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Garlic Powder Evaluation as Feed Additive on Nile Tilapia (*Oreochromis niloticus* L.) Growth Performance, Feed Utilization, Gill Parasitic Treatment, and Monogenean Diversity

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Abstract: The present study evaluates garlic powder (GP) effects on growth performance, feed utilization, gill parasitic treatment, and monogenean diversity. Thus, a trial was performed under controlled conditions with 84 juvenile Nile tilapia, *Oreochromis niloticus* (39.8 ± 8.8 g initial weight), from culture ponds with monogenean parasite presence for 30 days. Four balanced diets in protein (32.5%) and lipids (6.4%) with GP inclusion levels of 0%, 1%, 2%, and 3% were formulated, manufactured, and supplied daily at approximately 6.5% body weight/tank. The GP diets, compared to the Control (without GP), indicated that the three inclusion levels did not affect the water quality, survival, growth performance, and feed utilization parameters ($p > 0.05$). No differences were observed in the parasitological index of prevalence (20–25%), mean intensity (9.6–28), and mean abundance (2.7–5.3) among the experimental diets ($p > 0.05$), evidencing no effect by inclusion level. Efficacy among GP diets indicated a potential decrease in parasite number (13.4–45.6%) but not all monogenean gill parasites. In conclusion, GP diets did not affect the Nile tilapia survival, growth performance, and feed utilization parameters; therefore, its use is suggested as a preventive alternative for monogenean gill parasites.

Keywords: aquaculture; *Allium sativum*; *Oreochromis niloticus*; monogenean parasites; genetic diversity

Key Contribution: Garlic is defined as a functional food that has uses in various fish disease treatments, including parasitism. The present study successfully uses garlic powder (GP) at inclusion levels of 0%, 1%, 2%, and 3% without affecting water quality, survival, growth performance, and feed utilization parameters of juvenile Nile tilapia, *Oreochromis niloticus*. As a novel contribution, six monogenean parasite species were found in Nile tilapia gills after feeding GP diets for thirty days, which suggests the survival capacity of these parasites and opens possibilities for new research regarding the well-being–health relationship. Additionally, the present research may be the first monogenean genetic diversity report for the Western Aquaculture Region from Mexico.

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1. Introduction

The world apparent fish consumption is expected to increase over the next decade, reaching 21.2 kg in per capita terms by 2032, reflecting demand for fish aquaculture [1]. In parallel, over the next decade, capture fishery production for fishmeal and fish oil could be fluctuating between lows of 15.9 million metric tons (MT) in El Niño Southern Oscillation (ENSO) years [1]. Therefore, evaluating the potential of new functional ingredients is relevant to replace, fortify, and complement the nutritional quality of the aquafeed used for aquatic organism culture from the point of view of the nutrition–health relationship and sustainability [2,3]. Nile tilapia (*Oreochromis niloticus* L.) is the second most widely farmed freshwater fish group in aquaculture worldwide statistics, which recorded a volume of 4.51 MT for inland and coastal production according to data in 2020 [4]. Nevertheless, these production levels create abnormal conditions, in which parasitic infections with pathogenic microorganisms represent a high probability level of affecting the productive performance in tilapia culture, with infestation levels recorded up to 35% in some reports [5,6].

Cichlidogyrus spp. (Paperna, 1960) are small ectoparasitic monogeneans (100–200 µm long) that colonize buccal-opercular cavities, characterized by possessing a haptor that uses hooks to stay attached to fish gills. At least 22 species of the genus *Cichlidogyrus* (*C. dossoui*, *C. tiberianus*, *C. halli*, *C. agnesi*, *C. philander*, *C. thurstonae*, *C. tilapiae*, *C. levequei*, *C. longicornis*, *C. quaestio*, *C. rognoni*, *C. mbirizei*, *C. papernastrema*, *C. guineensis*, *C. sclerosus*, *C. berminenesis*, *C. flagellum*, *C. cubitus*, *C. flexicolpos*, *C. lobus*, *C. berradae*, and *C. maeander*) that infect important tilapia species are potentially associated with aquaculture or fishery mortalities [7]. Monogeneans perforate the gill epithelium, increasing fish susceptibility to bacterial infection, blood cell puncture, distortion, and sometimes penetration of the extracellular cartilaginous matrix in the gills, surface deformation of gill lamellae, erosion of epithelial cells, increased mucus production, neutrophil anemia, hyperplasia, and fusion of gill lamellae [7]. Paredes-Trujillo et al. [8] reported that high parasite burdens of the monogeneans *Gyrodactylus* spp. are correlated with a low host condition factor, with an estimated 12–15% decrease in the profit margin of Nile tilapia culture from Mexico. Therefore, studying this aspect is relevant to improve aquaculture profitability.

In general, garlic (*Allium sativum* L.) and its by-products have different bioactive compounds with functional properties for aquaculture feed, defined in several studies by their zootechnical growth performance and antiparasitic activities, as shown in Table 1 [9–14], as well as biological properties, such as antimicrobial, antiviral, antioxidant, hypolipidemic/hypocholesterolemic, hypotensive, hypoglycemic, hypothrombotic, and hypoatherogenic [15,16]. Moreover, garlic powder has different bioactive compounds, and among those that stand out are ajoene (0.17 mg/g), alliin (32.8 mg/g), and allicin (0.1 mg/g), as derived compounds with high bioavailability [17–20]. According to recent statistics, global garlic production recorded a volume of 33.4 MT in 2023, reflecting the fact that China represents the world's main producer [21]. Nile tilapia is host to potentially pathogenic monogenean parasites that can negatively impact aquaculture fish stocks. Furthermore, the in vivo studies performed on Nile tilapia indicate that the use of garlic has been focused on providing primarily health and well-being status [22–26]. However, the inclusion level effects of 1%, 2%, and 3% for commercial garlic powder products as a model for gill parasite control are still unknown on the *O. niloticus* population from cultivation systems in Western Mexico. Therefore, the main objective of the present study is to determine garlic powder effects as a feed additive on Nile tilapia growth performance, feed utilization, gill parasitic treatment, and monogenean species diversity.

Table 1. Data review of the garlic effects on zootechnical parameters and monogenean parasitic indices with respect to the Control diet response in *Oreochromis niloticus*.

