0.28 ± 0.06
18:4n-1	0.15 ± 0.15	0.55 ± 0.49	0.06 ± 0.01	0.08 ± 0.05	0.06 ± 0.04
20:00	0.41 ± 0.08	0.24 ± 0.34	0.33 ± 0.04	0.33 ± 0.13	0.27 ± 0.08
20:1n-9	0.22 ± 0.19	0.71 ± 0.54	0.10 ± 0.03	0.11 ± 0.03	0.10 ± 0.06
20:1n-7	0.99 ± 0.07	1.68 ± 0.68	0.91 ± 0.03	0.90 ± 0.25	0.77 ± 0.23
20.1n-5	0.21 ± 0.16	0.62 ± 0.54	0.09 ± 0.04	0.09 ± 0.04	0.07 ± 0.03
20:2n-9	0.16 ± 0.13	0.84 ± 0.38	0.17 ± 0.08	0.11 ± 0.03	0.09 ± 0.05
20:2n-6	1.13 ± 0.13	1.28 ± 0.55	0.96 ± 0.24	1.01 ± 0.28	0.98 ± 0.20
20:3n-9	1.11 ± 0.26	1.31 ± 0.35	0.95 ± 0.12	1.18 ± 0.14	0.99 ± 0.04
20:3n-6	0.25 ± 0.25	0.82 ± 0.30	0.10 ± 0.04	0.12 ± 0.08	0.10 ± 0.09
20:4n-6	2.85 ± 0.67	2.64 ± 0.75	2.53 ± 0.60	3.44 ± 0.49	3.03 ± 0.72
20:3n-3	1.94 ± 0.35	1.77 ± 0.07	1.85 ± 0.55	1.98 ± 0.47	1.99 ± 0.17
20:4n-3	0.38 ± 0.08	1.19 ± 0.90	0.36 ± 0.01	0.52 ± 0.12	0.38 ± 0.18
20:5n-3	0.79 ± 0.29	1.52 ± 0.09	0.88 ± 0.38	1.00 ± 0.05	0.92 ± 0.40
22:1n-11	0.36 ± 0.25	0.97 ± 0.68	0.24 ± 0.09	0.23 ± 0.09	0.21 ± 0.10
22:1n-9	0.73 ± 0.28	0.83 ± 0.49	0.40 ± 0.03	0.43 ± 0.19	0.45 ± 0.08
22:4n-6	1.17 ± 0.67	1.54 ± 0.91	0.79 ± 0.24	0.95 ± 0.14	0.75 ± 0.23
22:5n-6	1.47 ± 0.87	2.06 ± 0.87	1.01 ± 0.53	1.15 ± 0.14	1.01 ± 0.21
22:5n-3	3.29 ± 2.34	2.75 ± 0.94	2.43 ± 1.28	2.87 ± 0.29	3.02 ± 1.21
22:6n-3	17.58 ± 14.61	11.48 ± 7.91	14.33 ± 12.3	14.99 ± 0.97	17.38 ± 8.09
[1]Saturades	29.61 ± 12.06	24.21 ± 2.28	28.46 ± 7.61	24.4 ± 5.06	29.3 ± 7.43
[2]Monoenoics	19.96 ± 4.79	23.11 ± 1.29	22.49 ± 5.53	22.11 ± 2.48	19.05 ± 3.54
[3]∑n-3	30.82 ± 17.02	26.39 ± 8.57	28.84 ± 12.89	31.41 ± 1.97	32.25 ± 10.38
[4]∑n-6	16.90 ± 0.6	18.96 ± 0.23	18.3 ± 0.25	19.87 ± 1.89	17.21 ± 0.16
[5]∑n-9	14.95 ± 3.53	15.78 ± 1.07	17.73 ± 4.14	17.39 ± 2.31	14.48 ± 2.69
[6]∑n-3 HUFA	23.98 ± 17.53	18.7 ± 7.93	19.85 ± 14.51	21.36 ± 0.48	23.69 ± 9.65
ARA/EPA	3.70 ± 0.45	1.76 ± 0.59	3.01 ± 0.51	3.42 ± 0.34	3.60 ± 1.04
DHA/EPA	20.18 ± 9.83	7.71 ± 5.65	14.5 ± 6.17	14.98 ± 1.64	18.99 ± 3.5
DHA/ARA	5.68 ± 3.41	4.08 ± 1.84	5.17 ± 3.23	4.44 ± 0.91	5.51 ± 1.53
[7]Total PUFA	51.17 ± 16.75	53.51 ± 3.07	49.45 ± 13	53.92 ± 2.7	52.1 ± 10.22
n-3/n-6	1.81 ± 0.94	1.39 ± 0.47	1.57 ± 0.68	1.59 ± 0.21	1.88 ± 0.61

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

3.3. Histopathology

In Experiment I, quantitative morphometric analysis of the liver revealed a significant ($P < 0.05$) increase in hepatocellular area and maximum and minimum cell length in hepatocytes from fish fed with BP diets (Table 12). The fish-fed control diet had the smallest hepatocellular area, as well as maximum and minimum length, showing an eosinophilic

Table 9
Liver lipids, moisture and fatty acids content from tilapias fed with banana flower inclusion in Experiment II.

