Effect of the use of growth stimulators on performance indicators and morphoerythrocytic aspects evaluated in juveniles of *Centropomus undecimalis* (Bloch, 1792)

Efecto del uso de estimuladores de crecimiento sobre los indicadores de desempeño y aspectos morfoeritrocitarios evaluados en juveniles de *Centropomus undecimalis* (Bloch, 1792)

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Abstract

This study provided evidence of the effects of vitamin C and probiotic treatment on the productive performance and hematological profile in juvenile snook (*Centropomus undecimalis*). This species is emblematic of the American tropics and holds significant economic value in the market. Ninety specimens measuring 17.8 ± 0.5 cm in total length and weighing 42.5 ± 3.0 g were used in the trials. Three treatments (vitamin C, probiotic, and control) with three replicates were evaluated, randomly distributing 10 fish per tank. Water quality was maintained under controlled conditions. Performance indicators and hematological aspects were assessed for each treatment. Erythrocyte behavior, particularly cell size and shape, suggested benefits from supplementing diets with probiotics and vitamin C. However, when the diet was supplemented with vitamin C, changes in nucleus morphology, cell length, and width indicated a decrease in cytoplasmic space, influencing mean corpuscular volume and mean corpuscular hemoglobin concentration, thereby affecting oxygen transport in the blood. After 63 days of experimentation, fish supplemented with probiotics showed better responses in terms of zootechnical performance indices and immune response compared to both the control group and those supplemented with vitamin C.

Key words: *Centropomus undecimalis*; nutrition; probiotic; ascorbic acid; hematology

Resumen

El presente estudio proporcionó evidencias de los efectos del tratamiento con vitamina C y probióticos sobre el desempeño productivo y el perfil hematológico en juveniles de róbalo (*Centropomus undecimalis*), una especie emblemática del trópico americano que cuenta con gran valor económico en el mercado. En los ensayos se utilizaron 90 especímenes de 17,8±0,5 cm de longitud total y 42,5±3,0 g de peso. Se evaluaron tres tratamientos (vitamina C, probiótico y control) con tres réplicas, distribuyendo al azar 10 peces por tanque. La calidad del agua se mantuvo en condiciones controladas. Se estimaron indicadores del desempeño y aspectos hematológicos para cada tratamiento. El comportamiento eritrocitario, particularmente el tamaño de las células y el factor de forma, sugiere un beneficio por el suplemento de las dietas con probióticos y vitamina C. No obstante, la morfología del núcleo y la longitud y el ancho de la célula, cuando se utiliza vitamina C, indica una disminución del espacio citoplasmático, que influye en el volumen corpuscular medio y la concentración media de hemoglobina corpuscular, lo cual afecta el transporte y, consecuentemente, el oxígeno en la sangre. Después de los 63 días de experimentación, los peces suplementados con el probiótico presentaron mejores respuestas en los índices de desempeño zootécnicos y respuesta inmune que los del grupo control y los suplementados con vitamina C.

Palabras clave: róbalo común; nutrición; probiótico; ácido ascórbico; hematología

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Editor: Victor Macias
Recibido: 08 de mayo 2024
Aceptado: 07 de junio de 2024
Publicación en línea: 25 de agosto de 2024
Citar como: Gaitán-Ibarra, S., Benítez-Polo, Z., Pulgar-Bacca, A., Blanco-Cervantes, G. y López-Patiño, D. (2024). Efecto del uso de estimuladores de crecimiento sobre los indicadores de desempeño y aspectos morfoeritrocitarios evaluados en juveniles de *Centropomus undecimalis* (Bloch, 1792). *Intropica,* 19 (1). https://doi.org/10.21676/23897864.5890.

Introduction

The common snook *Centropomus undecimalis* (Bloch, 1792) is a demersal, migratory, and euryhaline fish of the Centropomidae family, with a wide geographic distribution that extends from the coasts of the southeastern United States to southern Brazil, including the Gulf of Mexico and the Caribbean Sea (Blewett *et al.,* 2009; Perera-García *et al*., 2011). This teleost maintains carnivorous habits, capturing small fish, crustaceans, and mollusks (Lira et al., 2017; Nascimento *et al*., 2021), can reach up to 140 cm in length (Robertson et al., 2023) and, as a protandric hermaphrodite species, part of the male population transitions to females (Muller and Taylor, 2006). Its natural populations along the Colombian Caribbean have been declining over the last decades due to overexploitation and habitat degradation, which has led to its listing in the Red Book of Marine Fish of Colombia as a threatened species in a vulnerable state (Grijalba-Bendeck *et al*., 2017).

Although conservation actions such as fishing prohibitions in protected areas in certain regions in Colombia (Duarte *et al*., 2018; Grijalba-Bendeck *et al*., 2017; Rodríguez *et al*., 2016), there has been an evident decrease in abundance in some functional groups of demersal fish, including common snook (García *et al.,* 2007). Worldwide, aquaculture consolidated as one of the most widely used strategies for conserving and recovering the populations of aquatic species under some degree of threat, allowing to guarantee the continuity of this natural resource, and providing food security before the global demand for fishery products caused by decreases in capture fishing (Froehlich *et al*., 2017; Ross *et al.,* 2008).

In this order of ideas, although *C. undecimalis* is a resource impacted by fishing, it is considered a species with great aquacultural potential due to its tolerance to wide variations in salinity and dissolved oxygen, high growth rates, good feed conversion, high stocking densities, and adaptability to captivity and commercial and formulated diets. Its meat also stands out for its excellent nutritional quality and high economic value in the market (Álvarez-Lajonchère and Tsuzuki, 2008; Contreras-Sánchez *et al.,* 2015; Gracia-López *et al.,* 2006; Peterson *et al.,* 1991; Polonía *et al.,* 2017b; Souza-Filho and Cerqueira, 2003)..

