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Growth, gut dimension, immune response and resistance to Aeromonas hydrophila in Nile Tilapia (*Oreochromis niloticus*) fed Onion (*Allium cepa*. L)

Crecimiento, dimensión intestinal, respuesta inmune y resistencia a Aeromonas hydrophila en tilapia del Nilo (*Oreochromis niloticus*) alimentada con cebolla (*Allium cepa*. L)

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ABSTRACT

This study evaluated the use of onion (*Allium cepa*) powder in *Oreochromis niloticus* diets, focusing on growth, gut area, immune response, and resistance to *Aeromonas hydrophila*. Six diets, OP_0, OP_1, OP_2, OP_3, OP_4, and OP_5 with onion powder inclusion from 0 % to 2.5 %, were formulated and fed to triplicate groups of fish (1.79±0.14g) for 10 weeks. Fish were then challenged with *A. hydrophila* and monitored for 14 d. While growth was not significantly altered by onion supplementation over 10 weeks, survival after *A. hydrophila* challenge was significantly improved. Bacterial diversity and total counts increased in gut onion-fed fish, with *Bacillus* and *Enterobacter* being the dominant species. Serum biochemistry indicated reduced alanine aminotransferase and aspartate aminotransferase levels in fish fed 0.5% to 1.5% onion diets, while alkaline phosphatase increased. Antioxidant enzyme activities were generally higher in onion-supplemented groups, with reduced levels of malondialdehyde. Villi height and width did not significantly differ across treatments. *A. hydrophila* challenge on Nile tilapia showed a significantly higher relative percentage survival (RPS) in onion-supplemented groups, with a record 100 % in groups fed 1.0 % and above. This study revealed that 1-1.5 % inclusion of onion in the diets of Nile tilapia resulted in enhanced immune response, gut health, and disease resistance.

Key words: Onion powder, *Oreochromis niloticus, Aeromonas hydrophila,* fish gut, fish blood.

RESUMEN

Este estudio evaluó el uso de polvo de cebolla (*Allium cepa*) en dietas de *Oreochromis niloticus*, centrándose en el crecimiento, el área intestinal, la respuesta inmune y la resistencia a *Aeromonas hydrophila*. Se formularon seis dietas, OP0, OP1, OP2, OP3, OP4 y OP5 con inclusión de polvo de cebolla de 0% a 2.5%, y se alimentaron a grupos triplicados de peces (1.79 ± 0.14 g) durante 10 semanas. Luego, los peces fueron desafiados con *A. hydrophila* y monitoreados durante 14 días. Si bien el crecimiento no se alteró significativamente con la suplementación con cebolla durante 10 semanas, la supervivencia después del desafío con *A. hydrophila* mejoró significativamente. La diversidad bacteriana y los recuentos totales aumentaron en los peces alimentados con cebolla intestinal, siendo *Bacillus* y *Enterobacter* las especies dominantes. La bioquímica sérica indicó niveles reducidos de alanina aminotransferasa y aspartato aminotransferasa en los peces alimentados con dietas de cebolla de 0.5 % a 1.5 %, mientras que la fosfatasa alcalina aumentó. La actividad de las enzimas antioxidantes fue generalmente mayor en los grupos suplementados con cebolla, con niveles reducidos de malondialdehído. La altura y el ancho de las vellosidades no mostraron diferencias significativas entre los tratamientos. El desafío con *A. hydrophila* en tilapia del Nilo mostró un porcentaje de supervivencia relativa (SPR) significativamente mayor en los grupos suplementados con cebolla, con un récord del 100 % en los grupos alimentados con una concentración del 1.0 % o superior. Este estudio reveló que la inclusión de cebolla del 1 % al 1.5 % en las dietas de tilapia del Nilo resultó en una mejor respuesta inmunitaria, salud intestinal y resistencia a enfermedades.

Palabras clave: Polvo de cebolla, *Oreochromis niloticus*, *Aeromonas hydrophila*, intestino de pescado, sangre de pescado.

INTRODUCTION

Aquaculture is crucial for global food security, and relies heavily on Nile tilapia (*Oreochromis niloticus*) as a key farmed species. The global production of this species, especially in tropical and subtropical riverine regions, reached seven million tons in 2024, a 4 - 5 % increase from last year (FAO, 2024). Sustainable aquaculture, which aims to produce high-quality fish for human consumption, relies on efficient nutrient utilization by farmed species, effective disease control, and eco-friendly culture practices. Ironically, intensive aquaculture practices can increase the susceptibility of tilapia to various diseases, including those caused by the bacterium *Aeromonas hydrophila*, posing significant challenges to sustainable production. Aquaculture practices have historically relied on the application of different synthetic compounds, including antibiotics, for growth enhancement, disease treatment and prevention. However, the rising prevalence of antibiotic-resistant bacterial strains, bioaccumulation of

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these compounds within cultured species, and the adverse ecological impacts on adjacent aquatic environments have led to regulatory restrictions, broadly prohibiting their use (Upadhaya and Kim, 2017). As a result, it is imperative to identify and implement more effective and sustainable approaches to pathogen management in aquaculture.