Diet	Control	BF0.2	BF0.5	BF1	BF3
Lipid (% dw)	17.02 ± 2.53	21.22 ± 4.45	24.28 ± 2.12	20.76 ± 2.35	22.84 ± 1.42
Moisture (% dw)	70.71 ± 5.33	68.06 ± 3.47	72.25 ± 5.60	70.86 ± 1.19	71.77 ± 1.01
Fatty acid (%)					
14:00	0.74 ± 0.37	0.91 ± 0.19	1.27 ± 0.63	1.49 ± 0.09	1.09 ± 0.49
14:1n-7	0.14 ± 0.22	0.03 ± 0.01	0.05 ± 0.03	0.06 ± 0.01	0.04 ± 0.02
14:1n-5	0.24 ± 0.35	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.02	0.04 ± 0.02
15:00	0.21 ± 0.18	0.08 ± 0.01	0.13 ± 0.07	0.13 ± 0.03	0.15 ± 0.08
15:1n-5	0.11 ± 0.17	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.05 ± 0.02
16:OISO	0.03 ± 0.03	0.06 ± 0.03	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
16:00	9.38 ± 0.98	10.42 ± 2.69	12.99 ± 3.67	13.46 ± 0.53	10.91 ± 2.03
16:1n-7	1.81 ± 0.79	3.15 ± 0.56	3.37 ± 1.72	3.98 ± 0.19	2.76 ± 1.00
16:1n-5	0.28 ± 0.11	0.13 ± 0.03	0.13 ± 0.03	0.21 ± 0.06	0.19 ± 0.05
16:2n-4	0.09 ± 0.07	0.14 ± 0.00	0.13 ± 0.01	0.14 ± 0.02	0.16 ± 0.02
17:00	0.14 ± 0.17	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.00	0.06 ± 0.01
16:3n-4	0.29 ± 0.19	0.24 ± 0.01	0.25 ± 0.04	0.25 ± 0.05	0.24 ± 0.05
16:3n-3	0.23 ± 0.24[a]	0.08 ± 0.00[ab]	0.07 ± 0.01[ab]	0.07 ± 0.01[b]	0.07 ± 0.02[ab]
16:3n-1	0.19 ± 0.26[a]	0.05 ± 0.04[ab]	0.04 ± 0.01[ab]	0.03 ± 0.01[b]	0.03 ± 0.01[ab]
16:4n-3	0.25 ± 0.28[a]	0.05 ± 0.02[ab]	0.05 ± 0.02[ab]	0.06 ± 0.01[b]	0.06 ± 0.02[ab]
16:4n-1	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.02
18:00	10.88 ± 3.55	9.35 ± 2.11	8.07 ± 1.09	9.21 ± 0.38	9.18 ± 0.63
18:1n-9	18.28 ± 2.58	25.48 ± 0.73	27.2 ± 5.52	26.01 ± 1.45	22.86 ± 1.30
18:1n-7	2.35 ± 0.30	2.68 ± 0.10	2.49 ± 0.53	2.69 ± 0.16	2.29 ± 0.32
18.1n-5	0.23 ± 0.21	0.11 ± 0.02	0.09 ± 0.02	0.11 ± 0.01	0.10 ± 0.01
18:2n-9	0.25 ± 0.18	0.18 ± 0.00	0.17 ± 0.08	0.24 ± 0.04	0.14 ± 0.03
18.2n-6	8.71 ± 0.36	9.77 ± 0.70	10.50 ± 2.49	8.75 ± 0.71	11.16 ± 1.49
18:2n-4	0.18 ± 0.21	0.08 ± 0.03	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.02
18:3n-6	0.77 ± 0.07	0.7 ± 0.06	0.69 ± 0.21	0.70 ± 0.11	0.70 ± 0.15
18:3n-4	0.16 ± 0.18	0.08 ± 0.02	0.08 ± 0.02	0.05 ± 0.01	0.05 ± 0.02
18:3n-3	5.75 ± 0.58	7.21 ± 1.98	7.21 ± 2.19	5.9 ± 0.78	8.75 ± 2.00
18:4n-3	0.34 ± 0.21	0.25 ± 0.11	0.21 ± 0.10	0.19 ± 0.02	0.22 ± 0.08
18:4n-1	0.16 ± 0.24	0.05 ± 0.05	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02
20:00	0.43 ± 0.26	0.28 ± 0.02	0.25 ± 0.07	0.23 ± 0.02	0.25 ± 0.10
20:1n-9	0.26 ± 0.31	0.17 ± 0.08	0.15 ± 0.03	0.12 ± 0.01	0.12 ± 0.03
20:1n-7	1.17 ± 0.38	1.42 ± 0.11	1.25 ± 0.06	1.08 ± 0.13	1.04 ± 0.33
20.1n-5	0.17 ± 0.17	0.11 ± 0.04	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.03
20:2n-9	0.25 ± 0.31	0.16 ± 0.05	0.12 ± 0.04	0.13 ± 0.03	0.08 ± 0.03
20:2n-6	1.10 ± 0.12	0.93 ± 0.06	0.87 ± 0.39	0.71 ± 0.09	0.93 ± 0.21
20:3n-9	1.08 ± 0.09	1.14 ± 0.03	0.84 ± 0.13	1.01 ± 0.04	0.92 ± 0.11
20:3n-6	0.17 ± 0.21	0.07 ± 0.03	0.10 ± 0.03	0.07 ± 0.05	0.08 ± 0.02
20:4n-6	4.38 ± 1.25	3.14 ± 0.42	2.64 ± 0.63	3.29 ± 0.15	3.24 ± 0.24
20:3n-3	2.31 ± 0.71	2.15 ± 0.27	2.05 ± 1.01	1.76 ± 0.29	2.60 ± 0.33
20:4n-3	0.59 ± 0.07	0.59 ± 0.04	0.43 ± 0.14	0.51 ± 0.13	0.51 ± 0.11
20:5n-3	1.14 ± 0.17[a]	0.76 ± 0.05[b]	0.56 ± 0.06[b]	0.52 ± 0.06[b]	0.78 ± 0.16[ab]
22:1n-11	0.72 ± 1.04	0.19 ± 0.04	0.17 ± 0.05	0.10 ± 0.01	0.20 ± 0.17
22:1n-9	0.47 ± 0.68	0.12 ± 0.02	0.10 ± 0.03	0.10 ± 0.02	0.13 ± 0.05
22:4n-6	1.09 ± 0.43	0.70 ± 0.12	0.50 ± 0.14	0.72 ± 0.12	0.60 ± 0.17
22:5n-6	1.28 ± 0.39	0.73 ± 0.02	0.56 ± 0.19	0.72 ± 0.10	0.68 ± 0.29
22:5n-3	2.33 ± 0.11	2.04 ± 0.58	1.42 ± 0.30	1.92 ± 0.42	1.78 ± 0.38
22:6n-3	18.89 ± 4.67	13.92 ± 2.12	12.42 ± 4.08	12.94 ± 0.47	14.5 ± 3.47
[1]Saturades	21.80 ± 1.76	21.15 ± 4.93	22.82 ± 5.15	24.63 ± 0.27	21.70 ± 3.09
[2]Monoenoics	26.70 ± 6.99	33.78 ± 1.06	35.25 ± 7.50	34.71 ± 1.91	30.03 ± 2.08
[3]∑n-3	31.82 ± 5.36	27.03 ± 5.19	24.43 ± 6.57	23.87 ± 1.45	29.28 ± 1.86
[4]∑n-6	17.51 ± 0.74	16.03 ± 0.57	15.85 ± 3.39	14.97 ± 0.90	17.40 ± 0.83
[5]∑n-9	20.58 ± 3.54	27.24 ± 0.55	28.59 ± 5.57	27.60 ± 1.51	24.25 ± 1.51
[6]∑n-3 HUFA	25.25 ± 5.39	19.44 ± 3.07	16.9 ± 5.30	17.66 ± 0.68	20.18 ± 3.92
ARA/EPA	3.84 ± 1.37	4.15 ± 0.83	4.70 ± 1.20	6.29 ± 0.44	4.15 ± 0.64
DHA/EPA	16.53 ± 4.70	18.38 ± 1.56	22.12 ± 6.88	24.71 ± 3.33	18.54 ± 7.16
DHA/ARA	4.31 ± 0.35	4.43 ± 1.28	4.70 ± 0.57	3.93 ± 0.28	4.47 ± 0.99
[7]Total PUFA	51.98 ± 4.91	45.19 ± 6.01	42.03 ± 9.93	40.75 ± 1.93	48.4 ± 1.19
n-3/n-6	1.82 ± 0.23	1.69 ± 0.26	1.54 ± 0.10	1.59 ± 0.10	1.68 ± 0.19