Feed Additive or Ingredient Levels [a]	Experimental Conditions [b]	Feed Quality [c]	Growth Performance [d]				Feed Utilization [e]			Antiparasitic Activity [f]			References
			WG	SGR	HIS	FI	FCR	FE	PER	Genus	E	P	
GO = 0, 5, and 10 g/kg.	IBW = 14 g/fish, EU = earthen pond cages (2 × 4 × 1 m), SD = 50 fish/cage, WT = 27.1 °C, FR = 3% of BW/day, and EP = 120 days.	CP = 249.7 g/kg, L = 31.3 g/kg, and GE = 14.2 MJ/kg.	↑	↑	↓	↑	↓	NE	↑	NE	NE	NE	Hussein et al. [9].
FG = 0, 3, and 5 g/kg. DG = 0, 3, and 5 g/kg.	IBW = 0.26 g/fish, EU = concrete ponds (7.5 × 2.25 × 0.70 m), SD = 60 fish/replicate, FR = 4% of BW/day, and EP = 154 days.	CP = 330 g/kg, L = 111 g/kg, and GE = 18.8 MJ/kg.	↑	↑	↓	SR	↓	NE	↑	NE	NE	NE	Abdel-Hakim et al. [10].
GP = 150 mg/kg. GO = 32 g/kg.	IBW = 20–21 g/fish, FR = 3% of BW/day, SD = 10 fish/replicate, and EP = 90 days.	CP = 60.9 g/kg and L = 19.9 g/kg.	↑	↑	↓	↓	↓	↑	↑	NE	NE	NE	Metwally [11].
GP = 0, 10, 20, 30, and 40 g/kg.	EU = glass aquaria (75 × 40 × 50 cm) of 100 L, WT = 26–27 °C, SD = 20 fish/aquaria, FR = 3% of BW/day, and EP = 90 days.	CP = 340–352 g/kg, L = 83–88 g/kg, and GE = 18.8–18.9 MJ/kg.	↑	↑	SR	↑	↓	↑	↑	NE	NE	NE	Shalaby et al. [12].
GE = 0.0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16, and 0.18 µg/mL.	IBW = 40–60 g/fish, SD = 10 fish/replicate, EU = 96-L glass aquaria, and EP = 4 days.	NE	NE	NE	NE	NE	NE	NE	NE	*Dactylogyrus*	85.7–100	NE	Reda et al. [13].
GO = 1, 1.5, 2, 2.5, and 3 PPT. GC = 3 PPT and 300 mg/L.	IBW = 5-, 15-, and 30-day-old fries, SD = 5000 fish/pond, EU = hatchery earthen ponds (3 m length × 2 m width × 1 m water depth), and EP = 7 days.	NE	NE	NE	NE	NE	NE	NE	NE	*Gyrodactylus*	NE	17–29	Abd El-Galil and Aboelhadid [14].

([a]) GO = garlic oil; FG = fresh garlic; DG = dried garlic; GP = garlic powder; GE = garlic extract; GC = garlic cloves. ([b]) IBW = body weight; EU = experimental unit; SD = stocking density; WT = water temperature; FR = feeding rate; EP = experiment period. ([c]) CP = crude protein; L = lipids; GE = gross energy. ([d]) WG = weight gain; SGR = specific growth rate; HIS = hepatosomatic index; FI = feed intake. ([e]) FCR = factor conversion ratio; FE = feed efficiency; PER = protein efficiency ratio. ([f]) E = efficacy (%); P = prevalence (%). ↑ (statistically significant increase); ↓ (statistically significant decrease); SR = similar responses (no statistical differences); NE = not evaluated.

2. Materials and Methods

2.1. Ingredient Preparation and Laboratory Analyses

For the design of the experimental diets, commercial macro-ingredients from suppliers of Mexico with a particle size greater than 1 mm were pulverized in a hammer mill (Wiley, Philadelphia, PA, USA) then sieved through a 0.9 mm mesh before being analyzed for proximate composition and energy content. The tested ingredients were analyzed in triplicate, as follows: Dry matter was calculated by gravimetric analysis, following oven drying at 100 °C for 24 h. The Dumas procedure in the nitrogen Gerhardt–Dumatherm analyzer (Gerhardt GmbH & Co., Königswinter, Germany) was used to determine the crude protein content: %nitrogen × 6.25 (Method No. 990.03) [27]. Gross energy was determined by calorimetry using an isoperibolic calorimeter, model C-6000 (IKA Works, Inc., Wilmington, NC, USA), calibrated with benzoic acid tablets.

2.2. Formulation, Diet Preparation, and Laboratory Analyses

All diets were formulated using AFOS® (https://www.animalfeedsoftware.com/) (Five Horizons LLC., Dover, DE, USA) software. For the growth trial, four diets were designed based on *O. niloticus* juvenile requirements, as described by Gutiérrez-Leyva et al. [2], using a Control group as a reference diet and three diets containing 1%, 2%, and 3% of garlic powder (GP1%, GP2%, and GP3%, respectively). Diets were made as described by Gutiérrez-Leyva et al. [2]. Briefly, a first macro-ingredient mixture was made; then, a vitamin–mineral premix was added and mixed thoroughly in a food mixer before fish oil was added. Subsequently, a second mixture was consolidated by adding water (approximately 35% of the total "as-is" ingredient weight). The resulting mixture was pressure-extruded twice in a 1-HP meat grinder through a die with 2.2 mm-diameter holes. Extruded pellets were dried in a forced-air oven at 60 °C for 8 h until moisture decreased to 8–10%. Feeds were stored at 5 °C until use, and a posteriori selected samples were analyzed for nutritional and energetic content with the procedures described. The ingredients and nutritional and energetic composition of the experimental diets are shown in Table 2. Feeding conditions were carried out under diets that were isoproteic (32.1–33.5%), isolipidic (6.3–6.5%), and isoenergetic (18.7–18.9 MJ/kg), among the four experimental treatments (Table 2).

Table 2. Ingredients and nutritional and energetic composition of the experimental diets tested in the growth bioassay with *Oreochromis niloticus* (n = 3, mean ± SD).

Ingredients (% "as Is")	Control	GP1%	GP2%	GP3%
Garlic powder [1]	0	1	2	3
Soybean paste [2]	26	26	26	26
Wheat flour [3]	30	30	30	25
Sweet potato flour [4]	12	11	10	14
Tuna by-product silage [5]	20	20	20	20
Vitamin–mineral premix [6]	2	2	2	2
Salmon oil [5]	6	6	6	6
Unflavored gelatin powder (binder) [7]	4	4	4	4
Total	100	100	100	100
Dry matter (%)	91.7 ± 0.5	90.6 ± 0.4	89.8 ± 1.2	91.5 ± 0.6
Crude protein (%)	33.5 ± 5.7	32.1 ± 0.3	32.4 ± 1.5	32.1 ± 1.9
Ethereal extract (%)	6.3 ± 1.1	6.5 ± 2.6	6.3 ± 3.4	6.4 ± 0.2
Gross energy (MJ/kg)	18.7 ± 0.0	18.9 ± 0.1	18.8 ± 0.1	18.7 ± 0.1

Diet codes depending on garlic powder inclusion level: 0%, 1%, 2%, and 3% (Control, GP1%, GP2%, and GP3%, respectively). [1] Dehydrated and pulverized bulbs. Encapsuladoras México, S.A. de C.V. Lot AS7322. [2] Forrajes Barajas, S.A. de C.V. Tepic, Nayarit, Mexico. [3] Guadalupe extra-fine flour. Harinas Guadalupe, S.A. de C.V. Guadalajara, Jalisco, Mexico. [4] *Ipomoea batata*, Produced in San Pedro Lagunillas, Nayarit, Mexico, through organic production in 2022. [5] Proteinas Marinas y Agropecuarias, S.A. de C.V. Zapopan, Jalisco, Mexico. [6] Farmix C-3060® fortified vitamin and mineral premix consisting of mineral oil, vitamin A-acetate, vitamin D3, vitamin E-acetate, vitamin K3, vitamin B1, vitamin B2, vitamin B6, vitamin B12, biotin, folic acid, niacin, calcium D-pantothenate, choline chloride, monodicalcium phosphate, calcium carbonate, salt, copper, iron, E.D.D.I. (source of iodine), manganese, selenium, zinc, L-lysine HCl, DL-methionine, L-threonine, L-tryptophan, enzyme supplements (phytase and xylanase), and antioxidant ethoxyquin (ETQ). Trouw Nutrition of Nutreco Co. Guadalajara, Jalisco, Mexico. [7] D'Gari® S.A. de C.V. Querétaro, Querétaro, Mexico.