One potential strategy to enhance health and disease resistance of tilapia is through dietary supplementation with immune-stimulants. In response to the adverse effects of synthetic compounds, there is a growing focus on utilizing natural alternatives from plants, herbs, and their extracts to enhance fish growth and immunity without negatively impacting aquatic ecosystems (Abdel-Tawwab *et al*., 2018; Ajani *et al*., 2020; Orisasona *et al*., 2024). The regulatory roles of these natural products in fish metabolism are attributed to their diverse bioactive constituents, such as flavonoids, steroids, pigments, alkaloids, and phenolic compounds (Hodar *et al*., 2021). *Allium cepa* (Onion), a widely used spice, has been recognized for its medicinal properties and ability to boost the immune system (Elgendy *et al*., 2023) due to its rich content of bioactive compounds including phenolics and flavonoids. *Allium cepa* is a source of various bioactive compounds beneficial for aquaculture. Muhammad *et al.* (2020) reported that dark-colored onion bulbs exhibit higher levels of pyruvic acid, vitamin C, and flavonoids, while light-colored bulbs are richer in phenolic content. Additionally, Sami *et al*. (2021) documented the presence of vitamins B and C, as well as essential ions like calcium, copper, magnesium, and potassium, all crucial in regulating metabolic activities in fish. Traditionally, onion has been used to aid digestion and stimulate appetite to enhance growth in cultured fish (Vigneshpriya and Krisnaveni, 2016). Consistent with this, growth improvements have been observed in olive flounder, sea bass, and African catfish fed diets supplemented with onion (Cho and Lee, 2012; Bello *et al*., 2013; Saleh *et al*., 2015). While onion extracts and derivatives have been studied in some fish species, their potential benefits in tilapia culture remain underexplored. Specifically, there is limited scientific evidence on how onion-fortified diets influence growth performance, gut morphology, immune function, and disease resistance in Nile tilapia. Furthermore, the optimal inclusion levels of onions in fish diets and their mechanisms of action against *A. hydrophila* infection require investigation.

This study aimed to evaluate the impact of onion-fortified diets on the growth performance, gut morphology, immune response, and disease resistance of Nile tilapia against *Aeromonas hydrophila*. This is to enhance fish health and sustainable production, while reducing reliance on antibiotics in aquaculture.

MATERIALS AND METHODS

Fish Feed, Experimental Design and Culture Conditions

Dark-skinned fresh onion bulbs were thoroughly washed, finely chopped, sun-dried, and pulverized into a fine powder using a blender. Six experimental diets with 35% crude protein were formulated, using a base mixture of cellulose, soybean meal, fishmeal, groundnut oil, salt, lysine, methionine, maize, and a fish premix. Onion powder was incorporated into diets at levels of 0%, 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%, to produce OP_0, OP_1, OP_2, OP_3, OP_4 and OP_5 respectively (Table 1). All ingredients were thoroughly mixed and pelletized using a Hobart pelletizer (model A200, 2mm die). The pellets were air-dried, packaged in labeled bags, and stored at 4°C.

The proximate composition of each diet was determined in duplicate according to AOAC (2005) methods. Moisture content was measured by drying samples at 105°C for 24 h. Crude protein was determined using a semi-auto Kjeldahl system (KDN-04A, Shanghai Hua Rui Instrument Co., Ltd), with a conversion factor of 6.25. Crude lipid was extracted using the Soxhlet method. Ash content assay was done by incinerating samples at 600°C for 24 h in a muffle furnace.

For 14 d, the fish were acclimatized, after which 360 *Oreochromis niloticus* (1.79±0.14g/fish) were randomly allotted equally to 18 fibreglass tanks (40 L) in a flow-through system. Fish were fed the six diets two times daily at apparent satiation (8:20 am and 4:20 pm) for 10 weeks. Water flowed through the tanks at 0.3 L/min and was aerated by air-stones connected to air pumps. Weights were determined biweekly. Dissolved oxygen and temperature (mean values 5.01-5.20 mg/L and 25.8-26.15 °C respectively) was measured using YSA digital probe meter (Model 57; VWR Company, NJ). The potential of hydrogen (mean values 7.10-7.30) was measured using Pen type meter, while Aquasol AE-207 nitrite kit was used to determine nitrite (0.04 mg/L).

Growth and Nutrient Utilization in *Oreochromis niloticus* Fed Onions Fortified Diets

The effect of onions on Tilapia's growth and nutrient utilization was estimated using the initial and final weights of fish, the quantity of feed consumed, and nutrients diets.

Feed intake (g)=Sum of feed consumed during experimental period
Weight gain (g)=W2-W1
Specific growth rate (SGR)={[Log_e W2-Log_e W1]÷[T2-T1]}×100
Food conversion ratio (FCR)=Feed consumed (g)÷Gain in weight (g)
Survival rate=[No.of fish at end of trial T2÷No.of fish at start of trial T1]×100
Where W2 = final weight, W1 = initial weight, Log_e = Natural logarithm, T2 – T1 = experimental period in d, Protein intake = Feed intake × %Protein in diets

Gut Ecology of *Oreochromis niloticus* Fed Onions Fortified Diets for 70 D

Gut samples were collected from nine *O. niloticus* fish per treatment (3/unit) using sterile instruments and placed in sterile Petri dishes. For serial dilutions, 9 mL of sterile distilled water in foil-wrapped, cotton-plugged test tubes was autoclaved at 121°C for 15 minutes. From each gut sample, 1 mL stock solution was prepared, and serial dilutions from 10^{-1} to 10^{-10} were performed. Using a sterile syringe, 1 mL of the 10^{-4} dilution (Ajani *et al*., 2020) was dispensed into labeled sterile Petri dishes and molten sterile agar was aseptically poured. The plates were gently swirled to ensure even distribution of the inoculum (Narvaez *et al*., 2010). After solidification, the plates were incubated at 37°C for 24-48 h. To obtain pure iso-