Over the past decades, one of the biggest challenges in aquaculture has been feeding carnivorous fish with plant-based foods instead of fishmeal, as well as using feed supplements to stimulate and improve their productive growth, immune system, and feed absorption (Dawood *et al.,* 2017). Such is the case of probiotics: microorganisms (bacteria, yeasts, or fungi) that, when added to water, sediment, or feed, enter the gastrointestinal tract and aid in growth performance, disease resistance, immunity, health status, improving the integrity of the intestinal epithelial barrier and intestinal microbiome while increasing water quality by breaking down fish waste (El-Saadony *et al.,* 2021; Martínez *et al.,* 2012).

The isolation, implementation, and management of probiotics as part of the daily diet in culture systems are well-known in other species of the *Centropomus* genus (Barbosa *et al.,* 2011; De Jesus *et al.,* 2016; Zatán *et al.,* 2020). However, there are few studies about these practices in *C. undecimalis*, which are limited to its use in bacterial control (e.g., Vibrio alginolyticus) (Blain *et al.,* 1998), as a supplement in Bacillus sp. mixtures during the larval stage (Hauville *et al.,* 2013; Tarnecki *et al.,* 2019), and with monospecific probiotic Bacillus subtilis in juvenile common snook (Noffs *et al.,* 2015).

Another supplement used in aquaculture is vitamin C (ascorbic acid), which is critical for many biological processes such as collagen synthesis, wound healing, immune system enhancement, increased stress resistance, and survival in many fish species (Barros *et al.,* 2014; Lim *et al.,* 2002; Peng *et al.,* 2013). However, very few studies on this topic exist for the Centropomidae family and are limited to testing vitamin C supplementation in their diet and its possible effects (Phromkunthong *et al.,* 1994; Silvão and Nunes, 2017; Tucker, 1987).

Likewise, the development of studies about the hematological profile and its relationship with supplied foods has been gaining importance in the search for a better understanding of metabolic action and health status. Blood performs vital functions such as transporting oxygen and nutrients to cells, eliminating metabolic waste, immune response, coagulation, and intercellular action (Vázquez and Guerrero, 2007). Therefore, blood parameter evaluation provides valuable information on a species' well-being, the effectiveness of its immune system, and possible short and long-term effects on the conditions suggested for the development of organisms, water quality, and disease risks (Casanovas *et al.,* 2021). Likewise, hematological analysis emerges as a fundamental tool in aquaculture management for identifying and adequately addressing factors affecting the health and productivity of *C. undecimalis* in specific food culture systems.

Based on the above, it is necessary to evaluate the effect of the diet with the addition of probiotics and ascorbic acid as stimulants of productive performance and its relationship with the hematological profile in *C. undecimalis* juveniles in captivity conditions.

Materials and methods

Obtaining and acclimatizing common snook

Researchers captured a total of 90 juvenile *C. undecimalis* individuals with a cast net (mesh size of 1.5 cm) in the Ciénaga de Grande de Santa Marta (CGSM), Palmira sector (10°58'55.7"N and 74°18'29.1"W), and used plastic bags (30L capacity) during transport with a density of 30 g (biomass)/L of water from the capture site, and mixed with 7 µL of eugenol (96%, Eufar®, Bogotá, Colombia)/L of water as an anesthetic solution (Cruz-Botto *et al.,* 2018; Villamizar *et al.,* 2021) and oxygen (240 mg/L). The bags were then sealed with elastic bands.

The specimens were transported to the CDPAT (Taganga Fisheries and Aquaculture Development Center) of the Universidad del Magdalena (11°15'58.35"N and 74°11'23.00"W) following the methodology described by Polonía et al. (2017a). Once in the laboratory, individuals were subjected to a prophylactic bath with seawater at 37psu for one minute to eliminate microbial load from the natural environment. Subsequently, the fish were thermally and osmotically acclimatized (27-28°C and 0-0.5psu), gradually increasing by 5psu every three hours until reaching 35psu (Polonía *et al.,* 2017a). The individuals were kept in filtered and UV-irradiated water.

Experimental design

Once the common snook specimens acclimatized to laboratory conditions, researchers provided a diet containing 55 % crude protein (CP) with different growth stimulants: one with the mixed probiotic Bactercol Plus® (*B. subtilis, Bacillus megaterium, Bacillus amyloliquefaciens, Bacillus spp., Lactobacillus*, and yeasts) at a concentration of 1x109 cfu/kg of feed; another with ascorbic acid AC99 vitamin C® at 800 µg/kg of feed; and a control group without the addition of stimulant. Each treatment had three replicates.

Fish were randomly distributed in 2,000 L tanks at a stocking density of 10 fish/tank (17.8±0.5 cm total length and 42.5±3.0 g weight). Tanks were connected to a continuous flow system of filtered, aerated, and UV-irradiated seawater, with daily exchanges of up to 50%. Seawater was kept at 27.8±0.2°C, a salinity of 34.8±0.1 psu, oxygen saturation of 70%, and pH of 8.0. Researchers recorded measurements of these parameters daily and supplied feed twice a day, at apparent satiation at 5% of biomass for 62 days; they performed biometry every 21 days to adjust food rations and evaluated the behavior of growth indicators. Blood samples were taken for hematological analysis at the end of the experiment.

Diet characterization

The diet was formulated and prepared at 55 % CP using fish meal, shrimp meal, soybean meal, and rice flour, as well as the respective growth stimulators (mixed probiotic Bactercol Plus® and ascorbic acid AC99 vitamin C®), which were incorporated into the diets at the time of mixing each treatment's ingredients, according to Gaitán et al. (2023). The proximal feed analysis was carried out with NIRS DA1650 equipment (Table 1). The bromatological evaluations of formulated diets reported values close to the theoretical formulation.

