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ANIMAL SCIENCE

Curcuma longa hydrolate improves Nile tilapia survival in a recirculation rearing system, maintaining the animal homeostasis and modulating the gut microbial community

MARINA O. PEREIRA, JULIA D. HESS, JULIO CESAR B. RODHERMEL, DANIEL R. FARIAS, DELANO D. SCHLEDER, LUCIANO ALVES, FABIANO C. BERTOLDI, AMANDA CHABAN, JAQUELINE I.A. DE ANDRADE & ADOLFO JATOBÁ

Abstract: This study aimed to evaluate the effects of the dietary supplementation *Curcuma longa* hydrolate on Nile tilapia (*Oreochromis niloticus*) reared in a recirculation system. Hemato-immunological parameters, growth performance, nitrogen and phosphorus retention, as well as body composition and its interaction with the intestinal microbiota, were studied. Nile tilapia fingerlings (120) were distributed randomly in 8 polyethylene tanks (40 L). The experimental units were divided into two treatments, in quadruplicate: commercial diet supplemented with 2.5% of *C. longa* hydrolate and commercial diet without supplementation (control). After 45 days, the treatment supplemented hydrolate showed higher survival than the control group, 95.25% and 82.22%, receptively. In the blood profile, fish supplemented with hydrolate had a higher count of total leukocytes and neutrophils, as well as mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, than control group. The hydrolate group showed a substantial increase in the relative abundance of *Cetobacterium* and *Romboutsia*, as well as lower diversity in gut microbiota. The dietary addition of *C. longa* hydrolate for Nile tilapia seems to have a beneficial effect on gut microbiota, in addition to a likely positive effect on the physiological performance of Nile tilapia by maintaining intestinal homeostasis and promoting survival in reared conditions.

Key words: aquaculture, curcumin, herbal medicines immunomodulatory, *Oreochromis niloticus*.

INTRODUCTION

Aquaculture has become the main activity for the provision of fishery, increasing in production by 60% between 2007 and 2017 and ensuring an adequate supply of aquatic animal protein to the growing world population (Ocde/Fao). The worldwide production rose from 50 million / t / year to 80 million / t, while Brazilian production reached 722,560 tons in 2018 (Anuário Peixe-BR 2021).

Fish farming in Brazil produced 802 thousand tons in 2020, Nile tilapia (*Oreochromis niloticus*) is the most produced species in Brazil, representing more than 55.0% of national production, thereby consolidating the country as the fourth largest tilapia producer in the world (Anuário Peixe-BR 2021). This growth in production could be associated with the fast growth Nile tilapia, its ability to adapt to artificial food and intensive production systems, in addition to its acceptance by the consumer market based on being considered a source

of healthy animal protein. (Lunkes et al. 2018, Vicente et al. 2014).

However, in recent years, the intensification of aquaculture systems associated with high stocking densities have contributed to increase outbreak disease and its transmission. As a treatment, chemotherapeutic substances are frequently used, but the indiscriminate use of these drugs, coupled with poor administration and failed management techniques, has resulted in the selection of microorganisms resistant to a wide variety of active principles (Cabello et al. 2013). This situation calls for a review and improvement of management techniques, especially preventive ones. Several simple options are possible, including liming, monitoring of water quality parameters, asepsis of the materials used in the tanks/ponds, as well as the application of vaccines, probiotics and food additives (Jatobá et al. 2016).

World associations, such as the World Organization for Animal Health and Codex Alimentarius, have called on all sectors of animal production to engage in a global campaign to reduce antimicrobials based on a unique concept of health which links humans, animals and the environment (Torres 2019). Concomitantly, demand has grown for certified products, ensuring that a given product has been reared within the standards of environmental and social responsibility, food safety, animal welfare and traceability of the entire production cycle (Bergleiter & Simon 2015).

Thus, it is important to search for alternative products, such as herbal medicines, that are of natural origin. They can be presented in the form of extracts, essential oils or hydrolate, all of which have some pharmacological properties like antimicrobial (De Souza et al. 2020), antiparasitic (Andrade et al. 2018) and immunomodulatory (Pereora et al. 2020) activity in fish.

