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The Effectiveness of *Cosmos caudatus* Enriched Pellets to Improve Growth and Survival of *Pangasianodon hypopthalmus* Reared in Dark Saline Media

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ABSTRACT
Cultured *Pangasianodon hypopthalmus* may face water quality related problems that weaken the fish and increase the risk of pathogen infection. Provision of health promoter plant *Cosmos caudatus* enriched pellets may improve the fish health. A CRD method with 4 treatments and 3 replications/treatment, namely Negative Control/ NC (no *C.caudatus*, no infection), Positive Control/PC (no *C. caudatus*, infected with *A. hydrophila*), T1, T2 and T3 (10g, 15g and 20g of *C. caudatus* powder in 1kg of feed pellets and infected with *A. hydrophila*) were applied. *P. hypopthalmus* fingerlings (4-6cm TL, 4-5g BW) were reared in dark plastic tanks filled with 100L of 5ppt saline water, 30 fishes/tank. They were reared for 60 days and fed 3 times/day, ad libitum. On the 61st day, they were infected with *A. hydrophila*. On the 75th day, the growth and survival of the fish were investigated. Results showed that provision of *C. caudatus* positively affects the fish's growth and survival. On the 60th day, the best growth was obtained in T1 (10g/ kg feed), 24.23g BW, 7.03cm absolute length, 3.16% specific growth rate, 83.11% FE and 1.20 FCR. There was no difference in the survival rate of all fish treated (91.11 to 96.66%). On the 75th day, however, the survival of PC was dropped into 38%, while that of the treated fish ranged from 78.89 – 92.22%. Data obtained indicated that the addition of *C. caudatus* was effective to improve growth and enhance the survival of *P. hypopthalmus* infected with *A. hydrophila*.

INTRODUCTION

The *Pangasianodon hypophthalmus* or the striped catfish is one of freshwater fish that has high economical value and commonly cultured in Riau, Indonesia **(Andriyani & Sumantriyadi, 2017; Kurniawan *et al.*, 2020; Windarti *et al.*, 2021)** This fish is able to grow quickly and to adapt to water quality changes **(Chowdhury & Roy, 2020; Akter *et al.*, 2021)**. *P. hypophthalmus* is one of the most favored fish in Riau and it is commonly sold as fresh, smoked and filleted fish. Many Riau's traditional cuisines used this fish as the main ingredient and nowadays the fish has been processed into many types of products such as "patin presto", nuggets and meatball **(Oktavianawati & Palupi, 2017)**.

Increasing the market's demand for this fish is causing the increase of fish culture activities. The culture of *P. hypopthalmus* is conducted in ponds as well as in the floating net cages that are placed in the river, lake and dam. In Riau, *P. hypopthalmus* is mainly produced from floating net cage fish culture in the Koto Panjang Dam. **Azzam (2020)** stated that there are 150 ha area used for *P. hypopthalmus* culture in the Koto Masjid Village that is located close to the Koto Panjang Dam. The amount of fish produced in that area is around 360 tons/harvest, while **Shalichaty *et al.* (2022)** stated that the fish production at the Kampar Regency in the year of 2022 was 32.402.126 tons. The fish from the Kampar Regency was then distributed to Riau, North Sumatra and West Sumatra Provinces.

To find out a method for improving the effectiveness of *P. hypopthalmus* culture, several researches have been conducted. A simple, cheap and environmental friendly effort that has been done to improve the growth of *P. hypopthalmus* is by culturing the fish under manipulation photoperiod or rearing the fish in dark condition. Several studies such as **Magwa *et al.* (2020)** and **Windarti *et al.* (2021)** reared nocturnal fishes such as *Ompok hypopthalmus* and *P. hypopthalmus* in dark condition or in short photoperiod condition. Results of those researches showed that the fishes grow better than fish reared in the natural or long photoperiod. Fish reared in continuous dark showed positive response in total length as well as in body weight. During the dark condition, the fish seem to be less active while swimming, but shown very good response to feed provided **(Windarti *et al.*, 2019)**. In these research, dark condition was achieved by placing the rearing tank under dark tarp tent or covered by dark colored tarp **(Magwa *et al.*, 2020; Windarti *et al.*, 2021)**. This method is very easy, cheap and it might be applied for conducting a small scaled or a household scaled fish culture.