Table 1. Gross and analyzed composition of experimental diets fortified with onions (g/100g DM) fed to *Oreochromis niloticus*.
Tabla 1. Composición bruta y analizada de dietas experimentales fortificadas con cebolla (g/100 g MS) alimentadas a *Oreochromis niloticus.*

Ingredients	OP_0	OP_1	OP_2	OP_3	OP_4	OP_5
Fish meal (72)	16.78	16.78	16.78	16.78	16.78	16.78
Soybean meal (44)	32.66	32.66	32.66	32.66	32.66	32.66
Ground nut cake (45)	16.78	16.78	16.78	16.78	16.78	16.78
Yellow Maize	15.25	15.25	15.25	15.25	15.25	15.25
Cellulose	15.25	14.75	14.25	13.75	13.25	12.75
Oil	2.00	2.00	2.00	2.00	2.00	2.00
Premix*	0.50	0.50	0.50	0.50	0.50	0.50
Lysine	0.20	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin C	0.03	0.03	0.03	0.03	0.03	0.03
Salt	0.25	0.25	0.25	0.25	0.25	0.25
DCP**	0.10	0.10	0.10	0.10	0.10	0.10
Onion powder	0.00	0.50	1.00	1.50	2.00	2.50
Total (g)	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)						
Crude protein	34.07	34.37	34.03	34.88	34.85	35.08
Ash content	8.30	7.70	8.25	7.35	7.45	8.50
Ether	8.07	7.65	7.25	6.80	6.35	6.90
Crude fibre	3.06	3.30	3.55	3.77	4.25	5.00
Moisture contents	7.50	8.00	7.84	8.22	7.55	7.85
NFE	39.00	38.98	39.08	38.98	39.55	36.67

Premix* = HI-MIX®AQUA 1 kg each contains; Folic acid, 800 mg; vitamin A, 4,000,000 IU; vitamin B6, 3,800 mg; vitamin B1, 4,000 mg; vitamin B2, 3,000 mg; vitamin K3, 1,600 mg; vitamin B12, 3 µg; vitamin D3, 8,00,000 IU; vitamin E, 40, 000 IU; Nicotinic acid 18000 mg; Biotin, 100 µg; Pantothenic acid, 8000 mg; Choline chloride 120,00.
***DCP= dicalcium phosphate,* NFE= Nitrogen free extract.

lates, distinct yellow colonies were repeatedly sub-cultured on nutrient agar. These isolates were Gram-stained and stored on nutrient agar slants in cryovials at 4°C for further analysis. 24 h old isolates were examined and characterized based on their cultural characteristics and biochemical properties (Olutiola *et al*., 2000).

Blood and Serum Biochemical Profiling of *Oreochromis niloticus* Fed Onion Based Diets

Nine fish per treatment were selected and bled serially using tuberculin syringes fitted with 24-gauge needle (Omitoyin *et al*., 2006). For each treatment, two tubes were used for blood collection. For red blood cells (RBC), packed cell volume (PCV), white blood cells (WBC), hemoglobin (Hb) and platelet counts, blood samples were collected in bottles containing 20 U/L sodium heparinate as coagulant. The second tubes without anticoagulant were used for enzyme activities and biochemical analysis. Blood in the group was allowed to clot at 4°C and centrifuged at 10,000 xg for 15 min to obtain the serum. Packed cell volume (PCV) was determined using a hematocrit reader (Adeyemo, 2005). Hemoglobin (Hb) concentration and red blood cell (RBC) counts were measured using Drabkin's solution and Rees and Ecker's diluent fluid, respectively (Adeyemo, 2005; Oresegun and Alegbeleye, 2001). A Neubauer hemocytometer was used for White blood cell (WBC) counts as described by Kaplow (1955). Platelet counts were determined using the Rees and Ecker direct method (Osuigwe *et al*., 2005). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated using the method of Reitman and Frankel (1957), and serum alkaline phosphatase (ALP) activity was determined according to Tietz *et al*. (1983).

Histological Processing and Gut Morphometry of *Oreochromis niloticus* Fed Onion Fortified Diets

For histological analysis, four fish were randomly selected per treatment and dissected, and the mid-gut was excised and fixed in 10 % buffered formalin. Samples were dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Sections, approximately 5 µm thick, were cut using a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin (Hopwood, 1996). Histomorphometric analysis was conducted following the method of Oladele *et al.* (2012). Five villi were randomly selected and measured from each section. Villi dimensions were measured in microns, converted to centimeters, using ToupView software on an AmScope MU900 camera, and the absorption area was calculated (Omitoyin *et al*., 2019).

Measurement of Antioxidant Biomarkers and Oxidative Enzyme Activities

The livers of six fish selected from each treatment were aseptically excised, cleaned, and blended in potassium phosphate buffer (0.1 M, pH 7.4). The samples were centrifuged at 10,000 rpm for 20 min. The resulting post-mitochondrial supernatants were maintained at -4°C until used for the biochemical assays. Protein concentration, Hydrogen peroxide (H_2O_2) and Lipid peroxidation were determined by the method of Gornal *et al*. (1949), Wolff (1994), and Varshney and Kale (1990), respectively. The concentrations of glutathione (GSH), Glutathione-S-transferase (GST) and Glutathione peroxidase (GPx) activities were estimated as described by Jollow *et al.* (1994), Habig *et al.* (1974), and Buetler *et al.* (1963), respectively.