Table 1. *Proximal analysis of the diets supplied to juveniles of C. undecimalis*

Diets	Protein	Moisture	Fat	Ash
Control	55,75±0,167	14,09±0,408	8,5±0,143	17,04±0,463
Probiotic	56,15±0,252	13,31±0,526	8,27±0,099	17,34±0,550
Vitamin C	55,52±0,211	14,62±0,620	8,47±0,198	17,15±0,663

Zootechnical parameter assignment

All specimens were measured and weighed during sampling to estimate weight gain (WG), growth rate (GR), specific growth rate (SGR), Fulton condition factor (K), feed conversion rate (FCR), protein efficiency ratio (PER) and survival (S). For this purpose, the following equations were used:

$$WG\ (g) = FW - IW, \qquad (1)$$

where FW is the final average weight and IW is the initial average weight;

$$GR\ (\%) = (WG/IW)*100, \qquad (2)$$

$$SGR\ (g/day) = 100*[Ln\ FW - Ln\ IW]/t, \qquad (3)$$

where Ln FW is the natural logarithm of the final weight, Ln IW is the natural logarithm of the initial weight, and t is time in days;

$$K = 100*(W / L3), \qquad (4)$$

where W is total weight in grams, and L is total length in centimeters:

$$FCR = \text{Total food consumed (g)}/WG, \qquad (5)$$

$$PER = WG/\text{Amount of consumed protein (g)}, \qquad (6)$$

$$S\ (\%) = (\text{Final No. of fish}/\text{Starting No. of fish})*100. \qquad (7)$$

Blood characterization

Researchers took four fish per treatment and extracted 0.5 mL blood samples from each individual by puncturing specimens' caudal veins, keeping each treatment's independence, and making three smears for each specimen. The plates were dried at room temperature and fixed with methanol for 5 min. Subsequently, they were stained with Giemsa (10%) for 10 min and rinsed following the methodology described by Noro and Wittwer (2012). Finally, samples were fixed with Canada balsam for later observation in a Leica DM500 optical microscope, where images were captured in a 40X field.

Researchers selected 50 erythrocytes from each fish and measured four parameters using theimage processor ImageJ (Schneider et al., 2012): size (SL), width (W), nucleus length (NL), and nuclear width (NW). They also took records of erythrocyte size or area (ES=(SL/2)*(WL/2)π), nucleus size or area (NS=(NL/2)(NW/2)π), shape factor or circularity index (CI=SL/WL) and nucleus cytoplasm ratio (TN/TC) (Ahmed *et al.,* 2021; Najiah *et al.,* 2008).

Statistical analysis

Researchers performed one-way variance analyses (NWOVA) to find significant differences in the productive performance variables (WG, GR, SGR, K, FCR, PER, and S) and describe the hematological characterization between the different treatments evaluated, followed by Tukey multiple comparison tests, with prior confirmation of the normality assumptions (Shapiro-Wilk) and homoscedasticity (Levene test) of the data. An α value of 0.05 was used for all significance values and the Statgraphics Centurion XVII software was used.

Results

The average WG of *C. undecimalis* juveniles ranged from 10.48 g to 23.85 g. Larger gains were recorded in the probiotic and vitamin C treatments, respectively (Figure 1a). On the other hand, GR varied between 29.18% and 55.26% (Figure 1b), and SGR between 0.41 g/day and 0.70 g/day (Figure 1c). In WG, GR, and SGR, significant high values were recorded for the estimates of the vitamin C and probiotic diets compared to the control group (gl=2, $p=0.0178$).