Turmeric (*Curcuma longa*), one of the precious spices of world cuisine, is also used to treat many diseases because it has antioxidant, immunomodulatory, anti-inflammatory and tumor cell suppression effects (Kocaadam & Şanlier 2017). As one of its main components, this plant has curcumin which has antimicrobial activity against pathological bacteria, as reported in many studies, including those focused on aquaculture (Singh et al. 2017). For instance, it has promoted higher tolerance to stress during the transport of yellow-tailed lambari (Ferreira et al. 2017) and better zootechnical and immune performance of Nile tilapia (Abdel-Tawwab & Abbass 2017).

In particular, hydrolate is the aqueous fraction that contains the emulsified essential oil, and it is usually discarded, but many studies have proven the efficiency of this by-product. Catão et al. (2018), for example, observed the fungicidal activity of *Lippia menosides* hydrolate on the storage of castor seeds (*Ricinus communis*). In addition, Mello et al. (2015) verified the effectiveness of garden rosemary hydrolate (*Rosmarinus officinalis*) to control ticks, while the essential oil of this perennial evergreen shrub with blue flowers has shown less acaricidal activity, validating the effectiveness of its hydrolate by-product, which has, so far, been neglected.

The intestine plays an important role for farm animals, including fish, as it acts directly on the digestion and absorption of nutrients, but it also has a relevant function in the immune system (Salinas & Parra 2015). Many infectious diseases are caused by pathogenic bacteria that break down intestinal homeostasis, affecting commensal bacteria in that area and affecting epithelial integrity. In their study with Nile tilapia that fed a diet supplemented with turmeric, Yusuf et al. (2017) evidenced an increase in intestinal villi, a decrease in the total

bacteria count and an increase in *Lactobacillus* spp. compared to the control group, observing a better zootechnical performance in the group supplemented with 2g / Kg of *curcuma longa* powder. Furthermore, *C. longa* hydrolate changed the hematological and immunological parameters of Nile tilapia (*O. niloticus*) fingerlings, keeping fish healthy (Pereira et al. 2020). Therefore, this study aimed to evaluate the effects of the dietary supplementation *Curcuma longa* hydrolate on Nile tilapia (*Oreochromis niloticus*) reared in a recirculation system. Hemato-immunological parameters, growth performance, nitrogen and phosphorus retention, as well as body composition and its interaction with the intestinal microbiota, were studied.

MATERIALS AND METHODS

This study was carried out in the Laboratório de Aquicultura (Laq) do Instituto Federal Catarinense (IFC), Campus Araquari, and it was approved by the National Council for the Control of Animal Experimentation (CONCEA) under protocol number 157/2016.

Plant material

The botanical species used in this work was grown in the Medical Plants Unit at the IFC, located at 26° 23' 33.6691" S and 48° 44' 18.3336" W, at 10.6 m above sea level, City of Araquari, Santa Catarina State, southern Brazil. Plants were cultivated in an agroecological system without the application of agrochemical products. Tumeric (*Curcuma longa*) was collected from approximately 50 individuals. A voucher specimen of the botanical material was deposited at the Botanical Museum Herbarium located in the Botanic Garden of Curitiba, PR, with the number 358970.

Obtaining the hydrolate

C. longa hydrolate, a by-product of EO distillation, was achieved by hydrodistillation (Venskutonis et al. 1997) using the Clevenger apparatus adapted to a 2000 mL flask, in which the mint leaves were placed together with 1000 mL of distilled water according to Coradi et al. (2018). Fresh *Curcuma longa* (150g) was used for each distillation. The extraction time was 90 min, starting from the time of boiling. After the hydrolate (mixture of oil and water) was obtained, separation of the essential oil was initiated using the organic solvent pentane (3 x50 mL) in a separating funnel. After a few minutes of resting, the solution was filtered and concentrated on a rotary evaporator at 40°C until the solvent volume was significantly reduced.