2.3. Fish Rearing and Growth Performance

Selected Nile tilapia offspring progeny (mean male weight 28 ± 8.8 g, n = 300) were obtained from Centro Acuícola San Cayetano, Tepic, Nayarit, Mexico (21°27′06″ N and 104°48′57″ W). They were acclimated to water laboratory conditions at the Aquaculture Nutrition Laboratory of the Universidad Autónoma de Nayarit, Mexico (temperature 27 ± 0.6 °C, and dissolved oxygen 6.0 ± 0.7 mg/mL), in a 2000 L fiberglass tank for 15 days. Fish were fed daily at 6% biomass/tank with a commercial feed containing 40% of crude protein (Nutripec-Purina®, Guadalajara, JAL, Mexico) until reaching the size required for the trial.

A total of 84 Nile tilapia (average initial weight 39.8 g) with the presence of parasites in the gills were used, individually weighed, and distributed in twelve 1100 L plastic tanks in a randomized design of four treatments in triplicate at a density of seven fish/tank. All tanks were equipped with air stones, 300 W submersible heaters (Model SGH-380, Sunny Aquarium, CN, USA), a drain system, and did not maintain water exchange in the study period between tanks to avoid parasite exchange. A natural photoperiod of 13:11 h (light:dark) was maintained throughout the experiment. Experimental groups were fed to visual apparent satiety twice daily at 08:00 and 18:00 h, with a mean feed ration of 6.5% of total biomass per tank that was adjusted every day regarding consumption + n for 30 days. The weekly water exchange rate of the rearing system was 95%, which was performed on the seventh day with an approximate water flow of 167 L/h provided by a ½ HP water pump. Water temperature and dissolved oxygen were monitored daily with a portable oximeter, model AR8406 (Intell Instruments Pro., Dongguan, China), and pH, nitrite, nitrate, and ammonia were determined every week using a commercial freshwater kit, API™ (Mars, Chalfont, PA, USA). Water quality parameters were monitored in clear water conditions of the rearing system for 30 days (without primary productivity), as shown in Table 3. They reflect uniformity with respect to the experimental conditions without significant differences in temperature, dissolved oxygen, pH, total ammonia-nitrogen, nitrite, and nitrate ($p > 0.05$; Table 3) in the recorded values.

Table 3. Water quality parameters of the rearing system for *Oreochromis niloticus* (n = 3, mean ± standard deviation (SD)).

	Control	GP1%	GP2%	GP3%	Mean
Temperature (°C)	26.3 ± 0.1	26.9 ± 0.3	26.9 ± 0.4	27.2 ± 0.2	26.8
Dissolved oxygen (mg/L)	6.1 ± 0.1	6.2 ± 0.1	5.9 ± 0.2	5.9 ± 0.1	6.0
pH	7.8 ± 0.2	7.8 ± 0.1	7.7 ± 0.2	7.6 ± 0.1	7.7
Total ammonia-nitrogen (mg/L)	0.21 ± 0.04	0.21 ± 0.08	0.18 ± 0.00	0.21 ± 0.04	0.20
Nitrite (mg/L)	0.36 ± 0.05	0.34 ± 0.08	0.25 ± 0.08	0.19 ± 0.13	0.29
Nitrate (mg/L)	13.3 ± 1.7	11.1 ± 4.8	11.1 ± 1.0	8.4 ± 3.3	11.0

Diet codes depending on garlic powder inclusion level: 0%, 1%, 2%, and 3% (Control, GP1%, GP2%, and GP3%, respectively).

All fish were weighed at the end of the growth trial. Total length was measured at the beginning and end of the trial. Survival, growth performance, and feed efficiency utilization were assessed by calculating as follows: survival (%) = (final number of fish/initial number of fish) × 100; weight gain (g) = (FW − IW), where FW is final fish weight (g) and IW is initial fish weight (g); specific growth rate (%/day) = [(ln FW − ln IW)/days] × 100, where IW is the initial fish weight (g) and FW is the final fish weight (g); apparent feed intake (g/fish/day) = [(feed intake (g))/(number of fish)]/days; feed conversion ratio = [feed intake (g)/fish weight gain (g)]; protein efficiency ratio = weight gain (g)/protein intake (g), where protein intake = [AFI (g/fish/day) × feed protein content]; condition factor = [100 ∗ weight (g)]/[total length (cm)]^3.

2.4. Sample Preparation and Conservation

Once the growth trial was completed, fish were anesthetized and sacrificed following the protocols of the NOM-033-ZOO-1995 [28] for humane sacrifice of wild animals valid in Mexico, recording biometric data on weight and total length. Detailed examinations were carried out on each of the fish, including detailed inspection of its body surface, oral cavity, eyes, fins, operculum cavity, and gills, with the aim of identifying the presence of parasites. Subsequently, the four gill arches were dissected and placed in Petri dishes with 70% ethyl alcohol for identification. The counting of monogeneans by isolation was carried out in each of the gill arches using dissecting needles and millimeter pipettes of 0.5–10.00 µL. For the identification of the parasites, fractions from each gill were mounted on slides using the proteolytic digestion technique of Harris and Cable [29] with proteinase *K*, adding 3 µL to each preparation, and after 5 min, sclerotized structures were studied.

2.5. Microscopy and Illustrations

The morphological determination of each monogenean was carried out using morphological keys and specialized literature based on their hard parts [30–34]. Species descriptions were the standardized center of attention in the sclerotized parts, i.e., ventral and dorsal haptor, ventral and dorsal bar, and male/female copulatory complex [30–34]. Parasite morphometric measurements and visualizations were made with a magnification power of 400× on an Olympus CX21 Phase Microscope (Olympus America Inc., Breinigsville, PA, USA). Photographs of the parasites were taken with a camera adapted to the Apple iPhone 12 (Apple Inc. One Apple Park Way, Cupertino, CA, USA; wide-angle lens of 26 mm, aperture of 1.6, and 12 megapixel 3024 × 4032) for recording. Finally, the preparations were sealed with transparent nail varnish for preservation in the UAMVZ-2023 collection of Nile tilapia parasites.

