UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

CRECIMIENTO, SOBREVIVENCIA Y RESISTENCIA AL ESTRÉS DE POSTLARVAS Y ALEVINOS DE PACO (*PIARACTUS BRACHYPOMUS, CUVIER 1818*) EN SISTEMAS CON Y SIN BIOFLOC

Beatriz Elena Angeles Escobar

Tese apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Doutor.

Prof.(a) Dr.(a) William Severi
Orientador

Prof.(a) Dr.(a) Suzianny Maria Bezerra Cabral da Silva
Co-orientador

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Prof. Dr. William Severi
Orientador - UFRPE

Prof. Dr. Luis Otavio Brito
Membro Interno – Titular – UFRPE

Prof. Dr. Alfredo Olivera Gálvez
Membro Interno – Titular – UFRPE

Profa. Dra. Patricia Liliana Gil Kodaka
Membro Externo – Titular –UFSC

Dra. Alitiene Moura Lemos Pereira
Membro Externo – Titular – EMBRAPA Meio Norte

Prof. Dr. Ranilson Bezerra
Membro Interno – Suplente – UFPE

Prof. Dr. Mauricio Laterça Martins
Membro Externo – Suplente – UNALM

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Resumo

El presente trabajo de tesis evalúa el uso de sistemas biofloc como alternativa para la mejora en el crecimiento, sobrevivencia y resistencia al estrés de post-larvas y alevinos de paco (*P. brachypomus*). El primer capítulo determina el crecimiento, los eritrocitos y las alteraciones branquiales de alevines de paco rojo (2,7 ± 0,5 g) expuestos de forma crónica (45 días), en acuarios de 50L (por cuadruplicado), a una concentración baja (T1: 200 – 300 mg.L^{-1}) y una media (T2: 400 – 600 mg.L^{-1}) de sólidos suspendidos totales (TSS) en biofloc, y los compara con un control sin sólidos (TC). Los resultados muestran que esta especie puede ser cultivada en sistemas biofloc en concentraciones entre 200 y 300 mg.L^{-1} de sólidos suspendidos totales, sin afectar su salud, sin embargo, a concentraciones mayores (400 a 600 mg.L^{-1}) se reduce el crecimiento, se alteran los parámetros hematológicos y se afecta la morfología branquial. El segundo capítulo evalúa el efecto de la densidad poblacional sobre el crecimiento, la producción y los parámetros hematológicos de alevines de paco rojo cultivados en sistemas biofloc como una alternativa de producción para mejorar la tolerancia al estrés. Los alevines de paco rojo (2.76 ± 0.74 g) fueron mantenidos por 50 días a densidades de 400 (D400), 800 (D800) y 1600 (D1600) peces.m^{-3} en unidades experimentales de 50L en sistema biofloc (por cuadruplicado) y fueron comparados con un control (sisema de recirculación) a una densidad de 400 peces.m^{-3}. Los resultados indican que esta especie puede crecer aún a una densidad de 1600 peces.m^{-3} en esta etapa, estando sus parámetros de crecimiento entre los valores reportados por otros trabajos en sistemas biofloc. Asimismo, los parámetros hematológicos en los tratamientos con biofloc a las diferentes densidades evaluadas eran similares a los reportados en otros sistemas semi-intensivos y extensivos, sin mostrar alteraciones en las proporciones o tipo de células blancas y rojas, indicando la alta tolerancia de esta especie a altas densidades en este tipo de sistema de cultivo.

Palavras-chave: pirapitinga, estrés, parámetros productivos, hematología.

Abstract

The aim of this thesis was to evaluate the use of biofloc systems as an alternative to improve growth, survival and resistance to stress of post-larvae and fry of red pacu (*Piaractus brachypomus*). The first chapter determines growth, red blood cells and gill alterations of red pacu fry (2.7 ± 0.5 g weight) exposed, for a 45-day period, to different biofloc total suspended solids (TSS) concentrations with low (T1: 200 – 300), and medium (T2: 400 – 600 mg/L) levels, and compared it to a treatment control without solids (TC) in 50 L experimental units (in quadruplicate). The results showed that this species can be cultivated in biofloc systems in concentrations between 200 and 300 $mg.L^{-1}$ of total suspended solids, without affecting its health, however, at higher concentrations (400 to 600 $mg.L^{-1}$) the growth is reduced, hematological parameters are altered and branchial morphology is affected. The second work evaluates the effect of high stocking density on growth, production and blood hematology of red pacu cultivated in biofloc systems. Red pacu fry (2.76 ± 0.74 g) were raised for 50 days at densities of 400 (D400), 800 (D800) and 1600 (D1600) $fish.m^{-3}$ into 50 L experimental units in biofloc treatments (quadruplicate), and 400 $fish.m^{-3}$ in control (recirculation) treatment. The results indicated that this species can still grow at a density of 1600 $fish.m^{-3}$ at this stage, its growth parameters being among the values reported by other works on biofloc systems. Likewise, the hematological parameters evaluated showed that the values in the biofloc systems at the different densities used were similar to those reported in other semi-intensive and extensive systems, without showing alterations in the proportionsor type of white and red cells, indicating the high tolerance of this species to high densities in this type of culture system.