Aeromonas hydrophila Challenge Test on *Oreochromis niloticus* Fed Onions Fortified Diets

A virulent strain of *Aeromonas hydrophila*, obtained from formalin-killed bacterin (Baba *et al*., 1988) at the Microbiology Department, University of Ibadan, was held overnight at room temperature. Sterility and safety tests were performed (Cardella and Eimers, 1990). The bacterial culture was then adjusted to a concentration of 1×10^7 CFU/mL in phosphate-buffered saline (PBS) (Omitoyin *et al*., 2019). For each treatment, 20 fish were randomly selected and divided into two groups (A and B) of 10 fish per aquarium. Group A, serving as the control, received an intraperitoneal injection of 0.5 mL PBS, while group B received 0.5 mL of the *A. hydrophila* suspension. Fish were monitored for 14 d post-injection, with dead fish removed immediately. Relative percentage survival (RPS) was calculated (Kocour *et al*., 2005) as follows;

$$RPS(\%) = \frac{No\ of\ surviving\ fish\ after\ challenge}{No\ of\ fish\ injected\ with\ bacteria} \times 100$$

Statistical Analysis

Bartlett's test for homogeneity of variances among treatments was carried out. Descriptive statistics and one way analysis of variance (ANOVA) was used to ascertain the effect of onion powder on fish using IBM Statistical Package for Social Science (SPSS) version 20. Mean differences were separated using Duncan's test at the 5 % probability level. The optimum inclusion level of *Curcumin longa* for growth was determined using polynomial regression.

RESULTS

Nutrient Utilization, Growth and Survival of *Oreochromis niloticus* Fed Onions Fortified Diets

The indices of growth and nutrient utilization in *O. niloticus* fed onion powder fortified diets are presented in Table 2. Growth indicators of weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR) did not vary significantly (P>0.05) across treatments. Feed intake was also not affected by the administration of treatments. However, the percentage of survival varied from 85% in OP0 to 96% in OP_2. The second-order polynomial regression showing the relationships between survival and onion powder inclusion level is presented in Figure 1. An optimal inclusion of 1.05% is recommended and $Y = 34.391X^3 - 161.84X^2 + 228.51X$ represents the regression equation.

Mean gut morphometry of *O. niloticus* fed with different onion powder inclusion is presented in Table 3. Area of absorption was significantly lower (P<0.05) in the control and OP_5 groups when compared to the others. The latter group generally resulted in the lowest values for all three measured parameters. The highest villi width and absorption area were recorded in OP_4.

Microbial Analysis of *O. niloticus* Gut Microflora

The most isolated bacteria in the gut of *O. niloticus* fed different inclusion levels of onion powder were Bacillus and Enterobacter species (Table 4). In this study, the diversity of bacteria was higher in fish fed diets fortified with onions. Similarly, total heterotrophic and coliform counts were higher in the groups fed onions fortified diets, as shown in Table 4.

Hematological and Serum Biochemical Indices

Packed cell volume was significantly higher (p<0.05) in groups fed 1.5% onion inclusion and above, while red blood and white blood cell counts did not vary significantly across treatments, as shown in Table 5. High hemoglobin values were observed in OP_1 and OP_4 (8.46 g/dL and 8.35 g/dL, respectively) while significantly lower values were recorded

Table 2. Mean growth performance parameters and feed utilization of *O. niloticus* fed with experimental diets.

Tabla 2. Parámetros de rendimiento de crecimiento medio y utilización de alimento de *O. niloticus* alimentado con dietas experimentales.

Treatment	Initial Weight (g)	Final Weight (g)	Weight Gain(g)	FCR	SGR (%/day)	FI (g)	PER (%)	Survival (%)
OP0	1.76±0.15^a	14.75±0.28^{ab}	12.99±0.18^{ab}	1.79± 0.19^a	2.53±0.05^a	23.24±0.42^a	1.59±0.51^a	85
OP_1	1.78±0.086^a	15.43±0.71^b	13.66±0.79^b	1.78±0.43^a	2.57±0.21^a	24.32±1.21^a	1.60±0.13^a	85
OP_2	1.73±0.19^a	14.19±0.13^a	12.46±0.15^a	1.92±0.47^a	2.50±0.11^a	23.98±0.63^a	1.48±0.10^a	96
OP_3	1.73±0.17^a	14.69±0.36^{ab}	12.96±0.36^{ab}	1.86±0.55^a	2.61±0.13^a	24.23±1.03^a	1.52±0.16^a	91
OP_4	1.84±0.13^a	14.89±0.63^{ab}	13.76±0.56^b	1.85±0.35^a	2.42±0.12^a	24.01±1.18^a	1.54±0.08^a	91
OP_5	1.78±0.14^a	14.79±0.41^{ab}	13.02±0.46^{ab}	1.87±0.44^a	2.51±0.15^a	24.34±0.35^a	1.52±0.13^a	95

Mean values with different superscripts along a column are significantly different (p<0.05).
NB: FCR; Feed conversion ratio; SGR, Specific growth rate; FI, Feed intake; PER, Protein efficiency ratio.