The hydrolate obtained from the hydrodistillation of *C. longa* was treated with chromatographic grade hexane, followed by performing a liquid-liquid extraction with approximately 2 ml of hexane for 100 mL of hydrolate in a separating funnel. A 500 µL fraction of the organic phase was collected to perform a chemical analysis of the volatile components of the hydrolate of *C. longa* by gas chromatography with a mass spectrometry detector (GCMS, Shimadzu, model GCMS - QP2010). A ZB-5MS capillary column (30 m x 0.25 mm x 0.25 µm film) was used. Injector temperature was 250 ° C, and helium carrier gas flow was 1.0 $mL.min^{-1}$. The chromatograph oven was optimized with an initial temperature of 60 °C for 4 minutes until reaching 210 °C and then remaining for 6 minutes, totaling 35 minutes in the complete chromatographic run. A fraction extracted from hydrolate was diluted 2 times in chromatographic grade hexane for subsequent injection of 1µL in GCMS. The quantification of each component was determined by normalizing the areas (%) of the peaks in the total ion chromatogram (TIC),

with the total area being the sum of all areas of the eluted peaks (100%).

Retention rates were calculated according to Dool & Kratz (1963) from n-alkane standards (C7-C30) under the same chromatographic conditions as samples of essential oils. The identification by GCMS was based on the comparison of mass spectra with NIST-05 data libraries and also by comparing the retention rates calculated with those found in the literature (databases - WebNIST, GMD).

Experimental design and management

The research was performed using 120 Nile tilapia fingerlings (*Oreochromis niloticus*) with a mean weight of 0.85 ± 0.08 g. Fingerlings were distributed randomly in 8 polyethylene tanks (40 L) equipped with constant water renewal in an aquaculture recirculation system. The experimental units were divided into two treatments, in quadruplicate: commercial diet supplemented with 2.5% of *C. longa* hydrolate, according to the protocols established by Pereira et al. (2020), and commercial diet without supplementation (control).

Fish were fed a commercial diet three times a day (08:00, 12:00 and 16:00) (GUABI®, 1.0mm, 45% crude protein and 8.0% ethereal extract, manufacturer's warranty levels), with 3% of their biomass, and biometrics was performed weekly for food management, while pH and ammonia were measured weekly. The water quality parameters were as follows: dissolved oxygen above 4.5 mg L^{-1} and temperature 22.21±1.71° C (YSI55 oximeter); alkalinity between 88.3 – 142.1 mg $CaCo_2$ total L^{-1}; ammonia total 0.22±0.09 mg L^{-1}; nitrite 0.05±0.06 mg L^{-1} and pH 7.36±0.09, all measured twice a week according to APHA (2005).

Data collection and growth performance

After 45 days of rearing and a 24 h period of starvation, 10 fish per experimental unit were anesthetized with Eugenol (50 mg.L^{-1}) and euthanized by cerebral concussion. Five were used for hematological and immunological assays, and the other five for body analysis. The first three animals per experimental unit used for blood sampling for hematological and immunological assays were also used to collect gut content for metagenomic analysis. The gut content from nine fish per treatment (three per experimental unit) was gathered to make a single sample pool (200 mg) prior to total DNA extraction and further analyses. In addition, a final biometry was performed, and hepatosomatic index (liver weight / body weight * 100), specific growth rate (final weight - initial weight / initial weight), protein efficiency rate (weight gain / total consumption of protein), yield and survival were determined according Jatobá et al. (2014).

Hematological and immunological analysis

For hematological analysis, approximately 0.5 mL of blood was drawn from the caudal vein of each fish for the preparation of blood smears, in duplicate, and the following hematological analyses were performed: determination of hematocrit by the standard microhematocrit method; glucose (G-TECH free®, Accumed-Glicomed, Brazil); total hemocyte count by Neubauer hemocytometer and hemoglobin concentration. Hematimetric absolute rates of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also obtained. Blood smear slides were stained with May-Grünwald-Giemsa (MGG) stain (Ranzani-Paiva et al. 2013) for total and differential leukocyte count (Jatobá et al. 2011).

From the same fish used in the hematological assay, total blood was also collected without anticoagulant to obtain the blood serum used for immunological analyses. Lysozyme activity was measured using spectrophotometry according to Ellis (1990), with lyophilized chicken egg white as standard. For concentration of total plasma protein, a commercial kit (Lab Test ®) was used, with bovine albumin for the standard curve. Total immunoglobulin concentration was assessed according to the method of Amar et al. (2000).