Key words: pirapitinga, stress, productive parameters, hematology

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1- Introdução

El paco (*Piaractus brachypomus,* Cuvier 1818), es una especie nativa de las cuencas del Orinoco y del Amazonas que pertenece a la familia Characidae. Es omnívora, reofílica y resistente al manejo en cautiverio, presenta alta docilidad, buen crecimiento, y soporta condiciones limnológicas desfavorables por períodos no prolongados (MESA et al., 2007). A nivel mundial esta especie es producida en américa latina y en varios países asiáticos alcanzando las 122 mil toneladas (FAO, 2021). En américa latina su producción alcanza las 37,8 mil toneladas, siendo Colombia el país con mayor producción en la región con 33 mil toneladas, seguida por el Perú con una producción de 2165 toneladas en el 2020 (FAO, 2021; PRODUCE, 2020). En Asia el mayor productor es China (59 mil t), seguido por Vietnam (23 mil t), países en los que se inició el cultivo de esta especie en la década del 2000-2010, teniendo un marcado crecimiento entre el 2005 al 2017, sin embargo, éste se ha reducido notablemente entre el 2018 y 2020 (FAO, 2021).

Esta es una especie reofílica, realizando una migración reproductiva en la época de lluvias entre los meses de octubre y marzo, teniendo un desove anual. En cautiverio, la reproducción en estanques de cultivo es inducida con hormonas, alcanzando tasas de fertilización y eclosión variables (CHAVEZ-MORENO et al., 2011). La sobrevivencia larval en sistemas de cultivo depende de las condiciones de calidad del agua así como de la primera alimentación la cual puede provenir de colectas de fito y zooplancton del ambiente natural, de organismos zoo planctónicos cultivados (rotíferos, cladóceros, copépodos y Artemia) o de alimentos pulverizados o microencapsulados (PRIETO e ATENCIO, 2008; SIPAÚBA-TAVARES et al., 2008), sin embargo se ha demostrado la necesidad de utilizar alimento vivo en la primera alimentación de esta especie (DAVID et al., 2011).

Los productores suelen sembrar a las postlarvas de paco, poco después de iniciada la alimentación exógena (3 días post eclosión), en estanques fertilizados hasta finalizar la etapa de alevinaje, lo que resulta en bajas tasas de sobrevivencia debido a su dependencia de las condiciones ambientales y sanitarias del estanque, dificultando la producción de alevinos a mayor escala (VERDI-OLIVARES et al., 2014). Sin embargo, el abastecimiento sostenido de alevines es un factor clave para el desarrollo de la acuicultura, y en la selva central del Perú es una necesidad creciente expresada por los productores, quienes indican que se requiere de la evaluación y estandarización de los protocolos de manejo de reproductores y producción de semilla de paco (*P. brachypomus*) con el fin de mejorar su oferta para el cultivo (PRIETO y ATENCIO, 2008).

1.1- Contextualização da pesquisa

1.1.1 La reproducción y alevinaje del paco (*Piaractus brachypomus*)

En el Perú, especialmente en la región amazónica, existe un interés creciente por el cultivo del paco (*P. brachypomus*) especie conocida también como pirapitinga (Brasil), cachama blanca (Colombia), morocoto (Venezuela) o red-belly pacu (China). Esta es una especie nativa de la cuenca amazónica que ha mostrado cualidades importantes para la actividad acuícola como ser omnívora, aceptar bien el alimento formulado, tener buen crecimiento y alta tolerancia al manejo, asimismo, poseer características atractivas para el consumidor como su color rojizo y plateado y menor dimensión de cabeza, lo que la convierten en una especie con gran potencial de crecimiento (VERDI-OLIVARES et al., 2014; RIBEIRO et al., 2016). Sin embargo, la reproducción inducida en cautiverio se encuentra limitada a la época natural de puesta de la especie y la etapa de alevinaje es aún muy dependiente de las condiciones ambientales y de manejo de cada centro de producción (ALARCON et al., 2015; COLLAZOS-LASSO et al., 2014) lo que limita la obtención sostenida de alevines que permitan incrementar la producción de esta especie.