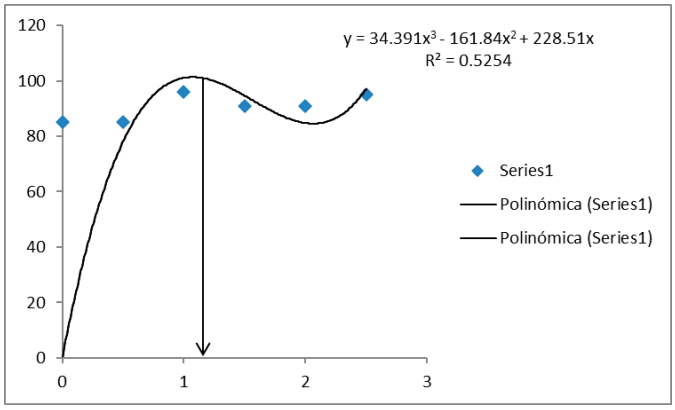

Figure 1. Polynomial regression curve showing the optimum inclusion level of onion powder in relation to survival.
Figura 1. Curva de regresión polinomial que muestra el nivel óptimo de inclusión de cebolla en polvo en relación con la supervivencia.

Table 3. Mean gut morphometrics of fish fed onion powder diets for 84 d.
Tabla 3. Morfometría intestinal media de peces alimentados con dietas de cebolla en polvo durante 84 días.

Treatment	Villi Height (µm)	Villi width (µm)	Area of Absorption (µm²)
OP_0	0.185^{b}	0.077^{abc}	0.014^{b}
OP_1	0.200^{c}	0.083^{c}	0.017^{cd}
OP_2	0.195^{bc}	0.075^{ab}	0.016^{cd}
OP_3	0.201^{c}	0.081^{bc}	0.017^{cd}
OP_4	0.193^{bc}	0.095^{d}	0.019^{d}
OP_5	0.165^{a}	0.070^{a}	0.011^{a}

Table 4. Summary of isolates and bacterial counts obtained from the gut of *O. niloticus* fed an onion powder-based diet.
Tabla 4. Resumen de aislamientos y recuentos de bacterias obtenidos del intestino de *O. niloticus* alimentado con una dieta a base de cebolla en polvo.

Bacteria	OP0	OP_1	OP_2	OP_3	OP_4	OP_5	Total
Bacillus sp.	1	1	3	2	1	2	10
Enterobacter sp.	--	1	1	3	4	1	9
Klebsiella sp.	--	--	--	--	1	--	1
Proteus sp.	--	--	--	--	--	1	1
Staphylococcus sp.	1	--	--	1	--	--	2
Flavobacterium sp.	--	3	--	--	--	1	4
Pseudomonas sp.	--	--	--	1	--	--	1
E. coli sp.	--	--	--	--	1	--	1
Bacteria count (x10³CFU/g)							
Total Coliform Count (TCC)	1.04	10.03	30.32	16.67	10.31	6.67	
Total Heterotrophic Count (THC)	0.63	0.83	1.50	4.63	5.83	3.63	

Note: -- = no growth; 1, 2, 3, 4, 9, and 10 are numbers of isolates.

Table 5. Mean blood and serum biochemical indices of *O. niloticus* fed onion supplemented diets**.**
Tabla 5. Índices bioquímicos sanguíneos y séricos medios de *O. niloticus* alimentados con dietas suplementadas con cebollas.

Parameters	OP_0	OP_1	OP_2	OP_3	OP_4	OP_5
PCV (g/dL)	20.66±0.33^{d}	22.49±0.49^{cd}	21.66±0.33^{cd}	24.55±1.12^{ab}	25.04±0.04^{a}	22.84±0.17^{bc}
Hb (g/dL)	7.05±0.05^{c}	8.46±0.03^{a}	6.92±0.09^{c}	6.93±0.16^{c}	8.35±0.05^{a}	7.60±0.10^{b}
RBC (x10⁶/µL)	1.68±0.00^{b}	2.83±0.07^{a}	2.15±0.15^{ab}	2.16±0.59^{ab}	2.61±0.01^{ab}	2.13±0.00^{ab}
WBC (x10⁹/µL)	1.47±0.20^{a}	1.40±0.00^{a}	1.50±0.09^{a}	1.45±0.11^{a}	1.45±0.01^{a}	1.42±0.00^{a}
Platelets (%)	14.61±0.01^{b}	12.33±0.00^{f}	13.76±0.06^{d}	13.33±0.03^{e}	16.05±0.05^{a}	14.28±0.05^{c}
Lymphocytes (%)	61.00±0.00^{c}	58.50±0.50^{d}	63.05±0.28^{b}	63.50±0.50^{b}	61.50±0.05^{c}	66.50±0.50^{a}
Heterophils (%)	31.62±0.05^{c}	34.63±0.03^{a}	30.71±0.04^{d}	28.36±0.03^{e}	32.33±0.05^{b}	28.36±0.03^{e}
Monocytes (%)	2.67±0.00^{c}	3.00±0.00^{b}	2.67±0.00^{c}	3.66±0.00^{a}	2.36±0.03^{d}	2.33±0.00^{d}
Eosinophils (%)	4.16±0.16^{a}	4.00±0.00^{a}	3.00±0.00^{b}	3.16±0.16^{b}	4.00±0.00^{a}	3.05±0.05^{b}
Basophils (%)	0.33±0.00^{a}	0.33±0.00^{a}	0.33±0.00^{a}	0.33±0.00^{a}	0.33±0.00^{a}	0.33±0.00^{a}
AST (µL)	194.95±0.72^{a}	189.00±1.00^{b}	192.43±0.23^{ab}	189.94±0.27^{ab}		193.57±3.24^{ab}
ALT (µL)	32.33±0.66^{a}	28.28±0.05^{bc}	28.00±0.00^{c}	28.55±0.45^{bc}	30.20±1.00^{b}	32.56±0.44^{a}
ALP (µL)	183.5±0.50^{c}	183.61±0.61^{c}	201.06±1.94^{a}	193.60±3.60^{ab}		185.20±2.00^{bc}