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

appearance with the presence of lipid droplets, and nuclear displacement to the cell periphery with a normal cell distribution showing prominent basophilic nuclei aligned around sinusoidal spaces (Fig. 1).

In fish fed with alternative diets from diets 2.5 onwards, cell size increased showing progressively more marked basophilia, cell consolidation and a greater presence of peri hepatocyte detachment. Hepatocytes with a more regular morphology were characterised by a considerable reduction in cytoplasmic vacuolation without nuclear displacement to the periphery, offering a more glucogenic content appearance. The presence of sinusoidal spaces decreased but the normal nuclear organisation was maintained. However, when feeding the BP20

Table 10
Muscle proximate composition and fatty acids content from tilapias fed with banana flower extract and the residue inclusion in Experiment III.

DIET	Control	BF3	BF3R	BF3E
Lipid (% dw)	8.00 ± 1.51	8.13 ± 1.67	9.79 ± 0.86	8.35 ± 0.39
Ash (%dw)	5.11 ± 1.09	6.01 ± 1.62	6.21 ± 1.33	7.48 ± 3.27
Protein (%dw)	92.51 ± 5.72	92.63 ± 5.84	89.19 ± 2.34	86.89 ± 11.94
Moisture (% dw)	79.77 ± 0.66	79.11 ± 0.60	79.58 ± 0.32	76.90 ± 3.89
Fatty acid (%)				
14:00	0.15 ± 0.05	0.20 ± 0.03	0.29 ± 0.12	0.09 ± 0.02
14:1n-5	0.05 ± 0.04	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
14:1n-7	0.06 ± 0.05	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.01
15:00	0.08 ± 0.00	0.06 ± 0.01	0.08 ± 0.02	0.03 ± 0.01
15:1n-5	0.04 ± 0.04	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
16:OISO	0.05 ± 0.03	0.01 ± 0.01	0.02 ± 0.00	0.01 ± 0.01
16:00	10.23 ± 4.54	12.39 ± 2.5	13.73 ± 1.74	7.31 ± 1.31
16:1n-7	1.01 ± 0.47	1.33 ± 0.01	1.31 ± 0.24	0.85 ± 0.07
16:1n-5	0.09 ± 0.00	0.07 ± 0.01	0.11 ± 0.02	0.08 ± 0.03
16:2n-4	0.05 ± 0.03	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
17:00	0.07 ± 0.03	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
16:3n-4	0.15 ± 0.01	0.13 ± 0.00	0.13 ± 0.01	0.13 ± 0.01
16:3n-3	0.08 ± 0.02	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01
16:3n-1	0.60 ± 0.10	0.50 ± 0.07	0.48 ± 0.05	0.49 ± 0.06
16:4n-3	0.96 ± 0.10	0.88 ± 0.19	0.88 ± 0.09	0.87 ± 0.04
16:4n-1	0.07 ± 0.01	0.06 ± 0.01	0.04 ± 0.03	0.06 ± 0.00
18:00	11.93 ± 0.64	9.28 ± 1.74	10.97 ± 1.35	10.41 ± 0.63
18:1n-9	24.46 ± 0.97[a]	20.34 ± 1.93[b]	22.87 ± 1.53[ab]	25.00 ± 0.77[a]
18:1n-7	3.47 ± 0.21	3.51 ± 0.79	3.32 ± 0.41	2.96 ± 0.03
18:1n-5	0.12 ± 0.03	0.07 ± 0.00	0.10 ± 0.01	0.07 ± 0.01
18:2n-9	0.23 ± 0.05	0.17 ± 0.02	0.22 ± 0.02	0.16 ± 0.03
18.2n-6	12.04 ± 0.18[a]	11.64 ± 0.80[a]	11.92 ± 0.51[a]	13.96 ± 0.26[ab]
18:2n-4	0.12 ± 0.04	0.08 ± 0.01	0.07 ± 0.01	0.11 ± 0.01
18:3n-6	0.68 ± 0.18	0.58 ± 0.01	0.55 ± 0.05	0.62 ± 0.02
18:3n-4	0.09 ± 0.04	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
18:3n-3	8.00 ± 0.28[a]	9.50 ± 1.02[a]	8.86 ± 0.51[a]	14.85 ± 1.10[b]
18.3n-1	0.05 ± 0.04	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.00
18:4n-3	0.28 ± 0.06	0.38 ± 0.07	0.23 ± 0.01	0.37 ± 0.04
18:4n-1	0.09 ± 0.06	0.02 ± 0.00	0.04 ± 0.02	0.04 ± 0.02
20:00	0.48 ± 0.11	0.30 ± 0.01	0.37 ± 0.05	0.39 ± 0.05
20:1n-9	0.18 ± 0.01	0.13 ± 0.05	0.12 ± 0.01	0.14 ± 0.02
20:1n-7	1.78 ± 0.09[a]	1.33 ± 0.16[b]	1.44 ± 0.14[b]	1.37 ± 0.13[b]
20.1n-5	0.11 ± 0.03	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.00
20:2n-9	0.18 ± 0.05	0.12 ± 0.03	0.12 ± 0.02	0.09 ± 0.01
20:2n-6	0.99 ± 0.11	0.7 ± 0.01	0.90 ± 0.11	0.83 ± 0.04
20:3n-9	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02
20:3n-6	1.07 ± 0.13	1.00 ± 0.04	1.12 ± 0.03	0.96 ± 0.02
20:4n-6	2.41 ± 0.43[ab]	2.78 ± 0.45[a]	2.36 ± 0.11[ab]	1.82 ± 0.10[b]
20:3n-3	1.54 ± 0.17[a]	1.30 ± 0.02[a]	1.53 ± 0.02[b]	1.98 ± 0.09[b]
20:4n-3	0.51 ± 0.10	0.54 ± 0.06	0.46 ± 0.04	0.66 ± 0.05
20:5n-3	1.04 ± 0.21	1.25 ± 0.03	0.84 ± 0.08	0.88 ± 0.08
22:1n-11	0.47 ± 0.03	0.40 ± 0.26	0.24 ± 0.02	0.24 ± 0.07
22:1n-9	0.46 ± 0.09	0.45 ± 0.04	0.40 ± 0.09	0.38 ± 0.03
22:4n-6	0.67 ± 0.13	0.76 ± 0.03	0.67 ± 0.06	0.45 ± 0.05
22:5n-6	0.82 ± 0.16[ab]	1.03 ± 0.04[a]	0.89 ± 0.10[a]	0.57 ± 0.05[b]
22:5n-3	1.99 ± 0.31[a]	2.70 ± 0.14[b]	2.14 ± 0.35[a]	2.15 ± 0.07[a]
22:6n-3	10.01 ± 2.06[ab]	13.73 ± 1.28[a]	9.89 ± 0.97[ab]	8.30 ± 0.51[b]
[1]Saturades	22.97 ± 3.78	22.28 ± 4.28	25.49 ± 0.47	18.27 ± 1.75
[2]Monoenoics	32.30 ± 0.91[a]	27.74 ± 1.64[b]	30.03 ± 1.73[ab]	31.21 ± 0.89[ab]
[3]∑n-3	24.39 ± 3.31[a]	30.31 ± 2.36[b]	24.87 ± 1.77[a]	30.09 ± 1.52[b]
[4]∑n-6	18.69 ± 0.95	18.50 ± 0.33	18.41 ± 0.41	19.22 ± 0.37
[5]∑n-9	25.52 ± 0.79[a]	21.23 ± 2.04[b]	23.74 ± 1.46[ab]	25.8 ± 0.82[a]
[6]∑n-3 HUFA	15.09 ± 2.85[ab]	19.51 ± 1.47[a]	14.87 ± 1.41[b]	13.96 ± 0.48[b]
ARA/EPA	2.32 ± 0.05[a]	2.22 ± 0.31[a]	2.82 ± 0.16[b]	2.08 ± 0.12[a]
DHA/EPA	9.60 ± 0.09	11.01 ± 1.26	11.77 ± 0.51	9.56 ± 1.34
DHA/ARA	4.14 ± 0.12	5.08 ± 1.30	4.18 ± 0.23	4.58 ± 0.46
[7]Total PUFA	44.58 ± 4.69	49.85 ± 2.65	44.34 ± 1.28	50.39 ± 1.24
n-3/n-6	1.30 ± 0.11[b]	1.64 ± 0.10[a]	1.35 ± 0.12[ab]	1.57 ± 0.10[ab]

