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Short Communication

Highly diluted bioactive compounds increase growth, survival, and condition factor in spotted rose snapper *Lutjanus guttatus* (Pisces: Lutjanidae) juveniles

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ABSTRACT. The spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869) is a commercially valuable and potentially cultivable species, but management stress compromises productivity. Some highly diluted bioactive compounds (HDBC) enhance relaxation and nutrition; thus, they were experimentally evaluated in 300 (n = 60: per treatment) juveniles (8.72 ± 4.07 g total weight, 8.47 ± 1.24 cm total length), temperature: 26.9 ± 1.5°C, salinity: 35, in 10 experimental flow-through units (120-L) for 30 days. Three HDBC treatments [PaV 31CH (Passival® MX), InM 3CH (RubioPharma® MX) and PaV+InM] and two controls [ET (Ethanol Similia® MX) and DW (distilled water] were sprinkled (5% volume/weight) in balanced food (Silver Cup® 45% protein) and administered five times a day to satiety. Treated juveniles presented higher productive performance with increases ($P < 0.05$) in weight and length with PaV (4.26 g and 0.95 cm), PaV+InM (5.34 g and 1.26 cm), and InM (3.91 g and 0.83 cm) *vs.* ET and DW (0.59 and 1.62 g, and 0.07 and 0.5 cm, respectively). Specific growth rate (1.54, 1.19, and 1.79% g d^{-1}), weight gain (0.141, 0.130, and 0.178 g d^{-1}), feed conversion rate (1.23 to 1.47), and Fulton's condition factor (K = 1.5) were higher ($P < 0.05$) in treated fish *vs.* controls. The average survival was higher in groups treated with PaV, InM, and PaV+InM with 90.0 ± 2.72, 93.3 ± 2.71, and 88.3 ± 1.36%, respectively, than ET and DW controls (65.0 ± 1.31 and 73.3 ± 5.44%), suggesting HDBC use could optimize *L. guttatus* sustainable farming.

Keywords: *Lutjanus guttatus*; food assimilation; physiological performance; feed conversion rate; sustainable aquaculture

INTRODUCTION

Marine fish culture has experienced fast growth, becoming a global industry of great importance, generating economic income, high-quality food products, and employing thousands of qualified and non-qualified workers. The world population is estimated to reach 9,700 million people by 2050, and aquaculture

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should play a crucial role in growing food demand (Godfray et al. 2010, FAO 2017). According to FAO (2020), mariculture was dominated by finfish (54.3 mt, US$139,700 million) from continental aquaculture (47 mt, US$104,300 million), as well as in marine and coastal aquaculture (7.3 mt, US$35,400 million) in 2018. Despite its rapid growth, the marine fish industry faces obstacles, such as novel protein sources as substitutes for fish-based meals, research for functional additives, and nutritional requirements in novel fish species susceptible to aquaculture. In this context, the aquaculture industry is exploring and developing equally efficient strategies and products with a long-term vision of sustainable management (Standen et al. 2013, García-Bernal et al. 2017, Mazón-Suástegui et al. 2017).

The use of highly diluted bioactive compounds (HDBC) has been applied successfully to promote growth and increase survival and digestive functionality in aquatic and terrestrial animal cultures (Mazón-Suástegui et al. 2020a, López-Carvallo et al. 2022). Their application is based on the hormesis principle, characterized by low-dose stimulation and high-dose inhibition of biological processes dependent on the chemical/physical agent, biological model, or treatment criterion applied (Calabrese & Baldwin 2003, Hercus et al. 2003, Mattson 2008, Bauer et al. 2018). Thus, a high dosage of an administered substance to a biological system generates a negative response that may be hazardous or lethal. In contrast, the low ultra-diluted dosage of the same substance may give place to a favorable and healing response, as in the case of 60Co gamma radiation dual effects in the marine mollusk *Haliotis discus discus* (Cho et al. 2016). According to the hormesis principle, the same bioactive compound generates a biphasic response at the physiological level in function of the dosage. For example, homeopathic medicine -a therapy founded in Germany by Doctor Samuel Hahnemann (1755-1843)- is based on the principle of similarities (*similia similibus curantur*) and hormesis (López-Carvallo et al. 2020, 2022). HDBC treatments increase food assimilation, energetic reserve accumulation (lipids and carbohydrates), and growth in marine organisms; some examples are mussel (*Modiolus capax*), octopus (*Octopus bimaculoides*), and longfin yellowtail (*Seriola rivoliana*) juveniles, survival and antioxidant response in white shrimp *Penaeus vannamei* and also favored reduction of intestinal coccidia. In contrast, immune response increased in juvenile marine fish *Lutjanus guttatus* (Ibarra-García 2018, Mazón-Suástegui et al. 2018a, 2020a, Rosero-García et al. 2019, López-Carvallo et al. 2022, García-Corona et al. 2024).