Mean values with same superscript across row are statistically similar (p>0.05).
NB: PCV= Packed Cell Volume; Hb = Hemoglobin concentration; RBC = Red Blood Cell; WBC = White Blood Cell; AST= Aspartate Aminotransferase; ALT = Alanine Aminotransferase; ALP = Alkaline Phosphate.

in OP_2 and OP_3 (6.92 g/dL and 6.93 g/dL, respectively). Platelets count across treatments varied significantly ($p<0.05$). There was a marginal reduction in the level of aspartate aminotransferase (AST) in fish fed diets fortified with onion powder. Alanine aminotransferase (ALT) was significantly lower in fish fed the onion supplement, except in OP_5, which was significantly similar to the value in the control group. However, alkaline phosphatase levels increased in fish fed onions-fortified diets.

Antioxidant Enzyme Activities and Oxidative Markers in *O. niloticus* Fed Onion Powder Based Diets

Examination of the oxidative markers and antioxidant enzyme activities from the kidneys of experimental fish showed no variation (>0.05) in total protein (TP), hydrogen peroxide (H_2O_2), glutathione S-transferase (GST), glutathione (GSH), and glutathione peroxidase (GPx) among experimental fish (Table 6). However, malondialdehyde (MDA) values were significantly lower in fish fed between 1.0 and 2.0 % onion powder fortified diets.

In the liver of fish, there was a significant reduction in the total protein values of fish fed onions fortified diets, except for the OP_5 group. As shown in Table 7, values of malondialdehyde, H_2O_2, and GPx were higher in the control compared to the others.

Resistance of *O. niloticus* to *Aeromonas hydrophila* after Feeding

The relative percentage survival (RPS) at the end of the 14 day post *A. hydrophila* injection was 30 % in the OP0 group, 80% in the OP_1 group and 100% in the other groups (Figure 2). The six control groups injected with saline water all had zero mortality recorded.

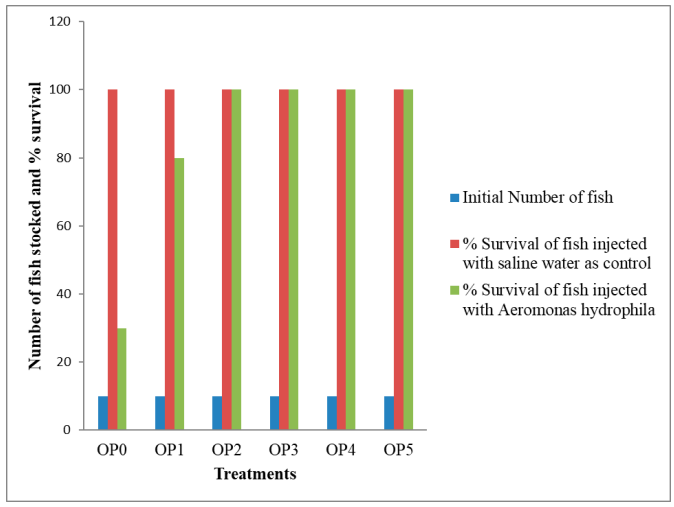

Figure 2. Survival of *O. niloticus* fed diets fortified with onion powder after challenging period.
Figura 2. Supervivencia de *O. niloticus* alimentados con dietas fortificadas con cebolla en polvo después del período de desafío.

DISCUSSION

In this study, results indicate that fortification of diets with onion (*Allium cepa*) powder did not impact growth and nutrient utilization in fish. Mean weight gain, feed conversion ratio and specific growth rate showed statistical similarity across all groups of experimental fish. Also, conversion of feed to flesh was not improved implying a no-effect of onion

Table 6. Oxidative stress indices in the kidney of *O. niloticus* fed Onion powder.
Tabla 6. Índices de estrés oxidativo en el riñón de *O. niloticus* alimentado con polvo de cebolla.

	Total protein (U/mg)	H_2O_2 (µmol/mg)	MDA (nmol/mg)	GSH (U/mg)	GPx (U/mg)	GST (µmol/mg)
OP_0	0.17±0.06^{ab}	47.73±14.32^{a}	5.20±2.02^{a}	83.11±3.07^{ab}	1354.21±448.71^{a}	0.65±0.38^{a}
OP_1	0.19±0.06^{ab}	44.02±9.05^{ab}	4.92±2.78^{a}	82.46±2.12^{ab}	1179.80±331.53^{a}	0.68±0.16^{a}
OP_2	0.21±0.08^{a}	37.48±7.68^{b}	2.51±1.23^{c}	83.43±2.14^{ab}	1116.79±445.66^{a}	0.49±0.37^{a}
OP_3	0.18±0.06^{ab}	38.62±8.28^{ab}	3.58±1.74^{bc}	82.60±5.66^{ab}	1209.98±393.08^{a}	0.53±0.32^{a}
OP_4	0.18±0.04^{ab}	43.73±11.92^{ab}	3.06±1.01^{bc}	80.63±2.83^{b}	1189.07±297.08^{a}	0.65±0.31^{a}
OP_5	0.15±0.04^{b}	42.67±12.36^{ab}	4.64±1.85^{ab}	86.78±13.04^{a}	1180.93±303.21^{a}	0.59±0.38^{a}

Mean values with same superscript along column are statistically similar ($p>0.05$).
Note: H_2O_2= hydrogen peroxide; MDA=malondialdehyde; GSH=glutathione; GPx=glutathione peroxidase; GST= glutathione S-transferase.