Other efforts to improve the effectiveness of *P. hypopthalmus* cultures are by providing natural sources that are able to improve the health of fish and enable fish to face the attack of any pathogen. The natural resources that have been used to improve the health of the cultured fish were turmeric **(Riauwaty *et al.*, 2021)** and *Moringa oleifera* **(Windarti *et al.*, 2023a, 2023b)**. Fish that was fed with *M. oleifera* enriched pellet had better immunity **(Helmiati *et al.*, 2011; Bbole *et al.*, 2016; Azhar *et al.*, 2021)** as well as better growth. The addition of 20% fermented *moringa leaves* in fish feed pellets increase the hematocrit and leukocryte levels, increase the phagocytosis activities, diminish the monocyte and improve the lymphocytes and plasma's protein. In fish culture, the addition of 7% Moringa leaves in fish feed pellets increases 0.12% of specific growth rate of *Oreochromis niloticus* **(Astiyani *et al.*, 2020)**. In *Mystus nemurus* (Bagriidae) fingerlings, provision of *Cosmos caudatus* enriched pellets improved growth as well as factor condition **(Rameli *et al.*, 2020)**. While, **Romadhoni *et al.* (2020)** stated that *Cosmos caudatus* extract provision significantly improved the survival rate and reduced stress due to salinity changing in *Litopaneus vannamei*.

To increase the production of *P. hypophthalmus*, this fish is also being cultured in saline media, making it suitable for coastal aquaculture. *P. hypopthalmus* can be cultured in brackish water (5ppt) and it grows better than the fish reared in freshwater **(Riswan *et al.*, 2022)**. Rearing media with less than 8ppt does not negatively affect the fish **(Abdel-latif *et al.*, 2023)** and this fact indicates that the fish is able to tolerate the salinity.

Based on the information stated above, it may be possible to rear *P. hypopthalmus* in the dark rearing tank with saline media in order to obtain an effective technique of culturing that fish. However, rearing freshwater fish in the saline media may affect the physiology of the fish in general. The osmoregulation process may be changed as the fish become hypotonic toward the media. The gill and kidney work to prevent the loss of salt and to remove the excess water in the freshwater environment. In the saline media, the gills prevent the entry of salt, while the kidney prevents the loss of water. If there are no difficulties in osmoregulation process, the fish might be healthy and hence grow well. In contrast, if there is a failure in osmoregulation process, the fish may be stressed, unhealthy and the growth as well as the immunity might be reduced **(Sun *et al.*, 2023)**.

In weak condition, the fish may be vulnerable to any attack of fish pathogen such as *Aeromonas hydrophila*. This bacteria may cause a MAS (*Motile Aeromonas Septicemia*) disease symptoms. The MAS disease symptoms include ulcers, tail rot, fin rot, and haemorrhagic septicaemia, which causes lesions that lead to scale shedding, haemorrhages in the gills and anal area, exophthalmia, abdominal swelling and might be fatal. To reduce the stress and to improve the immunity of the fish, they may be fed with a natural resource that has stress reducing effect toward salinity changing as well as improving the fish immunity, namely *C. caudatus*. To develop an effective, low-cost, and simple technique for *P. hypophthalmus* culture that leaves no harmful residues in the fish meat, the application of *C. caudatus* could be explored. Therefore, this study aimed to study the growth and survival of *Pangasianodon hypophthalmus* reared in saline media and fed with *Cosmos caudatus*-enriched pellets.

MATERIALS AND METHODS

This study was conducted from May to July 2024 at the Aquatic Biology Laboratory, Fisheries and Marine Science Faculty, Universitas Riau Pekanbaru. Results of the previous researches showed that *P. hypopthalmus* perform better growth in the rearing media with dark condition and 5ppt saline water. In this study, the fish were reared in darkened tanks containing 5ppt saline water. The experimental treatment involved feeding *Pangasianodon hypophthalmus* with pellets enriched at varying dosages of *Cosmos caudatus*. The CRD design was applied with 5 treatments and 3 replications in each treatment, namely:

Negative Control (NC) : commercial pellets with no *C. caudatus* and no *A. hydrophila* infection

Positive Control (C)	:	commercial pellets with no *C. caudatus* and infected with *A. hydrophila*
T1	:	10g of *C. caudatus* powder in 1kg fish feed pellets, infected with *A. hydrophila*
T2	:	15g of *C. caudatus* powder in 1kg fish feed pellets, infected with *A. hydrophila*
T3	:	20g of *C. caudatus* powder in 1kg fish feed pellets, infected with *A. hydrophila*