Regarding freshwater fish such as tilapia (*Oreochromis niloticus*), HDBC for human and veterinary use and even patented formulas have been successfully evaluated, obtaining improvements in morphofunctional response, overall performance, and cortisol levels, fatty acid and muscle performance, and even greater survival in masculinizing juveniles (Siena et al. 2010, Braccini et al. 2013, Standen et al. 2013, Andretto et al. 2014, Merlini et al. 2014, Díaz-Neto et al. 2017). Therefore, considering this background and the fact that these important bioactive compounds are efficient economic secondary effect-free for aquaculture and agriculture (Mazón-Suástegui et al. 2017, 2018a, 2020b, Ortiz-Cornejo et al. 2017), the present study aims to evaluate HDBC effects on growth, survival and condition factor on *L. guttatus* (Pisces: Lutjanidae) juveniles in a semi-controlled hatchery environment for 30 days.

A total of 300 *L. guttatus* juveniles (8.72 ± 4.07 g total weight [TW], 8.47 ± 1.24 cm total length [TL]) were obtained in Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD) Mazatlán Unit, Sinaloa, México. The organisms were transported to Centro Regional de Investigación Acuícola y Pesquera (CRIAP) Aquaculture Laboratory in La Paz, Baja California Sur, México, where the bioassay was performed. The experimental design included three treatments formulated with HDBC and 2 controls. Commercial medicines for humans (stock treatments) to formulate the experimental treatments were acquired from Similia®, National Homeopathic Pharmacy (CDMX, MX), and Rubiopharma® Pharmacy (Hermosillo, Sonora, MX). Treatment 1 (T1) (PaV) was obtained from the hydroalcoholic formula Passival® formulated by the 30 Centesimal Hahnemannian (30CH) dilution scale components of *Passiflora incarnata*, *Valeriana officinalis*, *Ignatia amara*, and *Zincum valerianicum* (Laboratorios Similia®, MX). These 30CH components were mixed 1:1:1:1, diluted 1:99 in distilled water, and intensively agitated for 2 min to obtain Passival® 31CH (PaV). Treatment 2 (T2) (InM) was obtained from injectable, homeopathic medicines for human use formulated by 2CH components of Cyme Heel®2, Gal Heel®, Heps Heel®, Mucs Heel®, and Chol Heel®. The components were mixed 1:1:1:1:1, diluted 1:99 in distilled water, and intensively agitated for 2 min to obtain InM® 3CH (PaV). Treatment 3 (T3) (PaV+InM) was a combination of T1 and T2 in a proportion of 1:1 v/v. The control treatments without HDBC were Treatment 4 (T4) Ethanol Similia® (ET): ethanol 87°GL (Similia®, MX) diluted with distilled water excipient to 30°GL and Treatment 5 (T5) (DW): only distilled water. Formulation of HDBC treatment and controls were

performed at the Experimental Laboratory of Aquaculture Homeopathy and Sea Seeds, Centro de Investigaciones Biológicas del Noroeste (CIBNOR) according to the methodologies proposed by López-Carvallo et al. (2019, 2020) and Mazón-Suástegui et al. (2017, 2020a).