Table 7. Oxidative stress indices in the liver of *O. niloticus* fed Onion powder-based diets.
Tabla 7. Índices de estrés oxidativo en el hígado de *O. niloticus* alimentados con dietas a base de cebolla en polvo.

Parameters	Total protein (U/mg)	H_2O_2 (µmol/mg)	MDA (nmol/mg)	GSH (U/mg)	GPx (U/mg)	GST (µmol/mg)
OP_0	0.25±0.00^{a}	49.95±0.06^{a}	5.60±0.06^{a}	78.47±0.07^{c}	1037.78±0.43^{b}	0.47±0.00^{c}
OP_1	0.24±0.00^{b}	37.88±0.01^{bc}	3.38±0.02^{f}	81.68±0.04^{a}	917.41±5.02^{d}	0.58±0.00^{b}
OP_2	0.23±0.00^{bc}	36.07±0.03^{c}	5.08±0.01^{b}	80.22±0.10^{b}	1091.83±1.51^{a}	0.89±0.00^{a}
OP_3	0.22±0.00^{c}	45.28±5.00^{ab}	4.47±0.01^{c}	71.27±0.22^{f}	983.32±4.89^{c}	0.58±0.00^{b}
OP_4	0.24±0.00^{b}	45.08±0.00^{ab}	4.28±0.00^{d}	74.70±0.16^{e}	882.22±0.66^{e}	0.47±0.01^{c}
OP_5	0.26±0.00^{a}	41.46±0.00^{abc}	3.95±0.03^{e}	76.30±0.09^{d}	792.27±0.95^{f}	0.41±0.00^{d}

Mean values with same superscript along column are statistically similar ($p>0.05$).
Note: H_2O_2= hydrogen peroxide; MDA=malondaldehyde; GSH=glutathione; GPx=glutathione peroxidase; GST= glutathione S-transferase.

powder on feed cost reduction or modulation in this study. This agrees with Taher *et al*. (2024) when onion powder was fed to *Cyprinus carpio* in earthen ponds, although Saleh *et al*. (2015) reported that onions have digestive properties and appetite enhancing abilities in sea bass. Onion powder fed as additives in earlier work was reported to cause growth improvement in brown-marbled grouper, *Epinephelus fuscoguttatus*, *Clarias gariepinus*, and *Oreochromis niloticus* (Aly and Mohamed, 2010; Apines-Amar *et al*., 2012; Agbebi *et al*., 2013). The results in the current study may be attributed to the possibility that the basal diet was already nutritionally adequate for growth, thus minimizing the potential benefits of fortification. Also, the effectiveness of onion may be influenced by the form and quality of the powder used. It is also possible that the tilapia developed tolerance to the phytogenic compounds in the onion's powder over time. Further studies using varied inclusion rates, and different onion preparations may help clarify the potential role as a growth promoter in tilapia diets.

The surface area for the absorption of nutrients in the gut of fish is dependent on the size and number of the villi, and there must be a balance in cell renewal and loss for the sustenance of the digestive and absorptive capacity of the intestine. According to Azevedo *et al.* (2016), disequilibrium in cell turnover resulting in a change in villi height may occur when the fish responds to anti-nutritional compounds in diets or pathogenic compounds. In the present study, villus height, width, and area absorption were significantly different ($P<0.05$) across the treatments, with groups fed onions fortified diets exhibiting higher values. Although this is similar to results of earlier work where villi length and width were significantly increased with the phytogenic extract additives, there was however, a non-significant difference in growth indices in the present study, which is at variance with the results of the previous studies (Mello *et al*., 2013; Omitoyin *et al*., 2019; Ajani *et al*., 2020). The non-alignment of the growth results in this present study to previous works may be due to species-specific responses, differences in onion dosage, form and quality, or already optimized diets that mask the effects of enhanced nutrient absorption. While onion powder improves gut structure, its impact on nutrient absorption may not be sufficient on its own to translate into measurable growth benefits under the present experimental conditions.

Fish fed onion powder had superior rates of survival. The result in this present study may be attributed to the presence of quercetin and organosulfur which are flavonoids in onion that have been suggested to improve the health status of fish. Also, immunomodulation properties are conferred on onions because of the predominance of S-propenyl-CSO in cysteine sulfoxide contained in them (Ostrowska *et al.*, 2004). According to Amar and Faisan (2011), sulfur containing compounds modulate the immune system in fish because of the S factor described as a component of antioxidant enzyme, glutathione peroxidase. The result of this study agrees with the findings of several authors where survival rates were improved in fish fed onion powder (Farahi, *et al*., 2010; Kalyankar *et al*., 2013; Saleh *et al*., 2015).