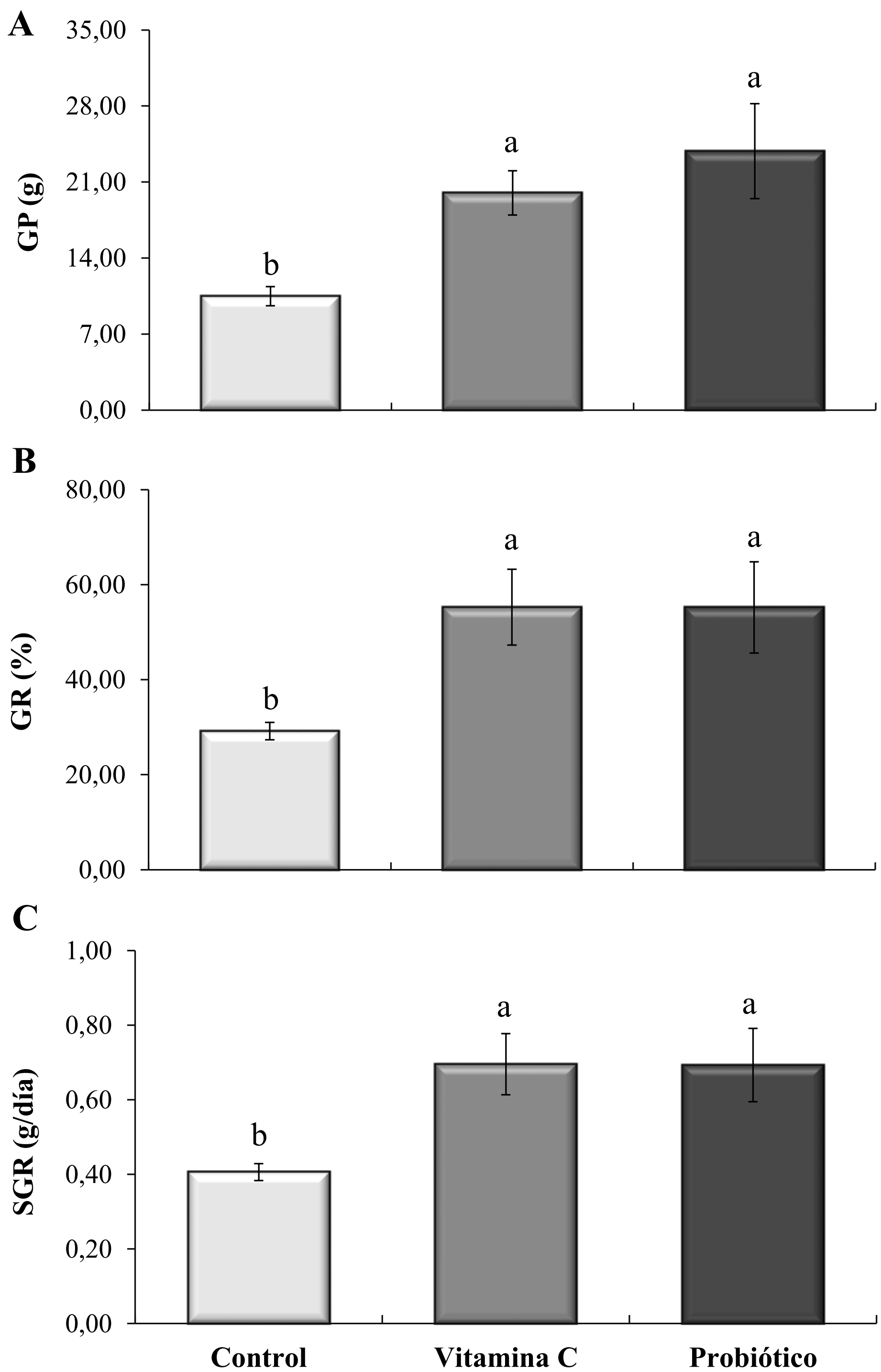

Figure 1. Effect of diet with productive performance stimulants in *C. undecimalis*: a) weight gain (WG); b) growth rate (GR); c) specific growth rate (SGR). Different letters indicate statistically significant differences (p<0.05). Vertical bars represent the standard error.

K factor remained between 0.74 and 0.76 (figure 2a), with the highest values in treatments with probiotics and the control group (gl=2, F=5.49, p=0.0378) compared to the one with vitamin C. The FCR ranged between 1.93 and 4.91 (figure 2b), and the highest results were recorded in the control treatment compared to the diet with stimulants (gl=2, F=170.57, p=0.0000), which

shows a better conversion in the latter.

The opposite occurred with PER, which varied between 0.37

and 0.96 (figure 2c), with significantly higher values in the treatment with probiotics (gl=2, F=45.16, p=0.0002), suggesting the protein assimilation process was more efficient in this case in terms of biomass.

Finally, the survival of the common snook at the end of the experiment was 100% in all the treatments (gl=2, F=0.00, p>0.0500).

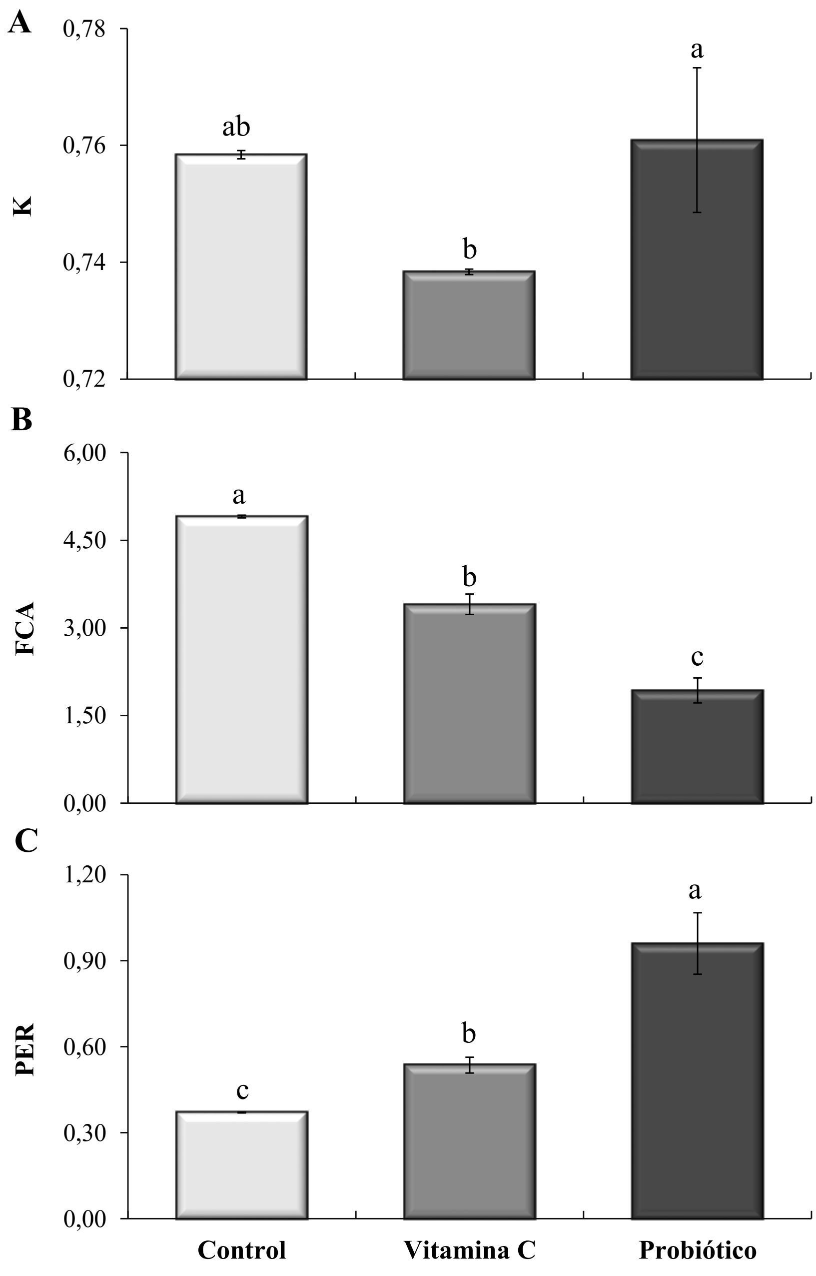

Figure 2. Effect of diet with added performance stimulants on *C. undecimalis*: a) condition factor (K); b) feed conversion ratio (FCR); c) specific growth rate (SGR). Different letters indicate statistically significant differences (p<0.05). Vertical bars represent the standard error.