Once in CRIAP, fish were acclimated in 5,000-L plastic tanks with filtered seawater at 5 µm, salinity 35, continuous aeration, temperature at 26.9 ± 1.5°C, and dissolved oxygen level at 5.7 ± 1.24 mg L^{-1} for 2 weeks. The organisms were fed commercial food with 45% protein (El Pedregal®, Silver Cup®; Toluca, MX) to satiety five times/day. Once *L. guttatus* juveniles were acclimated, they were randomly distributed in 10 experimental units (EU) (30 fish EU^{-1}) in 120-L cylindrical fiberglass tanks (60 fish per treatment). These units were disposed of in an external cylindrical container with a false plastic mesh bottom interconnected in such a way that they operate with ascending and recirculating water flux activated by an air siphon and continuous seawater flow (Lodeiros et al. 2021). The EU operated with filtered seawater (50 µm) at a temperature of 26.9 ± 1.5°C and 35 salinities with constant aeration and sufficient continuous water flow to ensure a daily exchange of 900% and dissolved oxygen levels of 7.8 ± 3.4 mg L^{-1}. Five groups were considered in the experimental design with 2 replicates, each one consisting of 3 treatments (T1, T2, and T3) and 2 controls (T4 and T5) sprinkled (5% volume/weight) in balanced feed (45% protein El Pedregal®, Silver Cup®; Toluca, Mexico), subsequently dried (23°C) and administered to satiety 5 times/day for 30 days. The EU had a slightly conical bottom and a drainage valve that allowed for the elimination of 50% of water and debris. Dead organisms were removed if necessary.

At the beginning and end of the bioassay, all fish in each replicate were counted, measured and weighed to estimate the average increase in TW, TL, Fulton's condition factor (K) (Leyton et al. 2015), [K = (TW TW^{-3}) × 100], specific growth rate (% weight d^{-1}) [SGR = (ln final weight - ln initial weight) × 100 / t in days], weight gain (g d^{-1}) [WG = (final weight - initial weight) / t in days], feed conversion rate [FCR = feed consumption weight (g) / (final biomass - initial biomass in g)] and survival [(number of surviving fish / number of initial fish) ×100]. The normality of data was analyzed using the Kolmogorov-Smirnov test and Levene's homogeneity of variance test (Sokal & Rohlf 1981). When needed, data were transformed (log, $1/\chi$, or $\sqrt{\chi}$) before the analysis to meet *a priori* assumptions. Before the analysis, an angular transformation (arcsine $\sqrt{P}$) was applied to the values expressed in percentages; a one-way analysis of variance (ANOVA) and Tukey's comparison of means were used to detect significant differences in size, weight gain, and survival percentage among the different experimental treatments. The differences were considered significant for a $P < 0.05$ level. The increase in average weight and length of *L. guttatus* juveniles treated with HDBC was significantly higher than in the control groups (Fig. 1). The ET control group organisms had the lowest total length values (Fig. 1).

Survival of the juveniles treated with PaV and InM was (90.0 ± 2.72 and 93 ± 1.36%, respectively), significantly greater than the PaV+InM (88.3 ± 1.33%) combination and ET and DW (<73%) controls (Table 1). The K was significantly greater in the groups treated with HDBC (K > 1.50), whereas the fish treated with ET and DW showed the lowest K values (1.37). The SGR and WG were significantly greater in fish treated with PaV, InM, and their combination (PaV+InM) (>1.19 g d^{-1} and 0.13%, respectively) when compared with the control groups (<1.37 g d^{-1} and 0.05% respectively); FCR was significantly lower in the HDBC groups (<1.47) and greater in the control groups (>3), indicating better feed uses in HDBC treated organisms (Table 1).