The microbial population also influences the survival of fish in the gut. Studies have shown that functional feed additives enhance the natural defense process by controlling the commensal gut microbiota, inhibiting disease-causing microbes from entering the organism and by directly activating the innate (nonspecific) and adaptive immune systems (Thepot *et al*., 2021). These gut microbes produce some physiologically active compounds (enzymes, amino acids, vitamins) beneficial to the host, and they also help in the breakdown of nutrients, while the host ensures the required metabolic activities for the sustenance of the microbes. In this study, eight isolated bacteria species were obtained (*Proteus sp.*, *Flavobacterium sp.*, *Staphylococcus sp.*, *Bacillus sp.*, *Klebsiella sp.*, *Enterobacter sp.*, *E. coli*, *Pseudomonas sp.*), with *Bacillus sp.* present in all samples examined. The bacterial diversity was higher in the gut of fish fed onion powder. Diets generally have an impact on the gut microbiota of fish. According to Givens *et al*. (2015), feed variation resulted in significant variability in the microbiota of fifteen different species. Similarly, the microbial gut components in rainbow trout and salmon fed different diets caused a shift in specific bacterial genera, for instance, an increase in the occurrence of *Staphylococcus* and *Lactobacillus* in grain-based diet (Wong *et al*., 2013; Schmidt *et al*., 2016).

Monitoring hematological parameters in cultured species can be used to ascertain their health and physiological status (Nwanna *et al*., 2013). Although no significant differences were observed in parameters such as red blood cells, haemoglobin and white blood cells, the increase recorded in packed cell volume at 1.5 % and above inclusion levels shows that onion has a positive health effect on fed fish. This is more so because all the other blood indices fall within the optimal range for cultured fish (Hrubec *et al*., 2000). The similarity observed in the RBC, Hb and WBC levels in both treated fish and the control group is at variance with the report of Saleh *et al.* (2015), where the fish blood had increased Hb and WBC when administered diets with garlic or onion powder and Kalyankar *et al*. (2013), where Swordtail, *Xiphophorus helleri* showed significant increase in all blood indices when fed 1.5% garlic in diet.

The marginal reduction in the level of aspartate aminotransferase (AST) in fish fed up to 1.5 % onion supplements and the significantly lower ($p<0.05$) values of Alanine aminotransferase (ALT) signify that fish organs are functioning at levels devoid of physiological stress. The release of these two enzymes is indicative of cellular damage in the presence of a stressor (Soosean *et al*., 2010). Omitoyin *et al*. (2019) observed a reduction in the value of AST and ALT when *O. niloticus* were fed diets with guava leaf extracts as a supplement. Following the postulation in Omitoyin *et al.* (2019), it may be stated that the presence of alkaloids in onion protects the hepatic tissue of fish. A significant increase in the values of alkaline phosphatase is a signal that *Allium cepa* supplemented diets enhance the health status of *O. niloticus.* Omitoyin *et al.* (2019) stated that increased ALP is an indication of better absorption, better build-up and proper functioning of

cells. The significant increase in alkaline phosphatase activity observed in *O. niloticus* fed *Allium cepa* supplemented diets may indicate enhanced liver metabolic activity and improved physiological function. When considered alongside other health indices, this suggests that *A. cepa* supplementation could contribute positively to fish health.

Oxidative stress causes the production of high levels of reactive oxygen species (ROS) that result in damage to proteins, lipids, and changes in glutathione and antioxidant enzymes. Quantification of oxidative stress in cultured fish is achieved by determining membrane damage by lipid peroxidation and evaluating the antioxidant defense (Ajani *et al*., 2020). Although the kidney's Total protein (TP), hydrogen peroxide (H_2O_2), glutathione S-transferase (GST), glutathione (GSH), and glutathione peroxidase (GPx) were statistically similar ($p>0.05$) among experimental fish, there was however a reduction in malondialdehyde (MDA) values in OP_2, OP_3 and OP_4 groups. Also, TP, MDA, H_2O_2 and GPx were reduced significantly in the liver of fish fed diets fortified with onions. A decrease in the products of oxygen free radical peroxidation of lipids is indicative of cellular integrity in the fish fed onion powder diets. The changes observed in the liver biomarkers in *O. niloticus* may be attributed to the combined antioxidant and metabolic regulatory effects of quercetin, allicin, and phenolic acids present in it. These bioactive compounds help reduce oxidative stress and modulate enzyme activity, and therefore enhance liver health and systemic physiology in fish. This assertion is supported by Yagi (1984) and Abdel-Tawwab and Abass (2017) where the mop up of superoxide anions by flavonoids in phytogenic is reported to stimulate immune-modulatory activity in fish. A similar trend of reduced MDA and H_2O_2 is reported in Xu *et al*. (2015) when hybrid tilapia was fed yeast nucleotides as an additive.

A challenge test was conducted to confirm the effect of onion powder on the innate immune system of fish fed experimental diets. After the 14-day challenge with *A. hydrophila*, the relative percentage survival was higher in *O. niloticus* fed onions fortified diets. Fish fed 1.0 % and above *A. cepa* recorded 100% survival after 14 d of infection with *A. hydrophila*. This suggests that the innate immune system is enhanced by onion powder, which enables them to resist attacks from *A. hydrophila* and achieve a better survival rate. This can be attributed to the immunostimulatory, antimicrobial, and antioxidant properties of bioactive compounds in onion. This agrees with earlier reports in Abdel-Tawwab and Abbass (2017) (turmeric), Abdel-Tawwab *et al*. (2018) (Clove) and Omitoyin *et al*. (2019) (Guava leaf extract). The antibacterial properties of *Allium cepa* are reported in Bello *et al*. (2013).

CONCLUSION

The use of onion powder as a feed additive in the diet of *Oreochromis niloticus* did not affect growth and nutrient utilization, but caused improvement in the innate immune system therefore increasing the rate of survival.

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