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

Table 11
Liver lipids and moisture from tilapias fed with banana flower extract and residue inclusion in Experiment III.

Diet	Control	BF3	BF3R	BF3E
Lipids (%dw)	60.04 ± 18.19	40.99 ± 9.00	54.50 ± 7.81	49.81 ± 11.43
Moisture (% dw)	69.17 ± 0.96	61.50 ± 6.16	66.64 ± 9.03	65.99 ± 5.74

diet, phenomena of hepatocyte dissociation accompanied by foci of necrosis occur and debris presence, although without drastically modifying the nuclear morphology. The peri hepatocyte detachment was maintained but it was less evident due to the loss of structure that increases eosinophilia, reminding us of the control diet. The count was conditioned by the presence of cells without preserved limits, therefore limited to those hepatocytes with intact cytoplasmatic limits. This less preserved structure also affected the vacuolisation level counting.

With the banana flower diets in Experiment II, the size of the hepatocytes increases in general, but it showed variability inside animals that prevents to determine a significant tendency (Table 12). The changes in the liver morphology were less noticeable including those referred to vacuolisation grade (Fig. 1) (Fig. 2).

Finally, no changes were registered in the size, morphology, or cellular distribution during Experiment III with the Banana flower extract and residue diets although an increase was found with the banana flower residue in the size of hepatocytes (Table 12).

3.4. Osmotic challenge

The fish survival during the osmotic challenge in experiment II was not significantly affected by the flower inclusion diets, during the osmotic challenge compared with the control diet (Fig. 3), the BF1 diet was removed from the challenge due to a technical problem with the system that could not be solved in time to perform the challenge. In the first 24 h of the challenge, what was observed is that the control and BF0.2

Table 12
Histopathological analysis of the liver from the Experiments I, II and III.

	Diet				
Experiment I	Control	BP2.5	BP5	BP15	BP20
Hepatocelular Area (μm)	38.21 ± 14.56^{a}	107.46 ± 34.57^{b}	83.37 ± 23.00^{c}	95.74 ± 30.22^{b}	35.84 ± 11.13^{a}
Max Length(μm)	7.61 ± 1.69^{a}	13.00 ± 2.53^{b}	11.74 ± 1.97^{c}	12.62 ± 2.33b^{c}	7.68 ± 1.51^{a}
Min Length(μm)	5.58 ± 1.28^{a}	9.82 ± 1.81^{b}	8.49 ± 1.55^{c}	9.08 ± 1.84^{c}	5.58 ± 0.99^{a}
Hepatocytes vacuolisation grade	1.94^{a}	1.22^{b}	1.22^{b}	1.06^{b}	1.83^{a}
Experiment II	Control	BF0.2	BF0.5	BF1	BF3
Hepatocelular Area (μm)	147.19 ± 46.56^{b}	160.11 ± 59.22^{ab}	140.92 ± 37.64^{b}	164.30 ± 42.36^{a}	162.52 ± 37.53^{a}
Max Length (μm)	15.15 ± 2.73^{bc}	15.90 ± 3.15^{ba}	14.78 ± 2.03^{c}	16.67 ± 2.79^{a}	16.62 ± 2.40^{a}
Min Length (μm)	11.19 ± 2.17^{b}	11.74 ± 2.55^{ab}	11.36 ± 2.07^{ab}	11.95 ± 2.01^{a}	12.01 ± 2.09^{a}
Hepatocytes vacuolisation grade	1.83 ± 0.82	1.88 ± 0.75	2.00 ± 0.35	2.25 ± 0.5	1.42 ± 0.58
Experiment III	Control	BF3	BF3R	BF3E	
Hepatocelular Area (μm)	127.03 ± 34.76^{b}	123.80 ± 36.40^{b}	125.40 ± 32.62^{a}	110.63 ± 37.68^{b}	
Max Length (μm)	13.81 ± 2.17^{b}	13.45 ± 2.26^{ab}	14.06 ± 2.11^{a}	13.02 ± 2.40^{b}	
Min Length (μm)	9.95 ± 2.50^{a}	10.11 ± 2.33^{a}	8.28 ± 2.65^{a}	9.36 ± 2.36^{b}	
Hepatocytes vacuolisation grade	1.92 ± 0.49	1.63 ± 0.95	3.00 ± 0.00	2.70 ± 0.27	