2.6. Infection Parameters of Fish

The quantitative analysis of the parasite indices was expressed according to the following proposed by Bush et al. [35]: Prevalence (%) is the number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species. Mean abundance is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and not infected hosts). Mean intensity is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite. The efficacy of garlic powder was determined with the procedure described by Jatobá et al. [36], as follows: E = MNPCG − MNPTG × 100/MNPCG, in which E = efficacy, MNPCG = the mean number of parasites in the Control group, and MNPGT = the mean number of parasites in the treated group (GP diets).

2.7. Monogenean Genetic Diversity

2.7.1. DNA Extraction, PCR Conditions, and Sequencing of Amplicons

After the microscopic identification of *Cichlidogyrus* spp., DNA was extracted by the guanidine-thiocyanate and chloroform-isoamyl alcohol method, as described by Boom et al. [37] in selected fish of *O. niloticus* after a 30-day trial. The amplification of gene fragments was performed in a 50 µL reaction volume with 0.20 µM of dNTPs (VIVANTIS Technologies, Subang, Selangor Darul Ehsan, Malaysia), 1 UI of *Taq* Pol, 5 µL of 10× buffer, 3 mM of $MgCl_2$, 10 pmol of each primer (F 5′-GCT TGT ACC TGG GAT CGT GT-3′ and R 5′-GCC TTG GAT GGA GTT TAC CA-3′), as developed by Ek-Huchim et al. [38], and 30 to 60 ng of genomic DNA as a template. Reactions were performed in an Aeris (ESCO Micro Pte. Ltd., Changi City, Singapore) PCR System thermocycler. For the amplification of genes, the parameters used were as follows: an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of

denaturation at 94 °C for 30 s, alignment at 54 °C for 45 s, and extension at 72 °C for 45 s, then a final extension cycle at 72 °C for 7 min. PCR products were separated by electrophoresis and visualized in ethidium-bromide-stained agarose gels. Amplicons were purified using the GeneJET PCT purification kit (Thermo Fisher Scientific Inc., Waltham, MA, USA), and PCR products were sequenced at the Macrogen® (Seoul, Republic of Korea) sequencing services using an 3730XL DNA Analyzer (Applied Biosystem, Foster City, CA, USA).

2.7.2. Sequence Editing and Analysis

For a quality chromatogram, all sequences were cut, assembled and edited using the Sequencer 5.4.6 Program (Gene Codes Corporation® 2023, Ann Arbor, MI, USA). Phylogeny analysis was computed by the unweighted pair group method with arithmetic (UPGMA) mean [39]. The evolutionary distances were computed using the Kimura 2-parameter method [39] and are shown as the units of the number of base substitutions per site. Evolutionary analyses were conducted in the software MEGA12® developed by the National Institutes of Health (https://www.megasoftware.net/) [40].

2.8. Statistical Analyses

Experimental data were analyzed for normality and homogeneity of variance using Shapiro–Wilk's test and Bartlett's test, respectively. The parameters, defined as water quality, survival, growth performance, feed utilization, and parasite indices, were analyzed using the Statistica 6.0 software. Therefore, one-way analysis of variance (ANOVA) and, for differences of population means, a multiple comparison Tukey's test were applied (mean at a significance level of $p < 0.05$) according to Gutiérrez-Leyva et al. [2].

3. Results

3.1. Growth Trial

The parameters of survival, growth performance, and feed utilization after 30 days are presented in Table 4. The comparison of the Control with respect to GP diets with garlic powder inclusion levels of 1%, 2%, and 3% did not show a significant response effect of S, IW, FW, TL, WG, AFI, SGR, FCR, PER, and CF parameters ($p > 0.05$; Table 4).

Table 4. Survival, growth performance, and feed utilization parameters of *Oreochromis niloticus* after a 30-day trial (n = 3, mean ± standard deviation (SD)).

	Control	GP1%	GP2%	GP3%	Mean
Survival (%)	95.2 ± 8.3 [a]	100 ± 0.0 [a]	90.5 ± 0.0 [a]	100 ± 16.5 [a]	96.4
Initial weight (g)	38.7 ± 1.4 [a]	41.2 ± 1.6 [a]	40.5 ± 4.3 [a]	38.9 ± 1.4 [a]	39.8
Final weight (g)	82.9 ± 7.0 [a]	92.3 ± 5.2 [a]	82.6 ± 1.6 [a]	84.4 ± 2.5 [a]	85.6
Total length (cm)	15.2 ± 0.9 [a]	15.7 ± 0.3 [a]	15.2 ± 0.9 [a]	18.5 ± 5.9 [a]	16.1
Weight gain (g)	44.2 ± 6.3 [a]	51.1 ± 4.0 [a]	42.1 ± 2.9 [a]	45.6 ± 3.8 [a]	45.8
Apparent feed intake (g/fish/day)	4.0 ± 0.1 [a]	4.2 ± 0.1 [a]	4.2 ± 0.3 [a]	4.0 ± 0.1 [a]	4.1
Specific growth rate (%/day)	2.5 ± 0.2 [a]	2.7 ± 0.1 [a]	2.4 ± 0.3 [a]	2.6 ± 0.2 [a]	2.6
Feed conversion ratio	2.8 ± 0.3 [a]	2.5 ± 0.2 [a]	3.0 ± 0.5 [a]	2.7 ± 0.3 [a]	2.8
Protein efficiency ratio	1.1 ± 0.1 [a]	1.3 ± 0.1 [a]	1.0 ± 0.2 [a]	1.2 ± 0.1 [a]	1.2
Condition factor	2.4 ± 0.3 [a]	2.4 ± 0.2 [a]	2.4 ± 0.4 [a]	2.2 ± 0.4 [a]	2.3

Diet codes depending on garlic powder inclusion level: 0%, 1%, 2%, and 3% (Control, GP1%, GP2%, and GP3%, respectively). Means with similar superscripts within the same row are not significantly different ($p > 0.05$).

3.2. Parasite Infection Levels and Efficacy

After analyzing all the gill fish from each diet in triplicate, total specimen counts of 404, 285, 411, and 241 were recorded for the different species of monogenean identified in the Control, GP1%, GP2%, and GP3% diets, respectively. The prevalence, parasitological index, intensity, and abundance of the monogenean species at the beginning and end of the 30-day

trial are shown in Table 5. The order of prevalence from the highest to lowest mean values defined by experimental treatment were Control = GP3% > GP1% = GP2%, with respect to intensity, the order was Control > GP2% > GP1% > GP3%, and finally, with respect to abundance, the order was Control > GP2% > GP3% > GP1% (Table 5). Monogenean indices as a direct indicator of the garlic powder effect did not show significant differences in the averages recorded for prevalence, intensity, and abundance ($p > 0.05$). In total, six monogenean species were identified (Table 5).

Table 5. Monogenean gill parasite indices of *Oreochromis niloticus* analyzed at the beginning of and after a 30-day trial (n = 3, mean ± standard deviation (SD)).