Blood cell determination

Five different types of blood cells were present in the samples collected from *C. undecimalis* juveniles. The percentage of erythrocytes ranged from 93.10% to 95.55% (Figure 3a), with the highest values corresponding to the treatments that supplied vitamin C and probiotics relative to the control group (gl=2, F=19.60, p<0.0005). Meanwhile, lymphoid and basophil cells were between 1.38-3.05% and 0.20-1.85% in the control group and the diet with vitamin C, respectively (Figure 3b), with a significantly higher proportion in the control group than in the other treatments (gl=2, p≤0.0013).

Monocytes remained between 0.80% and 2.45% (gl=2, F=17.03, p=0.0009), and high values appeared in the vitamin C and control treatments compared to probiotics. Finally, the percentage of platelets varied between 0.45% and 0.88% and was significantly higher in the probiotics treatment than the control and vitamin C treatments (gl=2, F=5.78, p=0.0243).

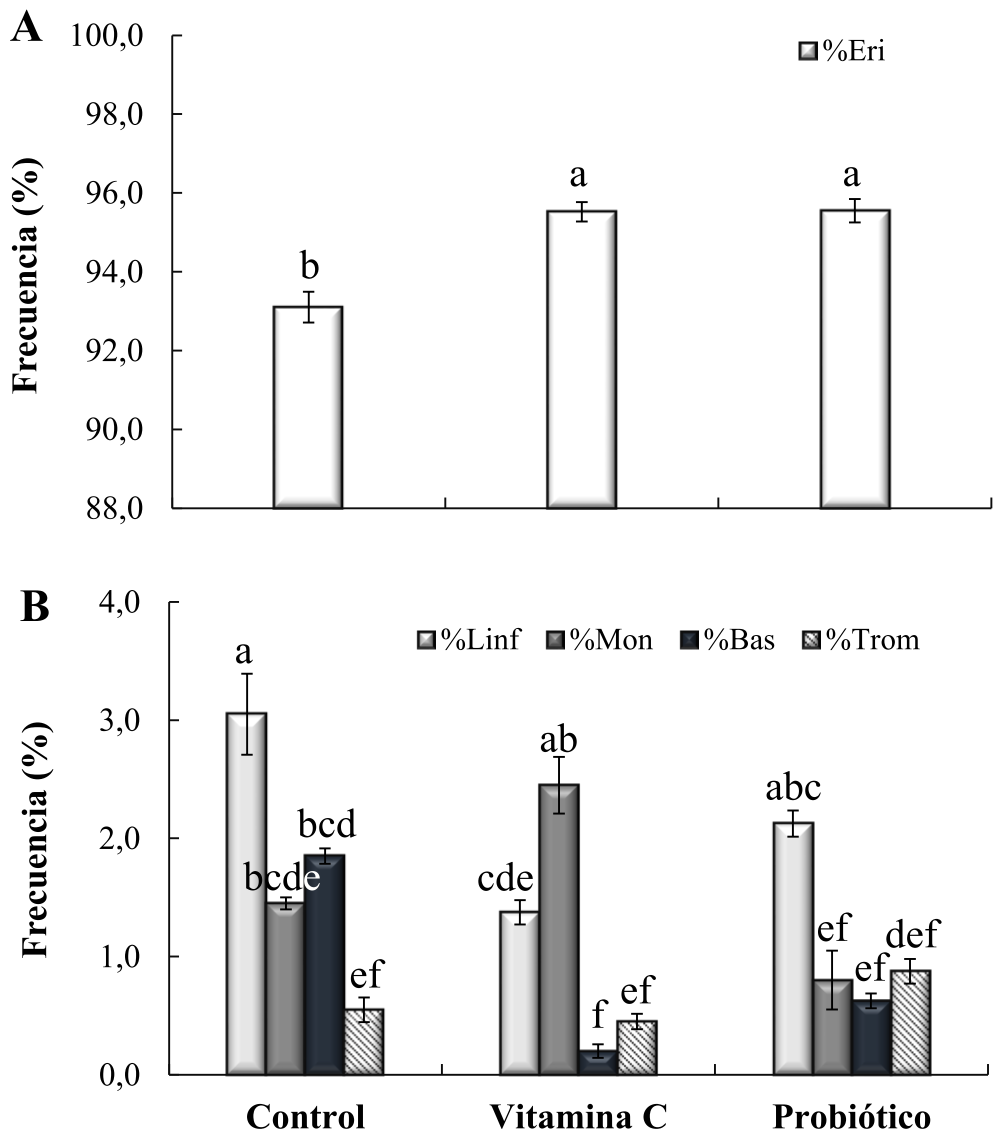

Figure 3. Blood cell frequencies in juvenile *C. undecimalis* exposed to diet with added growth stimulant: a) erythrocytes; b) lymphoid and basophil cells. Different letters indicate statistically significant differences (p<0.05). Vertical bars represent the standard error.

Characterization of erythrocyte morphometry

The erythrocytes in *C. undecimalis* presented an EL that ranged between 8,688 µm for the probiotics and 9,708 µm for the control treatment. The WE varied between 5,313 µm for the vitamin C and 6,196 µm for the probiotics treatment. Meanwhile, the NL was between 3,594 µm for the control and 4,474 µm for the vitamin C treatment. The WN fluctuated between 2,156 µm in the control and 2,988 µm for the vitamin C treatment.