The *P. hypopthalmus* fingerlings (around 4g BW and 6 - 8cm TL) were subjected to study. Specimens were placed in tanks, with 30 fishes/tank, and were fed with commercial pellets (type F999, produced by PT Central Proteina Prima) in the 1^{st} – 3^{rd} week and after the 3^{rd} week, the feed was replaced with F 781-1. The food was given to satiation, 2 times/day (in the morning and evening). Each rearing tan kwas supported with a circulatory pump and a filter system using palm fiber, coral and dacron filter.

The leaves of *C. caudatus* used in this study were purchased from local market. The stems were removed, while the leaves were taken, sun-dried, blended and sieved. The *C. caudatus* powder was then coated to the pellets using this following method: Around 50ml of water was mixed with 20 grams of tapioca powder. This mixture was then added with around 100ml of very hot boiling water and stirred well to form thick-sticky mixture. Then, the *C. caudatus* powder (as necessary dosages) was added and mixed well with the sticky tapioca mixture. The mixture was then added to 1kg of fish feed pellets and mixed well. The *C. caudatus* enriched pellets was sun-dried and then kept in containers, ready for feeding the fish.

In this study, parameters measured were the survival rate, growth rate, feed efficiency (FE) and food conversion rate (FCR). The fish were treated for 60 days. On the 61^{th} day, the fishes were infected with *A. hydrophila* culture (0.1ml, 10^6 cells/ml, and intra muscular). Fish survival was monitored 2 times/day, while dead fish were removed as soon as possible. Samplings for fish growth monitoring were conducted once/10 days. In each tank, three fishes were taken randomly. The total length of fish simples was measured using a ruler and their weight was weighed using a digital scale. After being infected with *A. hydrophila*, the clinical symptoms occurred were also investigated. The length, weight and general condition of the sampled fishes were noted.

The FCR of the fish in this study was calculated on the 60^{th} and 75^{th} days. The fish were fed till satiation, and the amount of feed given to the fish was monitored. A bottle filled with certain amount of feed pellets was provided for each tank. The amount or addition of feed pellets in the bottle was noted. At the end of the experiment, the remaining fish feed pellets in the bottle were weighed to calculate the total feed consumption during the trial period. This amount of feed was used as a basis for calculating the FCR of fish in each treatment.

Absolute growth of the fish was calculated using the following **(Windarti *et al.*, 2023)** formula:

$$Wm = Wt - Wo$$

Description:

Wm = Absolute growth (g)
Wt = Average weight of fish by the end of the research (g)
Wo = Average weight of fish in the initial day of the research (g)

Daily growth rate was calculated using the following **(Effendi *et al.*, 2025)** formula:

$$SGR = \frac{\ln Wt - \ln Wo}{t} \times 100\%$$

Description:

SGR = Specific growth rate (%/day),
Wo = Average weight of fish in the initial day of experiment (g),
Wt = Average weight of fish in the -t day (g),
T =Experiment duration (days).

Efficiency and feed conversion were calculated using the following **(Effendi *et al.*, 2025)** formula

$$FE = \frac{(Wt+D)-Wo}{F} \times 100\%$$

Description:

FE = Feed efficiency
F = Weight of food consumed during the fish rearing period
D = Weight of dead fish during the rearing period
Wt = Total weight of fish by the end of rearing period
Wo = Total weight of fish in the initial day of rearing period Feed

The conversion rate was calculated using the following **(Effendi *et al.*, 2025)** formula

$$FCR = \frac{F}{(Wt+D)-Wo}$$

Description:

FE = Feed efficiency
F = Weight of food consumed during the fish rearing period
D = Weight of dead fish during the rearing period
Wt = Total weight of fish by the end of rearing period
Wo = Total weight of fish in the initial day of rearing period