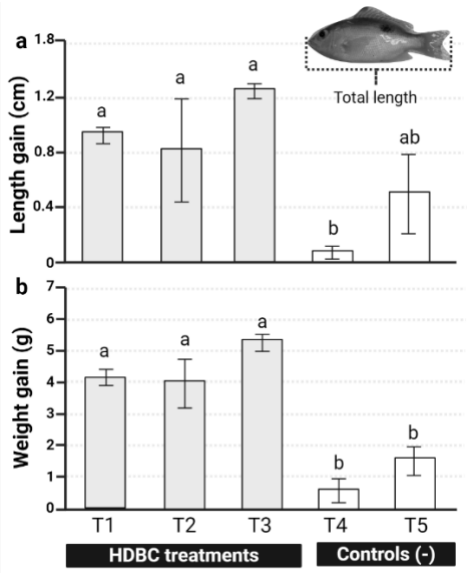

Figure 1. The average increase in a) total length and b) weight of *Lutjanus guttatus* juveniles that received different highly diluted bioactive compound (HDBC) treatments and 2 controls. Different letters on the bars indicate a significant difference between treatments (Tukey's test, $P < 0.05$).

Table 1. Survival, Fulton condition factor (K), specific growth rate (SGR in % weight g d^{-1}), weight gain (WG in g d^{-1}), and feed conversion rate (FCR) of juvenile spotted rose snapper *Lutjanus guttatus* with different highly diluted bioactive compound (HDBC) treatments: PaV, InM, PaV+InM and 2 control groups: Ethanol Similia® (ET) and distilled water (DW). The numbers represent the average ± standard deviation. Different letter in the same line indicates significant differences (Newman & Keuls test; $P < 0.05$).

	Treatment				
	T1 (PaV)	T2 (InM)	T3 (PaV+InM)	T4 (ET)	T5 (DW)
Survival (%)	90.0 ± 2.72[a]	93.3 ± 1.36[a]	88.3 ± 1.33[a]	65.0 ± 1.31[b]	73.3 ± 5.44[c]
K	1.50 ± 0.10[a]	1.53 ± 0.17[a]	1.52 ± 0.09[a]	1.37 ± 0.03[b]	1.37 ± 0.05[b]
SGR % (g d^{-1})	1.54 ± 0.325[a]	1.19 ± 0.19[a]	1.79 ± 0.47[a]	0.26 ± 0.29[b]	0.28 ± 0.354[b]
WG (g d^{-1})	0.14 ± 0.018[a]	0.13 ± 0.04[a]	0.18 ± 0.03[a]	0.02 ± 0.02[b]	0.05 ± 0.050[b]
FCR	1.28 ± 0.051[a]	1.47 ± 0.29[a]	1.23 ± 0.04[a]	5.57 ± 2.07[b]	3.03 ± 0.921[b]

In the present study, *L. guttatus* juveniles treated with PaV, InM, and a combination (PaV+InM) significantly increased weight, size, and K value for fish treated with both control seawater (DW) and ethanol (ET) (Fig. 1). These results suggest an improvement of the general condition and better feed assimilation when juveniles are treated with HDBC.

Alcalá-Carrillo et al. (2016) found K values of 1.3 for *L. guttatus* fed in the laboratory with the same commercial diet and similar environmental conditions (24°C and 35 of salinity) used in the present study. This value (K = 1.3) was lower than the results obtained in the present study with HDBC (K = 1.5) in juveniles of the same species. In contrast, lower values obtained in this study (K = 1.3) were similar to those obtained by these authors in similar experimental conditions, so HDBC application is assumed to maintain healthier organisms.