Treatment		**Parasitological Index**		
		Prevalence (%)	**Mean Intensity**	**Mean Abundance**
Initial point	mean ± SD	21.9 ± 28.2	26.8 ± 34.4	7.0 ± 9.0
Control	*C. thurstonae*	11	26	2.7
	C. tilapiae	11	56	5.9
	C. sclerosus	68	16.4	11.2
	C. halli	11	13.5	1.4
	mean ± SD	25 ± 28.5	28.0 ± 19.4	5.3 ± 4.4
GP1%	*C. thurstonae*	33	13.8	4.0
	C. sclerosus	33	9.7	2.8
	C. halli	22	22.8	4.3
	C. tubicirrus	6	41.0	2.0
	C. longicornis	6	12.0	0.6
	mean ± SD	20 ± 13.5	19.9 ± 18.2	2.7 ± 1.5
GP2%	*C. thurstonae*	10	25.0	2.4
	C. sclerosus	38	11.9	4.5
	C. halli	19	33.0	6.3
	C. tubicirrus	14	17.0	2.4
	C. longicornis	19	20.8	4.0
	mean ± SD	20 ± 10.7	21.5 ± 8.0	3.9 ± 1.6
GP3%	*C. thurstonae*	11	2.5	0.2
	C. sclerosus	53	16.9	8.0
	C. halli	16	9.0	1.3
	C. tubicirrus	21	10.0	1.9
	mean ± SD	25 ± 18.9	9.6 ± 5.9	2.9± 3.5

Diet codes depending on garlic powder inclusion level: 0%, 1%, 2%, and 3% (Control, GP1%, GP2%, and GP3%, respectively).

The efficacy of garlic powder by inclusion level is presented in Figure 1. In terms of percentages, the response from the highest to lowest value by experimental treatment indicated that GP3% > GP1% > GP2%; that is, of the total specimens collected in all the fish, the treatments with the lowest and highest amounts of monogenean parasites at the gill level were GP3% and GP1%, respectively. The GP2% treatment maintained an intermediate value to the responses of GP3% and GP1%, which agreed with respect to the total specimen counts per treatment mentioned (Table 5).

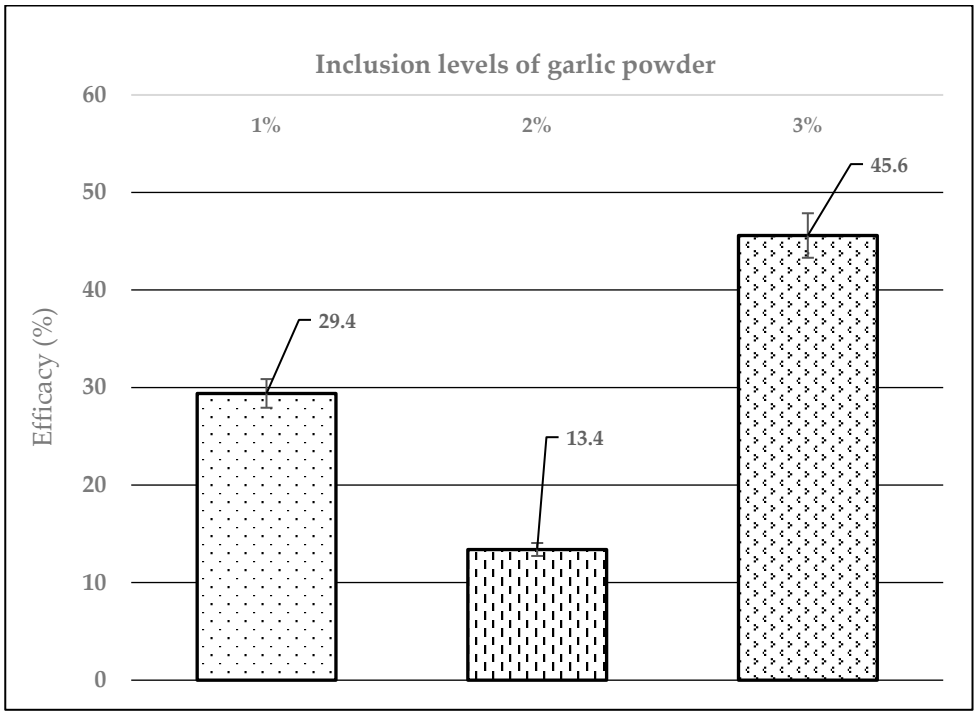
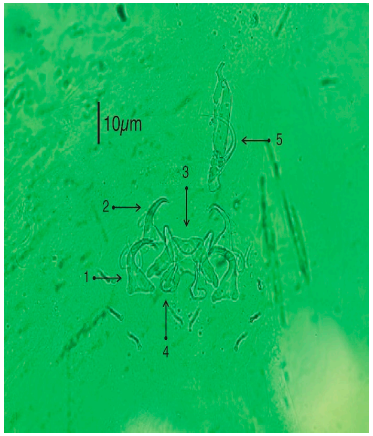


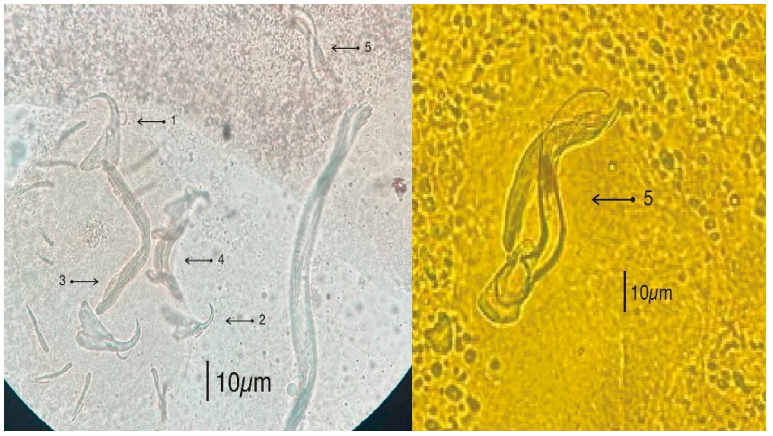
Figure 1. Efficacy of garlic powder as an antiparasitic agent against the mean number of gill monogeneans for *Oreochromis niloticus* after a 30-day trial (n = 3, mean ± standard deviation (SD)). Note: The efficacy calculations present at the inclusion levels of 1%, 2%, and 3% represent a reference value based on the result of the Control group [36].