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

diets have the highest survival, although it is decreasing. In contrast, the BF3 and BF0.5 diets showed a stabilisation of mortality that was maintained over time. At the end of the challenge (48 h), even though there were no significant differences, the diet with the highest survival was BF3 with 50% of the fish alive, followed by BF0.5 with 42% and the control diet and BF0.5 presented the lowest total survival with 25% and 17% each.

In experiment III, in the first phase of the challenge with 50% saltwater, as it was expected, no mortality was recorded after five days, that was the reason to increase salinity to better observed the impact of the diets on the mortality during the osmotic challenge. The results obtained in the second part of the challenge with salt water 100% showed no significant influence but BF3 and BF3R gave 10% and 12% lower mortality relative to the control diet (Fig. 4), and the BF3E diet showed a 10% higher mortality than the control diet.

3.5. Plasma parameters

No cortisol peak was observed at 3 h of exposure in brackish water (21ppt), but there was an increase at 24 h. The basal cortisol (t0) in experiment II presented a higher level in diets BF3 and BF1 but at 3 h, only control, BF0.2 and BF0.5 were increased, BF1 and BF3 decreased the cortisol levels compared to 0 h. BF0.5 maintained the cortisol level more stable during the challenge. Plasma glucose was increased as the time in brackish water increased. At 0 h, the BF0.2 diet has the highest plasma glucose level, at 3 h there were no differences between the diets and at 24 h the BF1 diet had the highest level (Table 13). In Experiment III, a similar effect was observed between the control diet and BF3 diet, although there were no significant differences in any of the diets in the basal cortisol level. As in the previous experiment, there were no differences in glucose between the diets (Table 15).

3.6. Muscle and liver oxidation in Experiment II (MDA)

At the end of the feeding trial (t0), no influence of the diets was observed on the muscle oxidation, but at 24 h of being in brackish water (21ppt), oxidation was higher in tilapia fed the BF0.2 and BF0.5 diet meanwhile, as the BF inclusion was increased, oxidation was improved compared to the control diet. At the same time, the liver at 24 h showed a higher concentration of MDA in the BF3 diet, while it decreased in the BF1 and BF0.5 diets (Table 14).

3.7. Liver catalase

Catalase activity was not affected by the diets during the osmotic challenge in experiment II until 24 h, where BF0.2 was significantly lower than BF3. Values were in the range between 1712 and 4493 nmol/min/mL (Fig. 2) (Table 15).

4. Discussion

The use of banana by-products to replace cornmeal in feed for tilapia has some previous studies, but none have been carried out with secondary by-products from the processing of banana stems or with the male and hermaphrodite flowers of the banana inflorescence. The studies presented in this paper are the first attempt, to our knowledge, to

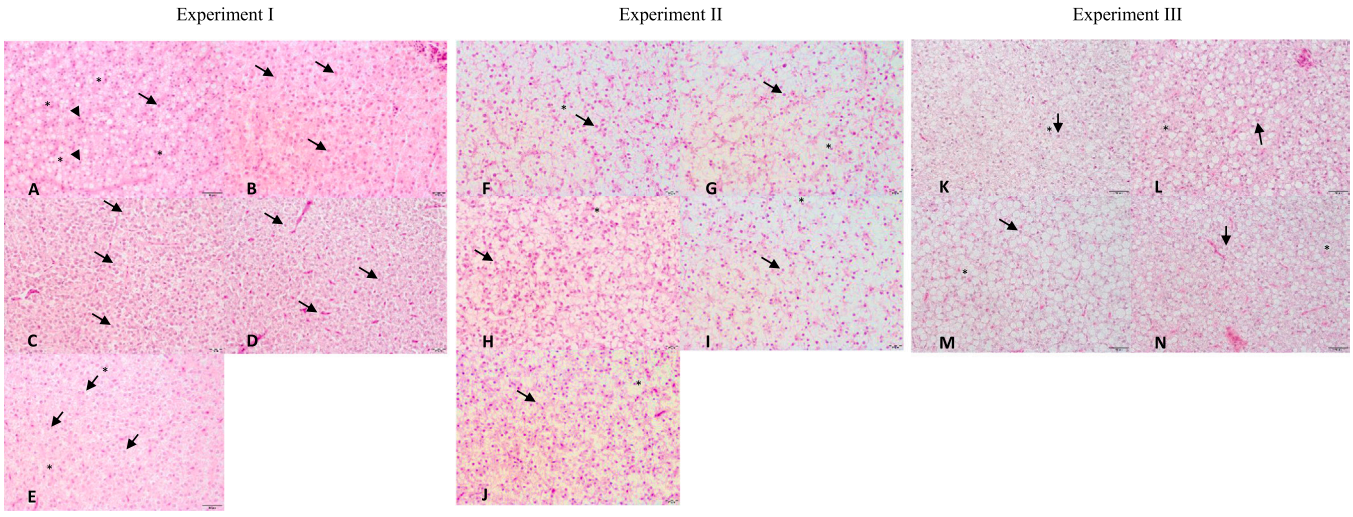

Fig. 1. Liver's micrograph (40x) from the three experiments. Experiment I: control diet (A), BP2.5 (B), BP5 (C), BP15 (d), BP20 (D). Experiment II: control diet (F), BF0.2 (G), BF0.5 (H), BF1 (I), BF3 (J). Experiment III: control diet (K), BF3 (L), BF3R (M), BF3E (N). Basophilic nuclei (▶), functional nuclei (→) and lipid droplets (*).