RESULTS

1. Survival rate of *P. hypopthalmus*

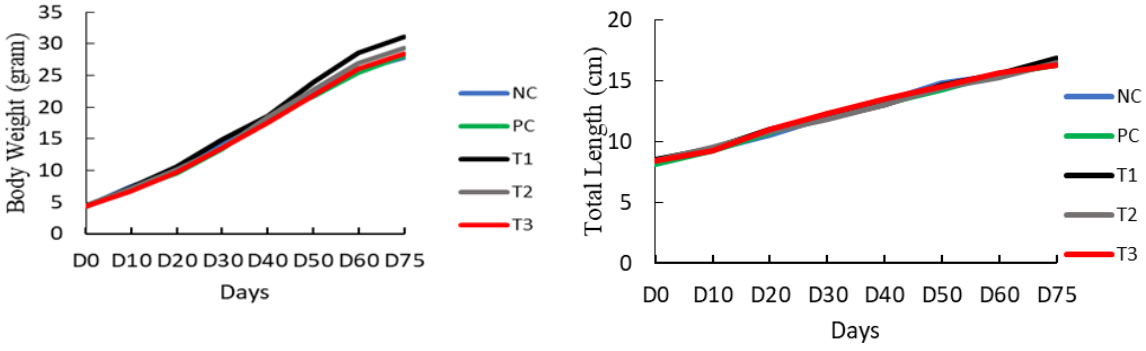
In this study, the survival rate of the fish was counted in the 60th and 75th day (the 24th day after infection with *A. hydrophila*). By the 60th day, the survival of fish in all treatments applied showed no differences. Survival rate of the Negative Control as well as the *C. caudatus* treated fish ranged from 91.11 – 96.66% (Table 1).

Table 1. Survival of *P. hypopthalmus* reared in dark saline media and fed with *C. caudatus* enriched pellets, on the 60th and 75th days

Parameters	Treatments				
	NC	PC	T1	T2	T3
60th day					
SR (%)	91.11±3.84[a]	92.22±1.92[a]	96.66±3.33[a]	96.66±3.33[a]	94.44±1.93[a]
75th day					
SR (%)	91.11±3.84[c]	38.88±5.09[a]	92.22±1.92[c]	83.33±8.82[bc]	78.89±1.92[b]

2. Growth rate of *P. hypopthalmus*

In this research, the growth (body length and body weight) of fish was monitored once/10 days. The data showed that fish in all treatment groups exhibited good growth up to day 60 and continued growing for 14 days post-infection with *A. hydrophila* (Figs. 1, 2).



(a) (b)

Fig. 1. Growth rate of *P. hypopthalmus* reared in dark saline media and fed with *C. caudatus* enriched pellets; a) Body weight; b) Total length

Table 2. Absolute weight and length of *P. hypopthalmus* reared in dark and saline media and fed with *C. caudatus* enriched pellet on the 60th and 75th days (14 days after *A. hydrophila* infection)

Parameters	Treatments (C. caudatus enriched pellets)				
	NC	PC	T1	T2	T3
60th day					
Absolute Weight (g)	20.99±0.21[a]	21.58±0.57[b]	24.23±0.17[d]	22.48±0.18[c]	21.73±0.29[b]
Absolute Length (cm)	6.87±0.20[a]	7.38±0.26[a]	7.03±0.27[a]	6.86±0.10[a]	7.23±0.2[a]
Specific Growth Rate (%/day)	2.92±0.10[a]	2.97±0.10[a]	3.16±0.02[b]	3.01±0.05[a]	2.99±0.01[a]
After infection (75th day)					
Absolute Weight (g)	23.75±0.23[a]	23.42±0.43[ab]	26.83±0.18[d]	24.90±0.20[c]	24.15±0.04[b]
Absolute Length (cm)	7.82±0.14[a]	8.11±0.29[ab]	8.35±0.78[b]	8.05±0.07[ab]	7.95±0.04[a]
Specific Growth Rate (%/day)	2.48±0.08[a]	2.47±0.06[a]	2.64±0.01[b]	2.52±0.04[a]	2.51±0.01[a]

3. Feed conversion rate and feed efficiency *P. hypopthalmus*

In this study, observations on feed conversion rate (FCR) and feed efficiency (EP) showed that fish treated with *C. caudatus* had a lower FCR and higher EP than those not treated with *C. caudatus* (Table 3).