Body centesimal composition and nutrient retention

For body analyses, 60 fish (20 initial fish, plus 5 per experimental unit, totaling 20 per treatmen) and diet samples were sent to CBO Análises Laboratoriais (Campinas, SP, Brazil) to measure crude protein contents using the Dumas nitrogen combustion method, crude phosphorus. Crude lipids were quantified using the ether extraction method, and ash and moisture were determined using AOAC (2005). These data were used for further analysis and calculation of nitrogen retention, phosphorus retention, and protein efficiency ratio, according to Silva et al. (2016).

Intestinal microbiota assay

Total DNA extraction, amplification of V3-V4 region of the 16S rRNA gene, and Ilumina MiSeq sequencing (300 bp single-end reads) were performed following the protocol established by Neoprospectra Microbiome Technologies, Brazil, and data analyses followed the method described by Schleder et al. (2020), using Usearch (version 10.0.240) in the R environment. The taxonomic assignment of bacterial sequences was conducted using the SILVA database at 90% identity (version 132).

Statistical analyses

All data were first subjected to Bartlett's analysis to verify the homogeneity of variance. Data were assessed by Student's *t*-test, with 5% level of significance. Gut microbial data were analyzed with R statistical environment (version 3.5.1).

RESULTS

Thirteen constituents of *C. longa* hydrolate were identified, representing approximately 93.50% of purity, the major compounds being ar-turmerone, α-tumerone and α-curcumene (Table I).

After 45 days, the treatment supplemented with *C. longa* hydrolate showed higher survival than the control group, while the other productive indices (final mean weight, productivity, specific growth rate, feed conversion, protein retention) and nutrient retention (N and P) did not differ between treatments (Table II). Viscerosomatic index, hepatosomatic index and body composition (moisture, crude protein, crude lipid and ash) also did not differ between treatments (Table III).

In the blood profile, fish supplemented with *C. longa* had a higher count of total leukocytes and neutrophils, as well as mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, compared to the control group. However, the control showed higher hematocrit, mean corpuscular volume and glucose than these same values in the supplemented group. The other hematological and immunological parameters did not differ between treatments (Table IV).

Raw reads were processed to remove low quality bases, adapters, and trimmed to a fixed length of 280 bp (Trimmomatic, version 0.36). Following quality filtering, 80.5% of the reads were retained for downstream analysis (average

Table I. Volatile profile of *Curcuma longa* hydrolate.

Components	RIc	RIl	Percentage (%)
α-Phellandrene	1007	1007	0.60
p-Cymene	1026	1025	0.87
1.8-cineol	1034	1033	3.73
Terpinolene	1086	1086	0.54
α-Curcumene	1484	1483	12.11
α-Zingiberene	1497	1495	6.58
β-Bisabolene	1511	1509	3.72
β.-Sesquiphellandrene	1527	1526	11.66
ar-tumerone	1666	1669	27.31
αTumerone	1672	1675	14.21
Germacrone	1700	1702	2.45
Curlone	1705	1705	9.72
Total identified	-	-	93.50

RIc = Retention index calculated, RIl = Retention index of literature, % = percentage of the component in the volatile fraction of the hydrolate.

of sequences per sample: 81.491). The rarefaction curve and the goods coverage index (min/max = 100.0) indicated complete sampling of the bacterial communities in the samples.

The hydrolate group showed a substantial increase in the relative abundance of *Cetobacterium* and *Romboutsia*, reflecting higher occurrence of the families Fusobacteraceae and Peptostreptococcaceae and the phyla Fusobacteria and Firmicutes, respectively, in this treatment. Additionally, hydrolate supplementation led to a decrease in the relative abundance of other bacteria, such as *Hyphomicrobium* and *Cryocola*, and, consequently, their families (Hyphomicrobiaceae and Microbacteriaceae, respectively) and phyla (Proteobacteria and Actinobacteria, respectively) (Figure 1).

Despite slightly higher richness, the gut microbiota from fish fed diets containing *C. longa* hydrolate showed lower diversity, most likely from a higher abundance of *Cetobacterium* and *Romboutsia* to the detriment to other bacterial groups (Table V).

DISCUSSION

The chemical composition of essential oils and hydrolates is determined by genetic factors of the plant; however, other factors can cause significant changes in the production of secondary metabolites, such as climate, altitude, nutrition, maturation stage and harvest time (Pettenazzi et al. 2019). In studies carried out by Majolo et al. (2014), αr-tumerone and α-tumerone were also observed as major compounds in tumeric oil, albeit in different percentages, 21.0% and 33.5%, respectively. These differences may have arisen from the use of hydrolate in the present work, whereas Majolo et al. (2014) used the essential oil that commonly has a higher concentration of these compounds.