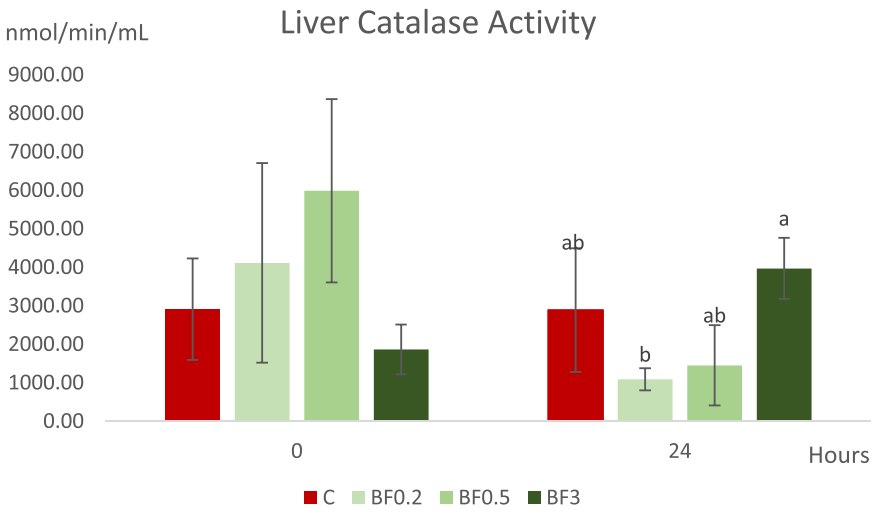

Fig. 2. Liver catalase activity at the beginning (t0) and at 24 h of the salinity challenge in the Experiment II (brackish water 21 ppt). Different letter indicates significant differences (p-value<0.05).

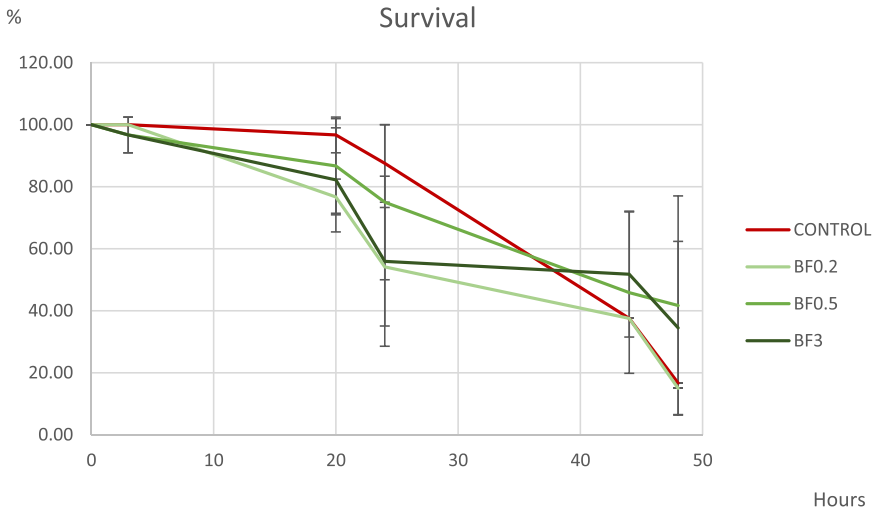

Fig. 3. Survival measured during salinity challenge (21ppt) in the Experiment II. Different letter indicates significant differences (p-value<0.05).

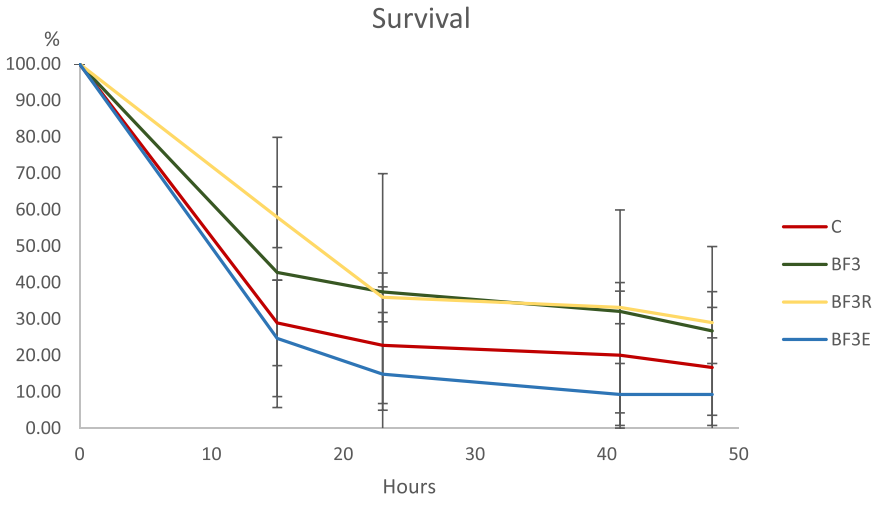

Fig. 4. Survival measured during the second phase of the salinity challenge (35 ppt) in the Experiment III.

Table 13
Plasma cortisol and glucose content during the osmotic challenge in the Experiment II (21 ppt salinity).

DIET	Control	BF0.2	BF0.5	BF3
0 h				
Cortisol (ug/dL)	10.85 ± 5.99[b]	9.35 ± 5.11[b]	16.02 ± 5.42[ab]	22.98 ± 6.61[a]
Glucose (mg/dL)	36.67 ± 4.46[b]	52.33 ± 3.33[a]	36.67 ± 5.72[b]	41.17 ± 8.30[b]
3 h				
Cortisol (ug/dL)	13.20 ± 9.87	18.98 ± 11.16	16.62 ± 17.94	11.39 ± 4.57
Glucose (mg/dL)	103.50 ± 20.54	114.40 ± 11.55	107.17 ± 43.47	104.80 ± 22.92
24 h				
Cortisol (ug/dL)	30.18 ± 4.28	26.07 ± 7.28	29.22 ± 9.95	26.02 ± 10.30
Glucose (mg/dL)	198.67 ± 53.45[a]	270.50 ± 54.45[a]	195.00 ± 7.07[b]	195.50 ± 55.94[ab]

Table 14
Muscle and liver MDA concentration in lipids, as indicator of oxidation status at the end of the feeding trial (0 h) and in muscle & liver after 24 h in brackish water (21ppt) in Experiment II.

	Control	BF0.2	BF0.5	BF3
Muscle				
0 h	82.44 ± 9.39	49.77 ± 32.75	119.73 ± 88.35	77.15 ± 59.02
24 h	95.73 ± 35.42[b]	148.41 ± 25.64[a]	166.23 ± 42.42[a]	135.84 ± 59.84[ab]
Liver				
24 h	73.25 ± 35.87[ab]	69.09 ± 62.99[ab]	34.66 ± 12.85[b]	123.44 ± 43.19[a]

Different letters indicate significant differences (p-value<0.05).

Table 15
Plasma cortisol and glucose content at the end of the feeding period of Experiment III, before the osmotic challenge.