Table 3. Feed conversion rate (FCR) and feed efficiency (FE) of *P. hypopthalmus* reared in dark and saline media and fed with *C. caudatus* enriched pellets on the 60th and 75th days (14 days after *A. hydrophila* infection)

Parameters	Treatments (C. caudatus enriched pellets)				
	NC		NC		NC
60th day					
FE (%)	57.62±2.68[a]	56.92±0.94[a]	83.11±1.81[d]	73.72±2.37[c]	68.77±0.39[b]
FCR	1.73±0.08[d]	1.75±0.03[d]	1.20±0.02[a]	1.36±0.46[b]	1.45±0.01[c]
After challenge test (75th day)					
FE (%)	42.32±2.20[a]	48.93±0.01[b]	73.66±2.44[e]	61.02±1.42[d]	55.24±0.54[c]
FCR	2.36±0.12[e]	2.04±0.00[d]	1.35±0.05[a]	1.63±0.04[b]	1.81±0.02[c]

DISCUSSION

In this study, the survival rate of the fish was counted on the 60th and 75th days (the 24th day after infection with *A. hydrophila*). By the 60th day, the survival of fish in all treatments applied showed no differences. Survival rate of the negative control group as well as the *C. caudatus* treated fish ranged from 91.11 – 96.66% (Table 1). This fact indicate that there was no negative impact of *C. caudatus* addition in the fish feed pellets on the fish survival. **Khadim and Al-Fartusie (2021)** stated that *C. caudatus* contain antioxidant that is useful to improve health in general, while **Cheng *et al.* (2015)** postulated that *C. caudatus* does not have any negative effects on health.

The ability of *C. caudatus* in improving the ability of fish to face the *A. hydrophila* attack is shown by the treated fish ability to survive after the infection of that pathogen.

On the 75th day or 14 days after being infected by that pathogen, both the *C. caudatus* treated fish and the positive control (PC) fish showed a different survival rate. The survival of the treated fish was higher than that of the PC fish. The *C. caudatus* treated fish (T1, T2 and T3) as well as the non-infected fish (NC) survival rate was 78.89 – 92.22%. In contrast, the survival of the PC fish (no *C. caudatus* and being infected with *A. hydrophila*) was low, 38.88%. Marked differences in clinical manifestations were observed between treatment groups. The positive control fish exhibited severe pathological responses to *A. hydrophila* infection, with injection sites becoming erythematous and swollen before progressing to necrotic furunculosis lesions. These fish showed progressive weakness, ceased feeding, and experienced high mortality rates. In marked contrast, *C. caudatus*-treated fish demonstrated remarkable resilience - while they developed initial wounds at injection sites, these lesions healed completely within 14 days, with fish maintaining normal feeding behavior and activity levels.

This striking difference in clinical outcomes clearly indicates that *C. caudatus* supplementation significantly enhances disease resistance in *P. hypophthalmus*. The herbal treatment appears to both mitigate *A. hydrophila* pathogenicity and reduce the severity of motile aeromonad septicemia symptoms. Most notably, the supplemented fish achieved complete wound epithelialization while maintaining growth performance, suggesting the treatment stimulates both immune function and tissue repair mechanisms.

These findings strongly support incorporating *C. caudatus*-enriched pellets (particularly at the 10g/kg feed dosage) into the striped catfish culture protocols. The treatment offers an effective, sustainable alternative to antibiotics for preventing *A. hydrophila* outbreaks, with the additional benefits of maintaining fish welfare and production performance during disease challenges. The rapid wound resolution observed in treated fish further suggests the supplement may enhance cellular immunity and inflammatory regulation, meriting further investigation into its precise modes of action.

The fish were successfully reared in dark conditions at 5ppt salinity with pre-challenge survival rates of 91.11-96.66%, demonstrating good adaptation to these environmental conditions. These survival rates are only slightly lower than the 100% survival achieved in freshwater dark-rearing systems reported by **Windarti *et al.* (2021)**, indicating that the combination of 5ppt salinity and darkness had minimal impact on fish viability. The results confirm that *P. hypophthalmus* maintains high survival rates in both brackish and freshwater systems under dark-rearing conditions. This environmental tolerance suggests the species' potential for culture in various light-limited, low-salinity aquaculture systems without compromising baseline survival performance. The comparable survival between 5ppt and freshwater conditions indicates that moderate salinity does not represent a significant stressor for this species when combined with dark-rearing practices.