Starting from the final length and weight data reported for *L. guttatus* cultivated in floating cages with a commercial diet (35% of protein) provided in daily rations 1.25% of biomass (Castillo-Vargasmachuca et al. 2007) obtained K = 1.4 and 1.5, values for fish with sizes from 24.6 to 29.5 cm of TL, respectively. Nonetheless, a natural diet has recorded higher K values (3.21 ± 0.080) (Avilés-Quevedo & Castelló-Orvay 2013). Similar or higher K values than those reported in the present study when using HDBC could be attributed to the culture technique and nutritional food value since higher general conditions can be obtained when floating cages are used compared to laboratory conditions (Shaik & Thuvanismail et al. 2024). Additionally, the higher fish conditions can be assessed using a natural diet compared to pellets, mainly in native species where specific diets are unavailable (Meyer et al. 2016). Although not completely comparable, population studies of juveniles in the laboratory (age, growth, and reproduction) would be interesting to confirm -at least as a point of reference- and know which of the K values obtained in the present study were higher than the maximum K values (1.03 and 1.39) reported for *L. guttatus* in Mexico and El Salvador by Arellano-Martínez et al. (2001) and Maravilla-Díaz (2001), respectively. The same authors reported that the maximum K values were associated with the peak of the reproductive period, decreasing in the rest of the season (Correa-Herrera & Jiménez-Segura 2013). Thus, a high K value in juveniles could be associated with good health and nutritious status (Leyton et al. 2015). The previous information confirms that maintaining optimal culture conditions and applying HDBC focused on increasing general culture conditions improves overall fish health

Investigations are underway to determine how PaV and InM enhance physiological status regarding growth and metabolism. Nevertheless, their use in marine fish, crustaceans, and mollusks has been associated with an increase in growth, weight, antioxidant digestive and enzymatic activities, energetic reserve accumulation, and oxidative stress reduction (López-Carvallo et al. 2022).

The SGR obtained with PaV, InM, and PaV+InM (SGR = 1.54, 1.19 and 1.79% g d^{-1}, respectively) were higher than those obtained by Olivares-Paulette & Boza-Abarca (1999), Alcalá-Carillo et al. (2016), Garduño-Dionate et al. (2016) for the same species (*L. guttatus* juveniles) in similar conditions (1.09, 1.02 and 0.9% g d^{-1}, respectively). In contrast, these last SGR values are similar to those obtained in *L. guttatus* juveniles cultured in floating cages with a commercial diet (1.2 to 1.0% g d^{-1}) according to Castillo-Vargasmachuca et al. (2007) and 1.59% g d^{-1} with a commercial diet and 1.68% g d^{-1} with a natural diet according to Banguera-Gil & Angulo-Sinisterra,

(2010). Alcalá-Carrillo et al. (2016) obtained WG of 0.05 g d^{-1} in the same condition of salinity and temperature of the present study, similar to WG obtained in DW in the control treatment, where PaV, InM, and PaV+InM obtained 0.141, 0.127, and 0.178 g d^{-1}, respectively. These last WG values have a closer or similar value than those obtained for this same species (1.87 and 1.7 g d^{-1}) by using discarded fish as fresh food (Gutiérrez & Durán 1999). Other WG values that have been reported for *L. guttatus* culture in cages with a balanced diet are 0.25 and 1.7 g d^{-1} (Avilés-Quevedo 2000, Avilés-Quevedo & Castelló-Orvay 2002, Avilés-Quevedo et al. 2008, Viveros 2008) and 1.47, 0.63, 0.45 and 0.33 g d^{-1} in laboratory condition with a balanced diet (Hernández-Martínez et al. 2007, Ochoa 2007, Plaza, 2007, González-Dcroz et al. 2014). Considering that fish cages and natural fresh food enhance growth in cultured fish compared to those reared in the laboratory (Meyer et al. 2016, Shaik & Thuvanismail 2024), the previous confirms that WG and SGR in fish depend on food quality and quantity provided besides environmental conditions. For example, temperature, salinity (Alcalá-Carrillo et al. 2016), and their increase due to HDBC make them an alternative to improve *L. guttatus* culture and fattening.

The present study obtained FCR values from 1.23 to 1.47 in *L. guttatus* juveniles with a dry commercial balanced diet (45% protein) and HDBC treatments (PaV, InM, and PaV+InM). For the same species other authors cite higher FCR values with a dry balanced diet: 2.3 to 1.8 (Alcalá-Carrillo et al. 2016), 2.9 (González-Dcroz et al. 2014), 2.5 (Banguera-Gil & Angulo-Sinisterra 2010), 1.86 (Olivares-Paulette & Boza-Abarca 1999), 1.77 (Gutiérrez & Durán 1999) and 1.4 (Garduño-Dionate et al. 2016). All these values are higher than those obtained in the present study, indicating that the HDBC application allows greater feed utilization, increasing the activity's profitability and reducing food waste in the system.