DIET	Control	BF3	BF3R	BF3E
Cortisol (ug/dL)	15.4 ± 5.85	23.81 ± 14.96	32.13 ± 8.26	22.53 ± 10.44
Glucose (mg/dL)	51.00 ± 6.99	41.29 ± 6.07	50.75 ± 6.99	45.50 ± 5.48

explore this pathway of by-product recirculation. The inclusion of dietary plant ingredients has been shown to enhance the growth performance of tilapia in most cases, such as Aloe vera, garlic or orange-peel oils (Metwally, 2009; Acar et al., 2015; Gabriel et al., 2015).

In the case of banana discharges, reported results in tilapia showed different behaviours depending not only on the type of by-product but on the species under study. Thus, Karaket et al. (2021) found that despite the good acceptance of the feed by the fish only up to 5% of ripe bananas did not affect the growth of hybrid tilapia, with a similar size to the fish from Experiments I and II, while Yossa et al. (2021) obtained good results up to 30% in the case of banana peel tested in GIFT tilapia slightly larger in size (22 g initial weight). In the same way, results in the present work in juvenile Nile tilapia depend on the materials under study, wherein the maximum dietary level with no damage in growth parameters seems to be 3% for banana flower and up to 5% for hydrolysed banana pseudo-stem with similar FCR but less growth of the fish in the later. A factor to consider in Experiment I is the high amount of ash present in the BP meal, which could be explained due to the salt generation as a result of the neutralisation process. Diet ash increases as the inclusion of BP increases, being from 15% higher than the control diet. The presence of this large amount of ash in the meal may be conditioning the response of the animals, thus with up to a maximum of 5% BP inclusion supported in the present study. According to Karaket et al. (2021) also in the tilapia trial, small levels of BP like 5% BP (about 10% whole dietary ash from which less than 2.5% comes from BP) in the present case may contribute to the whole dietary mineral balance, while over 5% BP levels (over 7.5% and 10% BP mineral contribution), the mineral content in diets increased and could prejudice the juveniles´ performance due to an excess of accessible minerals with high bioavailability that affected the digestibility of the nutrients in the diet (Lall and Kaushik, 2021). Moreover, the diluted sulphuric hydrolysis and final NaOH neutralisation process, used towards a better carbohydrate bioavailability for the banana pseudo-stem, may also change and affect the minerals profile and minerals availability, but also, increasing digestive the pH content by increase the final salt mineral complex (Hu et al., 2014), affecting the reported normal pH in the tilapia digestive tract (Payne, 1978).

Also, the 3% extract of banana flower and the residue did not negatively affect the growth performance in Experiment III, even when the size of the juveniles was reduced, to see if the smaller fish respond to the diets in the same way. Thus, results for this novel dietary by-product are in line with previous ones, with no high inclusion levels supported by the fish towards the fish growth. A similar observation was made in the tambaqui (*Colossoma macropomum*), for with over 16% banana meal inclusion reduced growth (Felix e Silva et al., 2020).

The liver does not appear to be damaged by the inclusion of any of the by-products, no lesions or signs of toxicity were observed in the treatments, and at least up to 15% banana pseudo-stem inclusion, which is consistent with results described in Nile tilapia fed mature banana (Palintorn et al., 2019). The only diet that showed some structural damage and necrosis foci was the 20% inclusion of banana pseudo-stem. The poor results obtained in this diet were conditioned by this fact, as the methodology is limited by the integrity of the cell structure at the time of measurement. Furthermore, these results were consistent with those observed with high soy feed (Khieokhajonkhet et al., 2021) in hybrid tilapia hepatocytes, where as the level of soy inclusion increased, the vacuolisation also increased. The decrease in the degree of vacuolisation as by-product inclusion increases seems to be related to the lower lipid content in the liver which may be a response to a poor lipid digestibility coefficient as has been described for banana peel (Yossa et al., 2021). The fibre provided by BP may decrease lipid adsorption which is reflected in the decrease of total lipids in both liver and muscle, which is in agreement with that found by Yossa et al. (2021), with a reduction of the lipid digestibility coefficient when feeding banana peel to GIFT tilapia. The undescribed amount of NSP which we assume to be high in BP, due to the chemical treatment that could facilitate the accessibility to the carbohydrates, influences juveniles' performance and lipid digestibility (Maas et al., 2020) related to possible modification of gut morphology and mucus viscosity, which is evident with inclusions higher than 5%. Lower inclusions, which show an increase in essential fatty acids, despite the decrease in total lipids in both muscle and fillet, do not show such a pronounced effect on growth, which according to Maas et al. (2020) may result from the fermentation of a fraction of the NSP present in the hydrolysed banana pseudo-stem, producing short fatty acids that could be rapidly absorbed and used to meet energy demands. A consequence of the decrease in total lipids is that the synthesis of LC n-3 PUFA from 18:2n-6 and 18:3n-3, especially EPA, ARA and DHA, increases (Olsen et al., 1990; Teoh et al., 2011; Chen et al., 2018), thus maintaining the proportions of these fatty acids despite the decrease in total lipids. Another sign of lipid mobilisation with both BP and flower extract and its residue is that VSI decreases with the inclusion of these ingredients. Even so, the inclusion of pseudo-stem can reduce the energy of diets affecting growth (Maina et al., 2003), which coincides with what was observed from diet BP15, where there is a decrease in growth. Furthermore, the BP20 diet fails to synthesise

enough DHA to compensate for the drop in total lipids. This may be a sign of loss of functionality, which coincides with the necrosis observed in the histopathological analysis for fish fed on this diet.

The non-effect of the flower on the fatty acid profile may be due to insufficient inclusion levels to reflect the DHA supply from the flower meal (Ramírez-Bolaños et al., 2021), so although not the main objective in the present case, studies with higher inclusion would be interesting to see if there is an influence of the flower on the fillet quality of the tilapia. Since no effects on liver fatty acids were observed for BF, fatty acid profiling was not considered necessary in experiment III. Interestingly, in muscle, the different results obtained depend on the flower extract which may indicate the nature of the bioactive causing these effects. The decrease in all diets with banana flower and derivates of 20:1n-7 fatty acid, and monoenoic acids in the BF3 diet may indicate the use of these fatty acids in the animal's energy demand (Varga et al., 2020). In the diet with the flower extract, the decrease in ARA and DHA and the high levels of LNA and ALA indicate that there is no stimulation of desaturases and elongases for the production of essential fatty acids (Teoh et al., 2011). In light of these results, it can be inferred that the bioactive that enhances the increase of DHA in fish fed with the flower diets is located in the extract residue (BF3R).