Analysis of animal growth performance is an important tool during rearing, and it can be used to develop new feeding strategies during the growth of fish, as well as increase yield and profitability. In Table II, average weight, specific growth rate, feed conversion rate, protein and nutrient retention (N and P) did not differ

Table II. Growth performance and nutrient retention of Nile tilapia (*Oreochromis niloticus*) fed a supplemented hydrolate diet from *Curcuma longa*.

Parameters	Hydrolate	Control	Significance (p)
Final mean weight (g)	14.52 ± 0.76	15.64 ± 1.76	0.222
Survival (%)	95.26 ± 5.29*	82.22 ± 7.20	0.036
Yield ($g.m^{-3}$)	260.91 ± 33.35	238.76 ± 7.46	0.195
Specific growth rate ($\%.day^{-1}$)	2.21 ± 0.05	2.27 ± 0.10	0.223
Feed conversation	1.10 ± 0.19	1.06 ± 0.07	0.385
Protein Retention	0.84 ± 0.05	0.83 ± 0.06	0.294
N Retention (%)	28.01 ± 1.54	27.84 ± 2.39	0.336
P Retention (%)	29.24 ± 3.86	30.52 ± 2.24	0.362

*** Indicates significant differences in the t-test.**

between treatments. This work showed that hydrolate supplementation did not present toxicity to fingerlings. In other words, hydrolate did not negatively affect growth performance. These results suggest that the *C. longa* hydrolate, at a concentration of 2.5%, can be used safely as a food additive. This result corroborates the study of Pereira et al. (2020), showing no differences in weight gain and specific growth rate in Nile tilapia (from 7.45 to 104.03 g) fed with a diet supplemented with same hydrolate used in this work, in different doses (0.0, 2.5, 7.5 and 10.0%).

The higher survival of the group supplemented with hydrolate could be supported by Al-Faragi & Hassan (2017), that fed juvenile common carp (*Cyprinus carpio*) with diet supplemented with *C. longa* powder obtained higher survival in reared condition and after infection with *Flexibacter columnaris*, a bacterium responsible for causing great losses in this life stage.

Fingerlings and new juveniles are very susceptible to several environmental factors that can lead to the proliferation of diseases and, consequently, economic losses. Moreover, fingerlings are sold by unit (Becker 2018), survival becomes one of the most important productive indexes to be evaluated during rearing, because total number or fish is more important than biomass or yield. During these initial phases, several practices can cause the breakdown of the animal's homeostasis, such as capture, biometrics and transport (Urbinati et al. 2014). With this in mind, the use of food additives that promote the survival of tilapia in these phases is extremely important, and the results of this work show the possibility of using *C. longa* hydrolate for this purpose.

About bromatological composition, the same result was observed in the use of essential oil of oregano as an additive for juvenile *Rhamdia quelen*, showing that the herbal medicine did not modify metabolism, keeping moisture, lipids, proteins or ash levels equal (Cararo et al. 2017). The same was observed for body indexes, which did not differ between treatments. This result supports a previous study that showed no statistical difference among different doses of *C. longa* hydrolate, demonstrating that this supplementation has no effect on hepatic metabolism or the accumulation of visceral fat (Pereira et al. 2020).

Hematological parameters are considered essential to assess the general health status of several fish species. Such analysis is considered the quickest way to detect symptoms of physiological stress and identify the presence of disease (Ranzani-Paiva et al. 2013), in addition

to evaluating the effect of food ingredients and additives.

Among the parameters are glucose, erythrocytes, hematocrit and hemoglobin, which can be affected by several factors, with diet being the most important, followed by fish stress and health status (Barton & Iwana 1991). These results agree with the study by Ferreira et al. (2017) who observed that supplementation with turmeric powder was effective in reducing stress responses and improving the antioxidant status of lambaris (*Astyanax bimaculatus*) after transport stress.