Consequently, the ES and NS values for the probiotic group ranged from 38.497 µm to 46.239 µm, and from 7.814 µm to

8.868 µm, respectively. On the other hand, in the control group, the ES and NS ranges were from 38.974 µm to 45.737 µm, and 6.255 µm to 8.021 µm, respectively. The cytoplasm size (CS=ES–NS), circularity index (CI=EL/EW), and nucleus-to-cytoplasm ratio (NS/CS) ranged from 30.006 µm to 38.426 µm; 1.548 µm to 1.804 µm; and 0.193µm and 0.480µm for the probiotic and vitamin C, respectively (Table 2).

and shape of red blood cells in parameters such as EW, NS, NW, and NS/CS ratio. The lowest values were recorded in the control group. The highest results were presented in the vitamin C treatment, followed by the probiotic treatment. The Kruskal-Wallis test showed significant differences between the vitamin C and control treatments, and between the vitamin C and probiotics treatments for NL (p=0.0097); between the vitamin C and the control treatments for NS (p=0.013); and between the vitamin C and the control treatments in NS/CS (p=0.043) (Figure 4).

Tabla 2. Parámetros morfométricos de eritrocitos en juveniles de *C. undecimalis*

Treatment	**EL**	**EW**	**NL**	**NW**	**ES**	**NS**	**CS**	**EL/EW**	**NS/CS**
Control									
N	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000
Mín.	9,079	5,469	3,594	2,156	38,974	6,255	32,592	1,633	0,193
Máx.	9,708	6,026	3,769	2,710	45,737	8,021	37,717	1,694	0,219
Average	9,384	5,726	3,662	2,450	42,104	7,104	35,000	1,662	0,207
Standard error	0,143	0,142	0,041	0,145	1,656	0,458	1,207	0,016	0,007
Est. deviation	0,286	0,284	0,082	0,290	3,312	0,915	2,414	0,032	0,013
Probiotics									
N	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000
Mín.	8,688	5,605	3,748	2,639	38,497	7,814	30,072	1,548	0,207
Máx.	9,519	6,196	4,048	2,766	46,239	8,868	38,426	1,602	0,480
Average	9,184	5,897	3,899	2,699	42,673	8,302	34,371	1,571	0,307
Standard error	0,185	0,125	0,079	0,028	1,660	0,226	1,820	0,012	0,061
Est. deviation	0,370	0,249	0,159	0,056	3,319	0,453	3,640	0,024	0,122
Vitamin C									
N	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000	4,000
Mín.	9,236	5,313	4,122	2,615	39,223	8,629	30,006	1,552	0,288
Máx.	9,502	6,107	4,474	2,988	45,242	10,463	35,299	1,804	0,343
Average	9,396	5,729	4,294	2,823	42,257	9,563	32,694	1,660	0,313
Standard error	0,057	0,213	0,082	0,079	1,630	0,403	1,286	0,063	0,011
Est. deviation	0,115	0,426	0,164	0,159	3,259	0,805	2,571	0,125	0,023

EL: red blood cell length; EW: red blood cell width; NL: nucleus length; NW: nucleus width; ES: red blood cell size; NS: nucleus size; CS: cytoplasm size; EL/EW: shape factor or ratio of red blood cell length/width; NS/CS: nucleus/cytoplasm ratio. All values are in microns (µm).