A salinity challenge is a good option to determine whether the test materials used in this experiment may improve the response and adaptation of tilapia under a challenge and to determine where the benefit is in the flower, by testing general indicators of the stress response such as the plasma cortisol level. Immersion in water with a salinity of 25 ppt for at least 24 h had an immunostimulatory effect on Mozambique tilapia (Jiang et al., 2008); after 3 h of exposure, cortisol increases in response to induced stress and may cause the observed changes in cell structure, allowing fish adaptation to that stress (Kammerer et al., 2010). In the present study, the differences observed in t0 for higher flower inclusion disappeared during the challenge. At t3 the cortisol levels among the treatments were equal, but the reaction of the fish fed with BF1 and BF3 diets was to reduce the cortisol compared to t0. At t24h, all the treatments increased the cortisol levels above basal, except for BF1 and BF3 which maintained cortisol at the same basal level. This trend in cortisol behaviour at the end of the feeding trial (equivalent to t0) was observed also in Experiment III, where all diets with banana flower, extract and residue slightly increased basal cortisol, being the residue the diet with the higher cortisol. This can be due to the presence of a component in the flower that could be in higher proportion at the residue. Based on the previous study performed with the banana flower characterisation, the high quantity of polyphenols present in the banana flower could influence the stress response, as it was established in other studies where the high amount of polyphenols was determined as the cause of the decreasing cortisol with pomegranate peel (Hamed and Abdel-Tawwab, 2021) and also with specific studies with ferulic acid inclusion where the basal cortisol was decreased from 20 mg/kg and after the heat stress, the lower values of cortisol corresponded to 80 mg/kg of ferulic acid inclusion (Dawood et al., 2020).

Glucose increased with time in brackish water. In Experiment II (21 ppt), the banana flower inclusion increased plasma glucose related to the control diet, contrary to what was observed in previous studies (Khan et al., 2022; Uma et al., 2022) with other plants by-products, but coincides with aloe effect as it increased serum glucose levels (Gabriel et al., 2015). Otherwise, at 18ppt in Experiment III, BF3 and extract confirm the tendency of decreasing plasm glucose level. This may be due to the presence of a large number of polyphenols in banana flower that could act as glucose regulators as pomegranate peel (Hamed and Abdel-Tawwab, 2021), and, specifically, protocatechuic acid, which is the main polyphenol identified in the banana flower and its extract (Ramírez-Bolaños et al., 2021), which has been observed to have a regulating effect on hyperglycaemia in mice (Talagavadi et al., 2016; D'Archivio et al., 2018). Furthermore, other bioactive that could be present in the banana flower may act as a glucose regulator (Hamed and Abdel-Tawwab, 2021). Also, a high glucose plasma level may indicate an increasing demand for energy in the cells to respond to the stress as was reported by Hassaan et al. (2021)).

According to these results, survival during the challenges was not significantly affected by the experimental diets, even the higher total survival observed at the end of the challenge in Experiment II with BF3 and BF0.5 (25% and 17% lower mortality concerning control diet), which may suggest that the benefits provided by the banana flower manifest itself over time. Other plant extracts like bougainvillaea and pineapple fibre increased survival in challenges with pathogens (Van Doan et al., 2021; Uma et al., 2022), so further and different studies must be carried out to clarify the possible benefits of banana flower under fish salinity resistance or even with other types of stress conditions, as it was done in Experiment III. The results obtained in Experiment III agreed with the previous one, despite the smaller size of the fish (7 g) and indicating again the possible advantage of the banana flower which may be located in the residue after the organic extraction. This brings up the possibility of increasing the amount of flower and residue to improve the potential benefits detected in these challenges. On the other hand, the extract seems to slightly impairs survival (10% lower compared to the control). Although not many studies are done with polyphenols absorption patron, the introduction of the feeding bioactive without a matrix containing them, may influences absorption and cause negative effects (Parada and Aguilera, 2007); this effect, not yet determined in fish, should be further studied.

Mannonaldheide concentration is an indicator of lipid oxidation status in the liver and in the muscle that increases with salinity (Sutthi and Thaimuangphol, 2020; Mohamed et al., 2021). Polyphenols present in pomegranate and dietary ferulic acid decrease oxidation (Dawood et al., 2020; Hamed and Abdel-Tawwab, 2021), which coincides with the results obtained in the current study, especially in the muscle, where the higher BF inclusions presented the best oxidation values with the control diet. CAT activity in the liver is an indicator of the hepatocytes reducing the reactive oxygen molecules in response to stress (Hegazi et al., 2010). At 24 h, CAT activity was higher in fish fed the BF3 diet, which is under the results obtained in tilapia with pomegranate, probably due to the polyphenols present in the peel (Hamed and Abdel-Tawwab, 2021). Concerning the compounds present in the banana flower, the role of polyphenols and other bioactive in the regulation of cortisol and glucose levels and the influence on the oxidative status of fish needs to be studied more closely.

In conclusion, BP and BF, which account for a high amount of the discharges from banana production, can be introduced up to 5% and 3%, respectively, in juvenile tilapia feeds. BP inclusion seems to reduce fish growth with similar FCR while reducing lipid contenct both in muscle and liver, from wich higher n-3 and n-3 HUFA was obtained in the first case concerning the control group. Over 5% BP can affect negatively in two ways, the high mineral content which can affect the mineral profile, digestive pH and nutrient digestibility, then, on the other hand, NSP and knowns effects on gut morphology and the absorption of lipids. Thus, BP processing must consider the bioaccessibility of carbohydrates and the amount and availability of minerals. BF did not affect fish growth or fish composition, with higher basal cortisol levels observed for BF3 fish. The inclusion of the 3% flower also provides some benefits in terms of survival over time under osmotic stress. In this regard, it is of interest to determine the bioactive present in the banana flower residue that appears to provide advantages during an osmotic challenge, as does the flower, and stimulate the activity of fatty acid elongases and desaturases. For BF opportunities for higher levels of inclusions should be assayed.

CRediT authorship contribution statement

Sara Ramírez-Bolaños: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Sara Díaz**: Investigation, Writing – review & editing. **Anais Ventura-Castellano**: Investigation, Writing – review & editing. **Raquel Quirós-Pozo**:

Investigation, Writing – review & editing. **Álvaro Rodríguez-Rodríguez**: Investigation. **Pedro Castro**: Supervision, Visualization, Writing – review & editing. **Lidia Robaina**: Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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