3.3. Morphological Identification of Monogenean

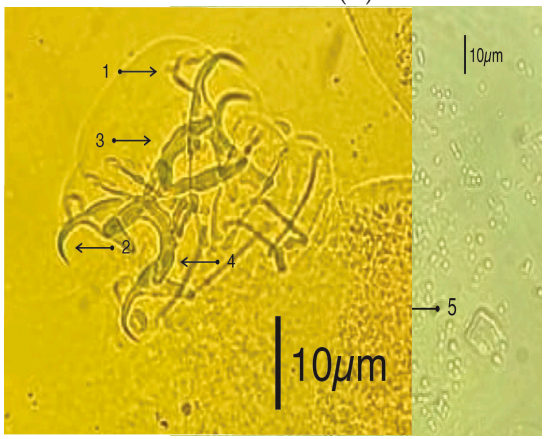
The morphological identification of Nile tilapia parasites is shown in Figure 2. Six species were identified: *Cichlidogyrus sclerosus* [31], *Cichlidogyrus halli* [32], *Cichlidogyrus thurstonae* [30], *Cichlidogyrus longicornis* [34], *Cichlidogyrus tilapiae* [31], and *Cichlidogyrus tubicirrus* [32,33], which correspond to the Figure 2A, Figure 2B, Figure 2C, Figure 2D, Figure 2E and Figure 2F, respectively.



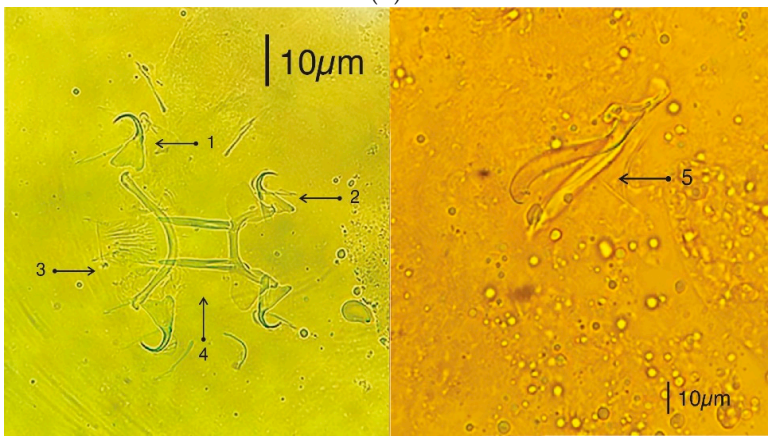
(**A**)



(**B**)

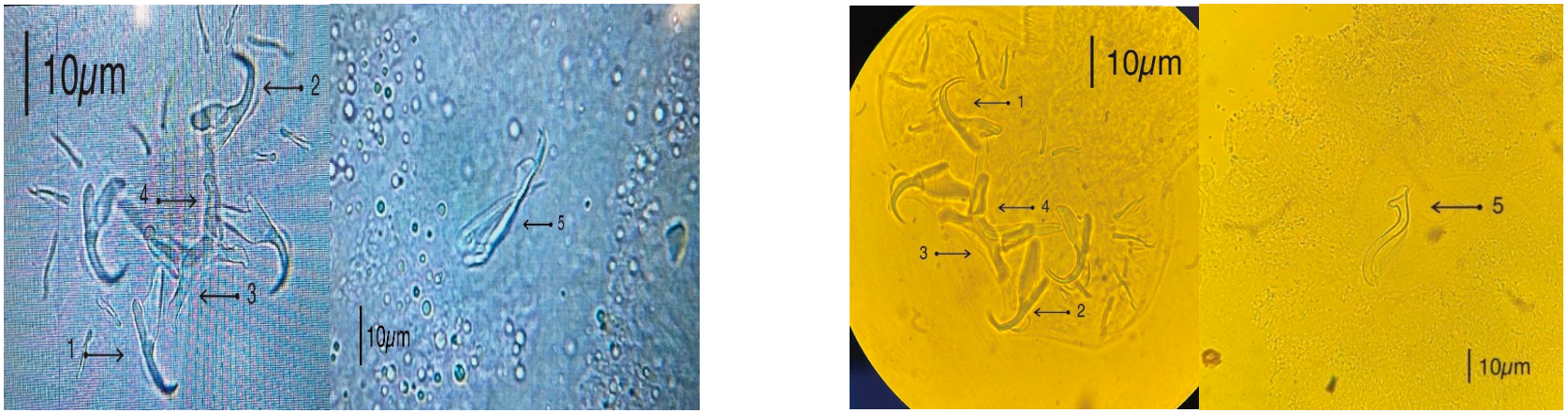


(**C**)



(**D**)

Figure 2. *Cont*.



(**E**) (**F**)

Figure 2. Descriptive graphic representation of monogenean parasites identified in the gills of *Oreochromis niloticus* after a 30-day trial. (**A**) Photograph of sclerotized structures of *Cichlidogyrus sclerosus*: (**1**) ventral hook, (**2**) dorsal hook, (**3**) ventral bar, (**4**) dorsal bar, and (**5**) male/female copulatory organ. (**B**) Photographs of sclerotized structures of *Cichlidogyrus halli*: (**1**) ventral hook, (**2**) dorsal hook, (**3**) ventral bar, (**4**) dorsal bar, and (**5**) male/female copulatory organ. (**C**) Photographs of sclerotized structures of *Cichlidogyrus thurstonae*: (**1**) ventral hook, (**2**) dorsal hook, (**3**) ventral bar, (**4**) dorsal bar, and (**5**) male/female copulatory organ. (**D**) Photographs of sclerotized structures of *Cichlidogyrus longicornis*: (**1**) ventral hook, (**2**) dorsal hook, (**3**) ventral bar, (**4**) dorsal bar, and (**5**) male/female copulatory organ. (**E**) Photographs of sclerotized structures of *Cichlidogyrus tilapiae*: (**1**) ventral hook, (**2**) dorsal hook, (**3**) ventral bar, (**4**) dorsal bar, and (**5**) male/female copulatory organ. (**F**) Photographs of sclerotized structures of *Cichlidogyrus tubicirrus*: (**1**) ventral hook, (**2**) dorsal hook, (**3**) ventral bar, (**4**) dorsal bar, and (**5**) male/female copulatory organ.

3.4. Molecular Identification and Genetic Diversity

In general, a distance matrix of selected 28S partial sequences of the six monogenean parasite isolates using the unweighted pair group method with arithmetic mean (UPGMA) is shown in Figure 3. The genetic diversity found was consistent with the morphological identification presented in Figure 3 when the analyzed sequences were compared. Thus, the tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic responses.

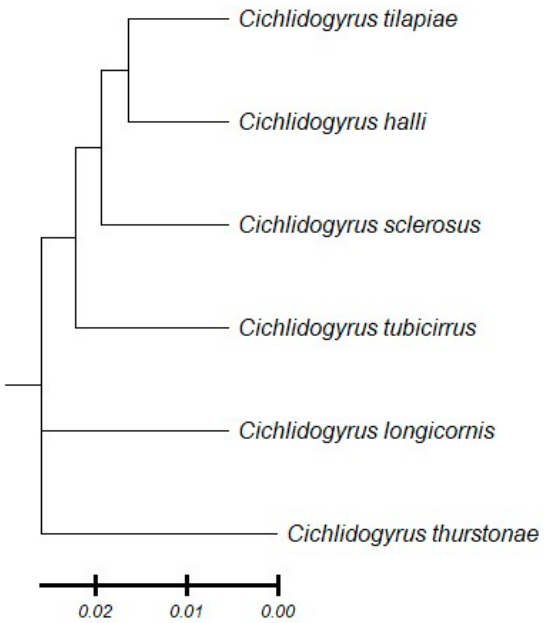


Figure 3. Phylogram of the six *Cichlidogyrus* species, derived from genetic distances obtained by UPGMA in selected specimens of *Oreochromis niloticus* after a 30-day trial.