The present study confirms the results of Rosero-García et al. (2019), who mentioned that HDBC, PaV, and InM improve nutrition and health in *L. guttatus* juveniles. The HDBC included in InM are injectable bioactive components (Rubiopharma®) used individually or combined for enzymatic disorder and infectious disease treatments to stimulate the organism defense and favor a general improvement in health, immunity, and nutrition (Mazón-Suástegui et al. 2017, 2018b, 2020a, 2021, Ortiz-Cornejo et al. 2017). Higher growth and health conditions produced by HDBC in marine organisms, including fish, have been attributed to the improvement of defense mechanisms against stressor factors, regulation of metabolic pathways (catabolism-anabolism), and regulation of the digestive system mechanisms (López-Carvallo et al. 2022). Stress may depress the defense system so that susceptibility to the disease is greater (Anderson 1990). Since aquatic organisms are constantly exposed to environmental fluctuations and pathogen attacks, favorable physiological and immunological changes are expected if relaxation is promoted and stress is reduced, which can translate into higher growth rates. The fish that received the relaxing PaV treatment presented an average survival of 90%. A similar result was obtained in the InM treatment (93%), which included medicine designed to favor immune response and stimulate the organism's defense. The sum or synergy of both treatments and their individual modes of action explain the good results obtained with the complex PaV+InM treatment in growth but not survival (Table 1). Nevertheless, higher survival was achieved using all HDBC compared to both controls.

In the present bioassay, the HDBC used in *L. guttatus* showed significantly better health conditions, considering the highest values obtained in growth and K, which is used in aquaculture as a good health indicator and associated with fat content in muscle. Furthermore, intensive fish culture systems rely on lowering fish feed costs by using enriched fish diets with high levels of lipids as an energy source, as well as meeting the growing requirements for cultured fish; thus, this factor differentiates wild fish from those cultured because of their higher degree of fat, which also implies greater growth and better use of the feed provided (Avilés-Quevedo & Castelló-Orvay 2004, 2013, Naiel et al. 2023). According to Mazón-Suástegui et al. (2017), HDBC application to Catarina scallop (*Argopecten ventricosus*) stimulated growth and favored survival when challenged to *Vibrio alginolyticus* a pathogenic and highly virulent strain (CAIM-57), indicating the enhancement of the overall health of HDBC treated organisms. The results obtained during the present research confirm that the formulated treatments with bioactive components act individually and/or synergistically and are a natural, innocuous, and eco-friendly alternative therapy to assist future challenges that the aquaculture marine fish-producing industry undoubtedly confronts to increase technical and economic efficiency with ecological sustainability.

The treatments formulated from HDBC authorized for human use, applied and evaluated during the experimental culture of *L. guttatus* juveniles and fed with a dry, balanced commercial diet are beneficial in

terms of a significant increase observed in growth, weight, length, specific growth rate, survival, and condition factor. The PaV, InM treatments, and their combination (PaV+InM) also improved the feed conversion factor, so more biomass with less feed was obtained and consequently less economic investment. The results of the present study are novel in marine species and show that HDBC is a potential alternative to sustainably improving the marine fish aquaculture industry.

Credit author contribution

J.M. Mazón-Suástegui: conceptualization, methodology, funding acquisition, formal analysis, writing-original draft; J.A. López-Carvallo: formal analysis, writing-original draft, review and editing; M.A. Avilés-Quevedo: methodology, formal analysis, review and editing; J. Salas-Leiva: formal analysis, review and editing; F. Castelló-Orvay: formal analysis, review and editing; M. García-Bernal: methodology, formal analysis, review and editing; F. Abasolo-Pacheco: methodology, formal analysis, review and editing; A.I. Campa-Córdoba: methodology, formal analysis, review and editing; D. Tovar-Ramírez, writing-original draft, formal analysis, review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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