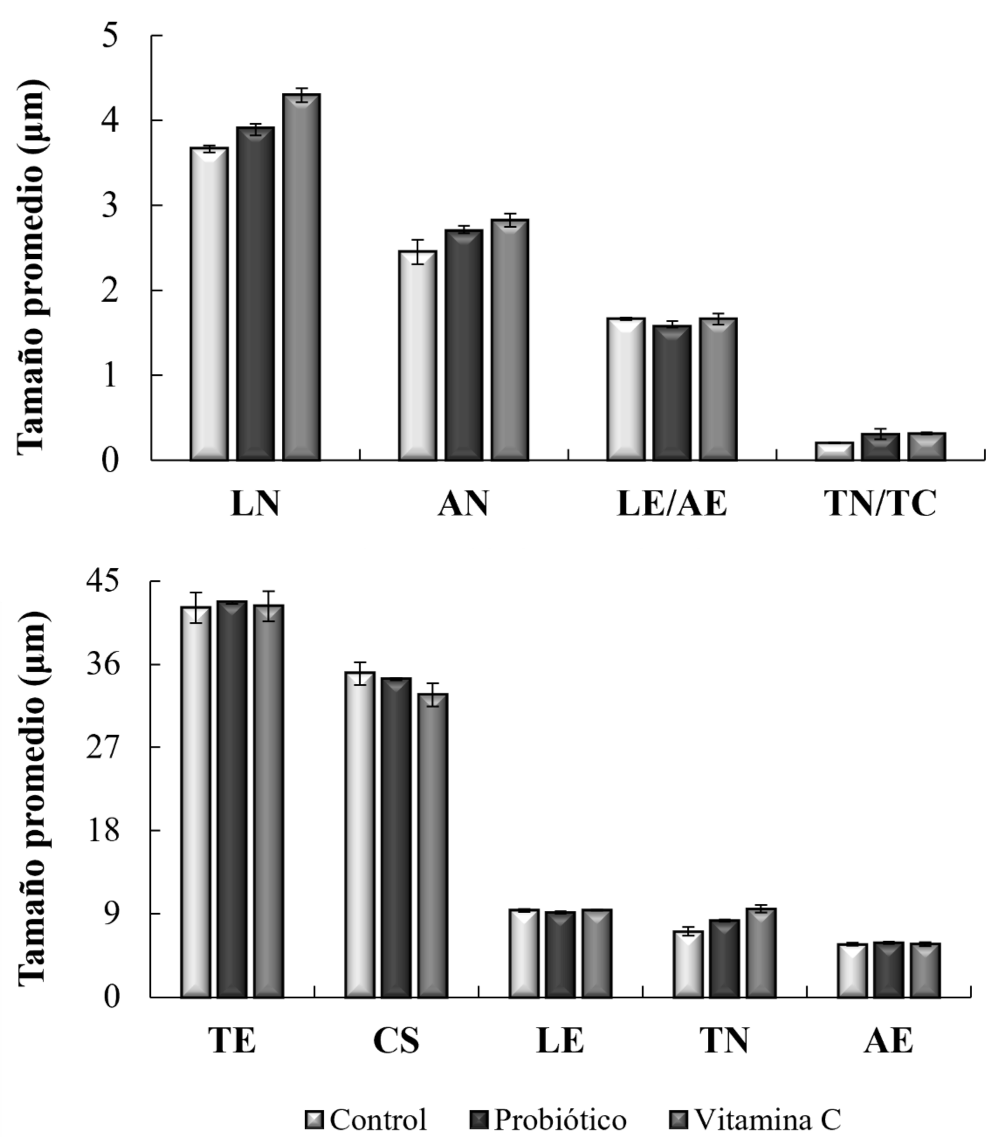

Figure 4. Effects of growth stimulator use in *C. undecimalis* diets on erythrocyte morphometric parameters. EL: erythrocyte length; EW: erythrocyte width; NL: nucleus length; NW: nucleus width; ES: erythrocyte area or size; NS: nucleus size; CS: cytoplasm size; EL/EW: shape factor or erythrocyte length/width ratio; NS/CS: nucleus/cytoplasm ratio. Different letters indicate statistically significant differences ($p<0.05$). Vertical bars represent standard error.

Discussion

The highest significant values of zootechnical growth indices (WG, GR, and SGR) estimated for *C. undecimalis juveniles* as an effect of supplying diets with probiotics and vitamin C are similar to those reported in other marine carnivorous fish such as Dicentrarchus labrax (Carnevali *et al.,* 2006), Lates calcarifer (Rengpipat *et al.,* 2008), Lateolabrax japonicus (Ai *et al.,* 2004), Psammoperca waigiensis (Luc *et al.,* 2021) and Trachinotus ovatus (Zhang *et al.,* 2019) and contrary to reports about *Centropomus parallelus* (Barbosa *et al.,* 2011), *C. undecimalis* (Noffs *et al.,* 2015), Dentex dentex (Hidalgo *et al.,* 2006) and Pseudosciaena crocea (Ai *et al.,* 2006). Probiotics influence digestion and nutrient absorption in these species by affecting the transcription of two genes that regulate body growth (IGF-I and myostatin MSTN). Thus, total body weight can increase up to 81% with the addition of autochthonous strains isolated from host fish, as well as decreasing cortisol levels in treated fish (Carnevali *et al.,* 2006; Rengpipat *et al.,* 2008). In this regard, this hormone is recognized as the main glucocorticoid released as a stress response in teleost fish and causes proteolytic effects because its synthesis can induce catabolic and antianabolic reactions that delay somatic growth, while causing deficiencies in immune system responses (Barton, 2002; Lemos *et al.,* 2023).

On the other hand, the lack of vitamin C (or ascorbic acid) in the diets of living organisms in nature has been documented. This nutrient's absence can lead to growth retardation, scoliosis, lordosis, fin erosion, darkening, anorexia, opercular deformation, severe liver atrophy, and high mortality rates observed in fish kept at different concentrations of vitamin C (Ai *et al.,* 2004; Wang *et al.,* 2003). Therefore, exogenous supplementation of this vitamin at adequate concentrations is necessary in carnivorous fish culture systems to ensure their normal growth and physiological functioning.

C. undecimalis exhibits positive allometric growth, that is, an increase in the body's relative thickness or fatness (Costa and De Mello, 2015); the availability and quality of food, seasonality, water quality conditions, and intrinsic genetic changes are some factors that can affect this process. In fish farming, the K factor is widely used as an indicator of a population's stage of development, maturity, reproduction, nutrition, and well-being (Froese, 2006). In this study, this variable's behavior displayed a difference between the control and the probiotics treatments concerning vitamin C, with values of 0.76 and 0.74, respectively. These results are within ranges reported in other nutrition and probiotic supplementation studies in the *Centropomus* genus (0.73-0.98) (Barbosa *et al.,* 2011; Noffs *et al.,* 2015; Polonía *et al.,* 2017b).

The FCR describes the relationship between the amount of feed consumed by the fish and the increase in body mass resulting from metabolic conversion or the fish's biological efficiency in transforming ingested nutrients into muscle tissue. Thus, low values indicate greater efficiency in feed conversion, which implies an optimal use of food resources for body growth. In this study, the most favorable results concerning FCR were found in fish supplemented with the mixed probiotic, which corresponds to better-performing growth indicators compared to the high FCR values found in the control and vitamin C groups.

These FCR results are well below those reported in *C. undecimalis* fingerlings under an alternating and continuous feed supply regime for 191 days with the probiotic strain B. subtilis at concentrations of 5x109 cfu/kg (12.6-19.6) (Noffs *et al.,* 2015). This variation between the same species could have been caused by the diet's nutritional content and the feed's presentation, the environmental conditions, and the fish's developmental stage or size (Fry *et al.,* 2018; Torrissen *et al.,* 2011).

Likewise, the high PER values in the diet with probiotics indicate the fish had a good absorption and assimilation process of the protein in the feed, up to 46% and 61% more than the control and the vitamin C groups. This finding is related to the ability of probiotics to bind to and improve intestinal function, resulting in better digestion and nutrient absorption (Eshak *et al.,* 2010; Hauville *et al.,* 2013). In this way, the protein profile of fish species such as Oncorhynchus mykiss (Kamgar and Ghane, 2014; Nargesi *et al.,* 2020) and D. labrax (Eissa *et al.,* 2022) improved. Likewise, good immune status in fish is indicated by increases in total protein, albumin, and globulin (Al-Dohail *et al.,* 2009), whose deficiency could trigger a series of liver diseases (Bernet *et al.,* 2001).