During the experiment, no integumentary lesions were observed and no difference in the count of monocytes, immunoglobulins and total plasma protein. More specifically, hydrolate did not cause the breakdown of homeostasis in fish, nor did it result in injury or stress, explaining the absence of changes in these cell counts. This result agrees with another study (Pereira et al. 2020) in which the thrombocyte count did not differ between treatments. Thrombocyte count is related to hemostasis, and these cells are typically present upon injury to an animal's integumentary system (Roberts 2012).

Herbal medicines have had a positive effect on the immune system of animals, modulating and exerting anti-inflammatory and anti-stress activities (Pu et al. 2017, Zhu 2020). As such, these compounds are commonly denoted as immunomodulators, i.e., factors that stimulate and modulate host immunity, presenting a nonspecific response against pathogens and increasing the response of humoral cells (Ferreira et al. 2017). In this work, supplementation with hydrolate stimulated the production of total leukocytes, especially lymphocytes and neutrophils, demonstrating an improvement in the immune system and explaining the higher survival during experimental time.

The dominance of *Cetobacterium* and *Romboutsia* in the gut of *C. longa* hydrolate-treated fish was reflected in lower microbial diversity in these animals. Despite the potential detrimental effect of reducing microbial diversity in the gut, these bacteria are broadly related to the enhancement of gut health and immune response in different vertebrate species (Chen & Yu 2020, Meng et al. 2019, Wu et al. 2020).

In Nile tilapia, Sakyi et al. (2020) assessed the effect of constant feeding, starvation and refeeding on gut microbiota, metabolism, and antioxidant response. The authors reported the increase of *Cetobacterium* abundance during feeding and refeeding periods, while *Romboutsia* increased during the starvation period. They suggested that the production of vitamin B by *Cetobacterium* is particularly important in fish feeding. Meanwhile, higher levels of *Romboutsia* during starvation suggested that its capacity to synthesize amino acids and vitamins, as well as utilize simple carbohydrates through different pathways, could have contributed to the physiological improvement observed in Nile tilapias during this period. Therefore, the authors concluded that the abundant variation of these bacteria was positively correlated with the improvement of physiological performance and the reduction of oxidative stress during starvation and re-feeding periods.

Based our results, the dietary addition of *C. longa* hydrolate for Nile tilapia had beneficial effects on the gut microbial community and, most likely, the physiological performance of Nile tilapia by maintaining fish homeostasis. All these effects ensured higher survival in rearing conditions. However, further studies performing a quantitative and more intensive analysis of the gut microbiota of Nile tilapias fed a diet containing *C. longa* hydrolate is needed in order further validate our present findings.

Table III. Body analyses of Nile tilapia (*Oreochromis niloticus*) fed a supplemented hydrolate diet from *Curcuma longa*.

	Hydrolate	Control	Significance (*p*)
Body indexes			
Hepatosomatic	2.25 ± 0.27	2.57 ± 0.25	0.384
Viscerosomatic	6.36 ± 0.56	6.32 ± 0.26	0.473
Body composition			
Moisture (%)	68.33 ± 0.94	67.29 ± 1.41	0.396
Crude Protein	16.82 ± 1.84	16.06 ± 2.32	0.365
Crude Lipid	11.03 ± 1.49	12.64 ± 2.32	0.299
Ash	2.62 ± 0.12	2.63 ± 0.09	0.356

Table IV. Blood profile of Nile tilapia (*Oreochromis niloticus*) fed a supplemented hydrolate diet from *Curcuma longa*.