4. Discussion

Biological effects of garlic powder have been demonstrated in different species from commercial species, such as broiler chicks [41], laying hens [42], ewes and lambs [43,44], and piglets [45], to mention some research on terrestrial animals. *O. niloticus* assessments of garlic's potential impacts on growth performance, feed efficiency, and antiparasitic activity parameters are scarce in the literature (Table 1), pointing out the importance of using garlic products for tilapia culture [9–14].

The survival and water quality parameters obtained in the present research were within the optimal range of water quality for Nile tilapia culture described by Gutiérrez-Leyva et al. [2,46] in similar research conditions. In this sense, the results obtained can be attributable to tilapia culture standard conditions in the area of Western Nayarit, Mexico.

Responses by *Oreochromis niloticus* were not affected by feed quality; consequently, growth performance and feed utilization parameters (FW, TL, WG, AFI, SGR, FCR, PER, and CF) were successfully and uniformly achieved among all the experimental treatments, which is a favorable element for the use of garlic powder at levels from 1% to 3% in feed design for tilapia. Another reason for these results was the feeding rate of the growth trial adjusted to biomass per tank (6.5% BW/day) for all the treatments, with the intention of evaluating uniformity in nutrient and energy intake. Some authors, such as Xie et al. [47], have shown that *O. niloticus* growth performance does not increase significantly when feeding rates are used to apparent satiation compared to the restricted feeding model, at feeding rates from 2% to 6%, respectively. Considering that the average feed consumption rate in the present research was adequate to maximize *O. niloticus* growth, the average mean value result of SGR, FCR, and PER parameters in Table 3 was moderate with re-

spect to those recorded by Gutiérrez-Leyva et al. [2] of 3.7–4.0%/day, 0.9–1.2%/day, and 2.7–3.4%/day, respectively, for juveniles of the same species. In this regard, a factor that could influence this trend is the fish parasitism degree at the initial time (day 0) shown in Figure 1. Nevertheless, fish species in freshwater environments are affected in their performance by parasites lodged in their digestive and respiratory systems [48]. In general, the apparent feed ingested by *O. niloticus* per treatment apparently denotes that no feed rejection was performed by fish due to garlic powder inclusion levels, since the general average value recorded (4.1) was even higher than the intake rates of 1.36–1.75 reported by Gutiérrez-Leyva et al. [2] for the same species under similar culture conditions. On the one hand, Paredes-Trujillo et al. [8] determined the condition factor (CF) distribution for the *O. niloticus* group with high ectoparasite burdens (295 $\pm$ 191 ectoparasites per fish; CF $\leq$ 0.25) with respect to the group with low ectoparasite burdens (45 $\pm$ 31 ectoparasites per fish; CF > 0.4). Both farms grow tilapia, where the most frequent and abundant monogenean species were *C. sclerosus* and *C. tilapiae* in the records of 29 tilapia farms located in Yucatan, Mexico.

Interestingly, under controlled culture conditions in the literature reports, the high inclusion levels of *A. sativum* (1% to 3%) significantly improved the growth performance of rainbow trout (*Oncorhynchus mykiss*) and African catfish (*Clarias gariepinus*) in the juvenile stage, according to Güroy et al. [48] and Ukenye et al. [49], respectively. However, in the present study, this phenomenon did not occur as a result.

Some studies have shown that as the garlic concentration in feed increases, the growth-promoting properties of natural garlic extract become evident in Nile tilapia specimens, with average initial and final weights of 7–22 g vs. 34.5–45.4 g according to Shalaby et al. [12]. However, other research, such as that of Diab et al. [50], did not report significant effects on the growth rate in juvenile offspring (3.5 $\pm$ 0.02 g) at stocking densities of 30 fry/hapa fed during the summer at a rate of 1% of body weight per day.

The mean infestation abundance value determined in the *C. tilapiae* (5.9) Control diet was similar to that reported by Vásquez-Ocmín et al. [51], regarding a 5.8 average value in *O. niloticus* specimens collected in a fishpond in the Peruvian Amazon. With respect to prevalence values, they were lower (11% vs. 73.3%), perhaps indicating that seasonal variations occur at the worldwide level when they are influenced by biotic and abiotic factors, in addition to the host–parasite genetic interaction, among other factors. When the parameters of the final weight and total length were compared, no trend was evident in prevalence, mean intensity, and mean abundance indices. A plausible explanation that could have influenced this is the sample size. In this regard, Marques and Cabral [52] mentioned that a size of $\geq$40 individuals analyzed per treatment is required. In this sense, a recommendation for future research is to use large-capacity ponds to validate representative sample sizes. Authors Marques and Cabral [52] mentioned that prevalence estimates are apparently independent of significant changes that depend on the sample size, since fish without parasite presence may be generally found. According to Khidr et al. [53], other features, such as water temperature, behavior, sex, age, resistance, or mortality, have an effect on monogenean parasite abundance peaks. Suliman and Al-Harbi [54] reported elevated levels of monogeneans on *O. niloticus* fish gills at mean values of 81.67%, 495.23, and 405.84 for prevalence, mean intensity, and mean abundance, respectively, for fish farms in the central region of Saudi Arabia. In the same manner, Paredes-Trujillo et al. [8] reported values of prevalence of 14–95%, mean abundance of 11–301.23, and mean intensity of 24.34–495.23 in 29 tilapia farms from Yucatan, Mexico, where the main species found were *C. sclerosus*, *C. tilapiae*, *C. dossoui*, *C. longicornis*, *C. quaestio*, and *C. halli*. These data as background suggest that the average values of this research represent a low ectoparasite burden (Table 5 and Figure 1). Additionally, in Nile tilapia, a negative relationship has been

demonstrated between the ectoparasitic burden and the relative condition factor values per fish, which shows that parasite control is relevant to improve the productive growth performance of *O. niloticus* [48].

The research hypothesis central role of garlic powder's effect on eliminating *O. niloticus* monogenean gill parasites could only be partially validated at the level under in vivo conditions of controlled feeding in 30 days. The efficacy of the GP diets recorded (13.4% to 45.6%) was concordant with Yavuzcan et al.'s research [55], studying the in vivo control of *Gyrodactylus elegans* in carp (*Cyprinus carpio*), and achieving only a partial reduction of the mean intensity indicator from 15.2 to 12.8 after 3 min of garlic extract exposure. In this regard, new research is required to determine if a higher efficacy level than 45.6% at in vivo conditions can be reached in commercial tilapia farming systems. Furthermore, the relationship between these results should be elucidated through specific statistics with Nile tilapia well-being and health in all aspects, such as immune-physiological parameters, profile hematological reproductive conditioning, stress indicators, biochemical profile, antioxidant status, and inflammatory gene expression.