Teleost fish cannot synthesize internally ascorbic acid, a vital micronutrient for their metabolism. However, after 63 days of experiment, survival in juvenile *C. undecimalis* in all tested treatments remained at 100%, with no apparent signs of deterioration. These results are similar to those found in other marine carnivorous fish with vitamin C concentrations between 11-400 mg/kg (Luc *et al.,* 2021; Zhang *et al.,* 2019). This parameter can fluctuate between 83% and 98% at concentrations between 234-489 mg/kg (Ai *et al.,* 2004, 2006) and between 60% and 75% for probiotic concentrations ≥1x109 cfu/kg (Barbosa *et al.,* 2011; Noffs *et al.,* 2015; Rengpipat *et al.,* 2008).

Although there were no differences in survival rates between treatments, researchers established that the best values of zootechnical productive performance of the common snook came from the mixed probiotic. However, the function of maintaining and increasing ascorbic acid is a phenomenon particularly associated with the tissue biosynthesis process, in addition to being essential for collagen synthesis, which is critical in the different stages of fish development (Sahoo and Mukherjee, 2003).

Hematological parameters are crucial measures reflecting the physiological well-being of fish populations and their variability can be influenced by several factors in aquaculture systems. Researchers observed significantly higher erythrocyte values in vitamin C and probiotics groups compared to the control. Similarly, Luc et al. (2021) and Barbosa et al. (2011) reported improvements in immune responses and oxygen transport capacity.

However, lymphocyte levels found in the supplemented treatments are contrary to the high values reported by Barbosa et al. (2011) and Noffs et al. (2015). These authors also reported high levels of platelets, which in fish are essential for homeostasis, defense, coagulation, and participation in phagocytic activity during an infection (Tavares-Dias *et al.,* 2002). Researchers observed this in the present investigation with the probiotics diet, so this supplement may have influenced greater platelet production.

On the other hand, the high percentage of monocyte cells in the control and vitamin C groups may be due to defense processes against an infectious evolution caused by viruses, bacteria, or other pathogenic agents in fish (Yang *et al.,* 2021). In this sense, it is necessary to remember that continuous exposure to high concentrations of probiotics or immunostimulating substances could decrease the immune system's activity to the point of immunosuppression due to resistance induced by the organism (Bricknell and Dalmo, 2005; Merrifield *et al.,* 2010; Noffs *et al.,* 2015).

Erythrocytes, which are essential cellular constituents of blood tissue in vertebrates, represent the most predominant fraction and circulate throughout the body, also providing valuable information on organ health (Farrell, 2011; Rahman and Baek, 2019; Ruas *et al.,* 2008). In this study, Erythrocyte length and width values were within the suggested normal ranges for poorly mobile fish (10–20µm EL and 6–10µm EW), and their coloration showed no apparent signs of anemia (Farrell, 2011; Fazio *et al.,* 2019). Likewise, no signs of nuclear or cellular deterioration were evident, which may indicate good health status.

The absence of significant differences in data related to the movement of erythrocytes through blood vessels, such as cell size (ES) and shape factor (EL/EW) could suggest the beneficial effect of diet supplementation with probiotics and vitamin C on erythrocyte parameters (Affonso *et al.,* 2007; Osman *et al.,* 2018; Taherpour *et al.,* 2023; Trichet *et al.,* 2015). However, the considerable increase in values related to nuclear morphometry, such as NL and NW, with diets supplemented with vitamin C may decrease the size of the cytoplasm available for hemoglobin, as shown by the also significant NS/CS ratio, which can be associated with erythrocyte hematological parameters such as hemoglobin, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) (Taherpour *et al.,* 2023).

These assessments are corroborated by the association found between NS/CS and hemoglobin, MCV, and MCHC in *Orechromis nilotius* and *Clarias gariepinus* (Osman *et al.,* 2018); which is consistent with contributions from nutritional supplementation with probiotics and vitamin C to fish's health status due to increases in hemoglobin concentration, erythrocytes, hematocrits, MCV, MCHC and mean corpuscular hemoglobin (MCH). These effects are reflected in less stress and better survival (Azarin *et al.,* 2015; Michael *et al.,* 2019).

In conclusion, the present study's results indicate that diets supplemented with mixed probiotics and vitamin C can improve the performance, growth, and immune response of the common snook. However, researchers highlight that using the mixed probiotic undoubtedly had better effects on the zootechnical and hematological performance indicators. However, researchers recommended using this supplement as an alternative or at different stages of the animal's development, to avoid resistance. Also, regarding vitamin C, they suggest evaluating lower concentrations than those used in this study to reduce the organism's immunosuppression and achieve higher values of growth, physiological development, and protection from diseases in common snook under culture conditions.

Conflicts of interest

The authors declare they have no conflict of interest related to this manuscript.

Authors' Contribution

Saeko Gaitán-Ibarra: conceptualization, experiment design, and manuscript writing.

Zamir Benítez-Polo: result analysis and manuscript writing.

Germán Blanco-Cervantes: laboratory design and analysis, information processing, and hematology-related writing.

Danny López-Patiño and Andrés Pulgar-Bacca: execution of the experimental design, laboratory analysis, and data collection.

Acknowledgments

The authors thank the staff of the Aquaculture Hangar of the Biodiversity and Applied Ecology Research Group (GIBEA) of the University of Magdalena for their technical support. This study was carried out within the framework of the project "Implementation of productive systems in marine fish farming of snook to promote its production in the department of Magdalena," with BPIN code 2020000100036, financed with resources from the General Royalties System (SGR) and the entity that was designated as the executor of these resources: the University of Magdalena. The research was developed under the permission of the National Aquaculture and Fisheries Authority (AUNAP), through Resolution No. 2433 of October 13, 2022.

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