Parameters	Hydrolate	Control	Significance (*p*)
Total and differential count			
Thrombocytes (x $10^3.\mu L^{-1}$)	12.81 ± 4.12	6.96 ± 3.15	0.127
Leukocytes (x $10^3.\mu L^{-1}$)	21.34 ± 0.34*	14.64 ± 0.07	0.046
Lymphocytes (x $10^3.\mu L^{-1}$)	19.64 ± 5.18*	13.7 ± 0.50	0.411
Monocytes (x $10^3.\mu L^{-1}$)	0.58 ± 0.29	0.47 ± 0.16	0.286
Neutrophils (x $10^3.\mu L^{-1}$)	1.12 ± 0.24*	0.46 ± 0.19	0.031
Hematimetric			
Erythrocytes ($10^6 \mu L^{-1}$)	1.31 ± 0.04	1.22 ± 0.14	0.248
Hematocrit (%)	24.90 ± 0.49	27.47 ± 0.41*	0.001
Hemoglobin (g/dL^{-1})	7.21 ± 0.79	12.37 ± 2.00*	0.014
Mean corpuscular volume (10^4.fL)	1.95 ± 0.04	2.43 ± 0.24*	0.023
Mean corpuscular hemoglobin (10^4.pg)	5.74 ± 0.09*	1.00 ± 0.20	0.031
Mean corpuscular hemoglobin concentration ($g.dL^{-1}$)	36.78 ± 5.71*	24.16 ± 3.22	0.024
Immunological			
Glucose ($mg.dL^{-1}$)	49.50 ± 2.35	54.33 ± 9.79*	0.026
Total Plasma Protein ($mg.L^{-1}$)	1047.87± 10.38	1050.73 ± 9.05	0.312
Total Plasma Immunoglobulin ($mg.L^{-1}$)	32.63 ± 2.15	34.72 ± 4.52	0.287