The work conducted by Maniat et al. [56] stands out in evaluating the binni (*Mesopotamichthys sharpeyi*) cyprinid fish species' growth performance and body composition, as well as Mohammad's research [57] on hematological, biochemical, and histopathological responses for the common carp (*Cyprinus carpio*). In turn, Ukenye et al. [49] reported growth performance and immunity analyses of catfish (*Clarias gariepinus*).

DNA analysis provides important information for the characterization of parasite strains [58]. The six monogenean parasite species identified involving their recognition have important epidemiological implications in tilapia culture in Mexico. Under normal culture conditions, hosts often show little or no signs of infection. However, the host response can be dramatic and cause significant disease due to organ dysfunction, with subsequent mortalities strongly influenced by the parasitism level [59]. Heavy gill *Cichlidogyrus* infestations could cause adverse effects, such as respiratory disease, anemia, growth loss, piping, severe gill destruction, and drastic economic losses, among other aspects [11,60]. Additionally, GP diets only partially reduce the number of parasites lodged at the gill level. Therefore, new research is required to elucidate the effect of garlic powder on the Nile tilapia welfare–health relationship, especially at high densities.

The present research suggested evaluating the effects at hematological, immunological, and histopathological levels of parasites in the gills of Nile tilapia. For this purpose, designing studies in production systems is necessary, where the diversity of monogeneans is modeled from a perspective of farmed and wild populations from a spatial analysis point of view to establish distribution models based on environmental and productive variables [11,61].

Monogeneans are notoriously difficult to control, as well as for various chemical treatments, because they pose associated problems, such as low efficacy, host toxicity, and health concerns [62,63]. The present research suggested beneficial properties of GP diets as a preventive alternative for monogenean gill parasites, which is represented in Figure 4, based on the most relevant functional compounds [64–67], biological properties [67,68] and mechanisms of action [68,69] for garlic-derived products.

Synergisms have been reported between parasitic pathogens and bacterial infestation in cultured Nile tilapia [68,69]. In the present analysis path, obtaining information on the severity of gill infection at the time of cultivation is crucial to prevent losses due to accumulated mortality in freshwater fish farming, since the results could show negative effects on parameters, such as growth performance, whole-body and hematobiochemical indices, digestive enzyme activity, immune responses, gene expression, and abnormal features in histopathology, among other aspects [70].

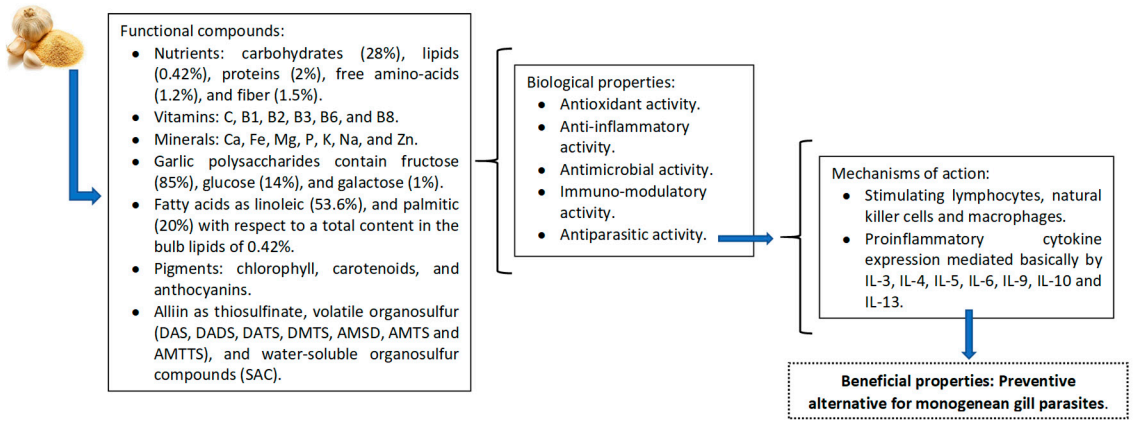


Figure 4. Proposed scheme for the most relevant functional garlic powder properties as a feed additive on Nile tilapia performance. DAS = diallyl sulfide; DADS = diallyl disulfide; DATS = diallyl trisulfide; DATTS = diallyl tetrasulfide; DMTS = dimethyl trisulfide; AMDS = allyl methyl disulfide; AMTS = allyl methyl trisulfide; AMTTS = allyl methyl tetrasulfide; SAC = γ-glutamyl-*S*-allyl-L-cysteine into *S*-allyl-L-cysteine; IL = interleukin.

Gill monogenean infestation causes significant mortalities in cultured fish as a result of respiratory manifestation [13]. Antiparasitic garlic (*Allium sativum*) activity against monogenean parasites has not been widely documented yet in *O. niloticus* as a feed additive, highlighting in this regard the research focused on garlic extract against *Dactylogyrus* spp. (0.02–0.18 µg/mL) [13] and garlic oil (1–3 PPT) and garlic cloves (3 PPT and 300 mg/L) against *Gyrodactylus* spp. [14]. In this path of analysis, the results found in the present research demonstrate the potential of garlic powder as a natural alternative for monogenean parasite infection at dietary levels of 1%, 2%, and 3%. Nevertheless, new research focused on bioeconomic models is required to determine the costs of implementing these strategies in Nile tilapia culture.

5. Conclusions

Garlic powder at inclusion levels of 1%, 2%, and 3% did not affect water quality, survival, growth performance, and feed utilization parameters of *Oreochromis niloticus*. Garlic powder use at inclusion levels of 1% to 3% only partially removed parasites from fish gills; therefore, we suggest that it could be used as a preventive alternative for monogenean gill parasites. Phylogenetic reconstruction represented the diversity of gill monogenean parasites of *O. niloticus* for the Western Aquaculture Region in Mexico, where six species of the genus *Cichlidogyrus* predominated under the experimental conditions used.

Author Contributions: Conceptualization, investigation, methodology, writing—original draft preparation, and funding acquisition, S.M.S.-M. and R.G.-L.; formal data analysis, methodology, and software, C.A.C.-G. and S.M.-G.; resources and supervision, J.C.R.-R.; project administration and supervision, C.O.D.L.C.-M.; writing—review and editing, J.J.F.B.-G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The growth trial was conducted following the principles of the European Food Safety Authority (2008) for animal health and welfare and the World Organization for Animal Health (WOAH) Terrestrial and Aquatic Animal Health Codes. Aspects of animal density and water quality were considered for transport, acclimation, and trial development based on

Oreochromis niloticus optimal growth requirements under laboratory conditions (Code: RBAP-2023-A; Approval Date: March 2023).

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are available upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest. All authors belong to the same educational institution and collaborate in an interdisciplinary manner between academic bodies in the generation and execution of research projects with the Universidad Autónoma de Nayarit.

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