The positive control (PC) fish exhibited compromised immunity, rendering them vulnerable to *A. hydrophila* infection. This pathogen produces multiple virulence factors including haemolysin, aerolysin, cytosine, gelatinase, and enterotoxins **(Semwal *et al.*, 2023)**, which collectively disrupt fish physiology and lead to mortality. In contrast, fish receiving *C. caudatus*-enriched feed demonstrated enhanced immune competence, attributed to the plant's antioxidant properties. The supplemented fish effectively controlled bacterial proliferation and prevented systemic infection, achieving significantly higher survival rates (78.89-92.22%) compared to the PC group.

C. caudatus appears to function through multiple bioactive compounds (flavonoids, alkaloids, terpenoids) that exhibit antimicrobial and immunostimulatory properties **(Semwal *et al.*, 2023)**. The treated fish not only survived but also showed remarkable wound healing capacity following *A. hydrophila* challenge, indicating robust innate immune activation. This aligns with the data reported in the study of **Smith *et al.* (2019)**, who described the innate immunity mechanisms involving physical barriers, phagocytic cells, and humoral factors. The results clearly demonstrate that dietary *C. caudatus* supplementation strengthens host defense systems in *P. hypophthalmus*, offering a practical strategy to mitigate *A. hydrophila*-related losses in aquaculture operations.

Growth rate of *P. hypopthalmus*

The fish showed consistent growth in both body weight and length when measured every 10 days, with all treatment groups demonstrating healthy development through day 60 of the study. While growth was relatively slow during the first 20 days, it accelerated significantly between days 20-60, particularly for the T1 group receiving 10g/kg of *C. caudatus* supplementation, which showed superior weight gain starting at day 50. Following *A. hydrophila* infection at day 60, the supplemented fish continued growing for the remaining 14 days of the study, with T1 fish maintaining the best growth performance in both weight and length measurements. In contrast, the positive control group (PC) without *C. caudatus* supplementation showed stunted growth after infection and suffered 62% mortality, while the T3 group maintained 80% survival despite more moderate growth rates. The similar length increments observed across all groups before infection suggest *C. caudatus* primarily influenced weight gain rather than linear growth, potentially through improved appetite and nutrient utilization. The maintained growth performance in supplemented fish after pathogen exposure indicates the treatment successfully mitigated the typical growth-suppressing effects of *A. hydrophila* infection. These findings align with previous research on antioxidant-rich diets in the rainbow trout **(Harsij *et al.*, 2021)**, demonstrating how phytogenic supplements can simultaneously enhance both growth performance and disease resistance in aquaculture species. The results particularly highlight the effectiveness of the 10g/kg *C. caudatus* formulation (T1) in promoting fish health and productivity under disease challenge conditions.

Feed conversion rate and feed efficiency of *P. hypopthalmus*

The study demonstrated that *C. caudatus* supplementation significantly improved feed utilization in *P. hypophthalmus*, with treated fish showing superior FCR (1.20-1.45) and FE (68.77-83.11%) compared to control groups (FCR:1.73-1.75; FE:~57%) by day 60. The T1 treatment (10g/kg feed) yielded optimal results with FCR 1.2 and FE 83.11%, suggesting enhanced nutrient assimilation likely mediated by the plant's antioxidant compounds, as supported by **Harsij *et al.* (2021)**. Following *A. hydrophila* challenge, unsupplemented fish exhibited dramatically worsened feed conversion (FCR:2.04; FE:48.93%) due to infection-induced stress, ulcerative septicemia **(Kari *et al.*, 2022)**, and metabolic disruption **(Semwal *et al.*, 2023)**, while supplemented groups maintained relatively stable feed performance, demonstrating greater disease resilience. These results indicate *C. caudatus*-enriched feed not only enhances baseline nutritional efficiency but also protects against the severe feed utilization decline- a characteristic of aeromonad infections- offering a practical solution to mitigate productivity losses in commercial aquaculture operations facing disease challenges. The maintained feed conversion efficiency during pathogen exposure highlights the treatment's dual benefits of improving both nutritional performance and disease resistance in cultured fish populations.

CONCLUSION

The administration of *Cosmos caudatus*-enriched pellets to *Pangasianodon hypophthalmus* reared in dark saline media did not significantly enhance growth performance but improved immune response, enabling the fish to resist *Aeromonas hydrophila* infection. Following pathogen exposure, a majority of *C. caudatus*-treated fish survived and maintained growth, whereas most untreated fish succumbed to the infection. The optimal results were observed in fish receiving feed supplemented with 10 g of *C. caudatus* powder per kg of feed (T1 treatment).

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