*** Indicates significant differences in the t-test.**

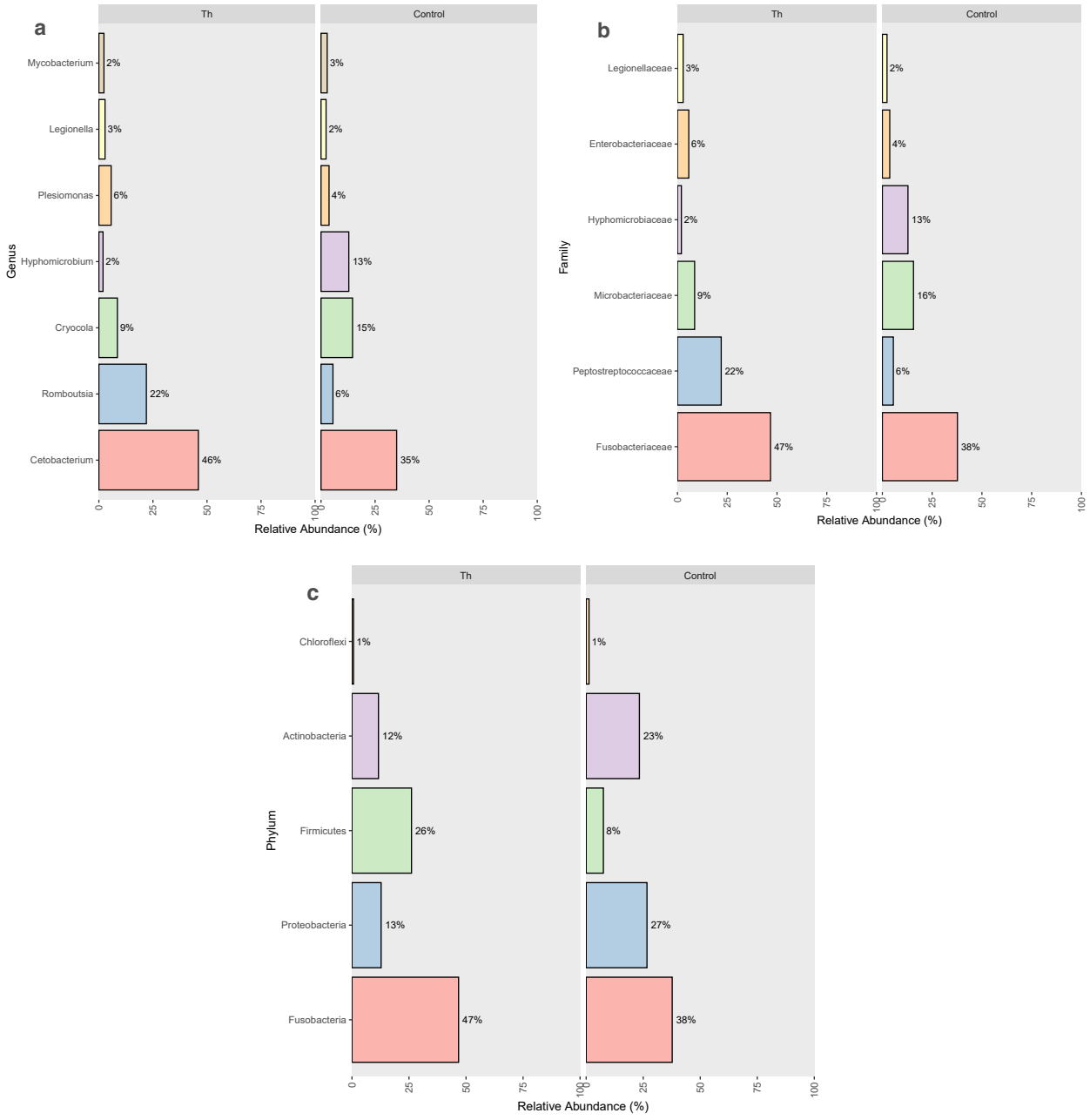

Figure 1. Bacterial relative abundance Nile tilapia (*Oreochromis niloticus*) fed a supplemented hydrolate diet from *Curcuma longa*. (Control). a, Genus; b, Families; c, Phyla.

Table V. Richness, Chao1 and the diversity indexes, Shannon and Inverse Simpson, of gut microbiota from Nile tilapia (*Oreochromis niloticus*) fed a diet supplemented with *Curcuma longa* hydrolate.

Treatments	No.seqs	Goods	Richness	Chao1	Inverse_simpson	Shannon
Control	72838	100.0	66.0	66.0	63.3	26.9
Hydrolate	72838	100.0	82.0	79.3	36.7	19.6

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MARINA O. PEREIRA[1]
https://orcid.org/0000-0003-1339-9543

JULIA D. HESS[2]
https://orcid.org/0000-0002-6089-3352

JULIO CESAR B. RODHERMEL[2]
https://orcid.org/0000-0002-1493-5101

DANIEL R. FARIAS[3]
https://orcid.org/0000-0003-4531-0195

DELANO D. SCHLEDER[4]
https://orcid.org/0000-0002-1318-8298

LUCIANO ALVES[5]
https://orcid.org/0000-0003-0565-5738

FABIANO C. BERTOLDI[6]
https://orcid.org/0000-0003-4750-5164

AMANDA CHABAN[7]
https://orcid.org/0000-0002-8066-5242

JAQUELINE I.A. DE ANDRADE[2]
https://orcid.org/0000-0002-9907-9948

ADOLFO JATOBÁ[4]
https://orcid.org/0000-0002-9470-4775

[1]GeneSeas Aquacultura, Rodovia Selviria/Aparecida do Taboado, Zona Rural, 79590-000 Selviria, MS, Brazil

[2]Instituto Federal Catarinense, Câmpus Araquari, Laboratório de Aquicultura, BR-280, Km 27, Caixa Postal 27, 89245-000 Araquari, SC, Brazil

[3]Programa de Pós-Graduação em Tecnologia e Ambiente, Instituto Federal Catarinense, Laboratório de Produção Vegetal, Câmpus Araquari, BR-280, Km 27, Caixa Postal 27, 89245-000 Araquari, SC, Brazil

[4]Programa de Pós-Graduação em Tecnologia Ambiental, Instituto Federal Catarinense, Laboratório de Aquicultura, Câmpus Araquari, BR-280, Km 27, Caixa Postal 27, 89245-000 Araquari, SC, Brazil

[5]Instituto Federal Catarinense, Laboratório de Microbiologia, Fitossanidade e Propagação Vegetal, Câmpus Araquari, BR-280, Km 27, Caixa Postal 21, 89245-000 Araquari, SC, Brazil

[6]Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina/EPAGRI, Laboratório de Óleos Essenciais, Av. Antônio Heil, 6800, Iaipava, 88316-002 Itajaí, SC, Brazil

[7]Instituto Federal Catarinense, Câmpus Araquari, BR-280, Km 27, Caixa Postal 27, 89245-000 Araquari, SC, Brazil

Correspondence to: **Adolfo Jatobá**
E-mail: jatobaadolfo@gmail.com

Author contributions

MOP and AJ participated collectively in all steps of the letter writting, editing, review and submission, which includes literature review, data collection, the presentation of its main results and discussion. MOP, JDH and JCBR were responsible for fish handling and data collection. DRF and DDS carried out intestinal microbiota assay. LA and FCB prepared the hydrolate for use. AC and JIAA carried out hematological and immunological analysis.