Díaz-Olarte et al. (2010) indican que los ovocitos de *P. brachypomus* al momento del desove miden entre 1000-1200 µm y son de color verde azulado, alcanzando un diámetro de 1680± 34 µm una vez fertilizados e hidratados. El desarrollo embrionario de esta especie se completa en aproximadamente en 12-14 horas después de la fecundación a una temperatura promedio de 27°C, momento en el que se inicia la eclosión (DIAZ-OLARTE et al., 2010). No se ha encontrado información sobre el momento de la reabsorción del saco vitelino, sin embargo los ensayos de alimentación con esta especie se inician entre las 36 y 72 horas post eclosión (hpe) ya sea con nauplios de artemia, zooplancton filtrado o alimento formulado (DAVID et al., 2011; COLLAZOS-LASSO et al., 2014), siendo posteriormente (3-7 días post eclosión) trasladadas las postlarvas a estanques de tierra fertilizados con abonos orgánicos e inorgánicos por un periodo de 30-45 días hasta su comercialización como alevinos (ATENCIO, 2001). Las postlarvas de *Colossoma* y *Piaractus* se alimentan principalmente de rotíferos, protozoos y crustáceos planctónicos en los primeros días de vida debido a que su sistema digestivo no se encuentra completamente desarrollado y maduro, requiriendo de las enzimas liberadas por éstos para ayudar en el proceso de digestión del alimento ingerido (WOYNAROVICH Y WOYNAROVICH, 1998; DAVID et al., 2011; KUBITZA, 2003, PRIETO et al., 2006).

Los trabajos publicados en el Perú sobre la producción de alevinos de *P. brachypomus* muestran sobrevivencias del 1,3 al 50 por ciento a los 32 días de cultivo empleando como fuente de alimento, principalmente, plancton proveniente de estanques fertilizados (ASCON, 1992; VERDI-OLIVARES et al., 2014). Sin embargo, existen vacíos de información respecto al desarrollo larval, postlarval y alevinaje de esta especie (PORTELLA et al., 2014), siendo necesario profundizar en los requerimientos y características de esta especie que permitan mejorar su manejo en esta etapa. En general la larvicultura en especies sudamericanas se realiza en sistemas semiintensivos (JOMORI et al., 2003) colocando a las larvas que ya abrieron la boca en estanques fertilizados hasta completar su desarrollo a juveniles (30-50 días post eclosión), estando sujetas a la variación de la cantidad y tipo de alimento, así como a la predación, lo que resulta en producciones impredecibles y altamente dependientes de las condiciones ambientales (PORTELLA et al., 2014). El uso de sistemas intensivos de larvicultura es una alternativa para incrementar la sobrevivencia de estas especies, permitiendo la crianza en condiciones de laboratorio por un número determinado de días o semanas empleando alimentos en cantidad y calidad adecuada que permitan que las larvas alcancen un estado de desarrollo avanzado antes de ser transferidas a los estanques de cultivo(JOMORI et al., 2003; PORTELLA et al., 2014).

1.1.2 Los sistemas de cultivo cero recambio o biofloc

Los sistemas de cultivos cero recambio o bioflocs (SBF) han sido empleados por numerosos investigadores y productores por sus potenciales beneficios y aplicaciones a peces y crustáceos. Esta tecnología permite la conversión de los desechos del medio de cultivo (principalmente el amonio) en biomasa microbiana que puede ser utilizada por los organismos en cultivo como fuente de alimento, permitiendo a la vez mantener los parámetros de calidad de agua dentro de los rangos adecuados para la especie (EKASARI et al., 2016; AVNIMELECH, 2009). De esta manera las sustancias de desecho que podrían ser tóxicas para los organismos en cultivo pueden ser mantenidas en bajas concentraciones y se puede incrementar la eficiencia en la utilización de nutrientes del alimento (EKASARI et al., 2016). Asimismo, se ha demostrado que este tipo de sistema tiene efectos positivos en la capacidad inmunológica y performance reproductiva de organismos de cultivo, incrementando la producción de larvas y la resistencia de éstas a diversos estresores y a algunas bacterias (EKASARI et al., 2015). La mejora en la capacidad reproductiva y en la sobrevivencia larval puede estar relacionada con la disponibilidad constante de los flocs, así como de los organismos de diverso tamaño (fitoplancton, bacterias, rotíferos, protozoos flagelados y copépodos), cuyo consumo brinda algunos nutrientes esenciales como aminoácidos, ácidos grasos esenciales, antioxidantes y vitaminas los cuales se encuentran contenidos éstos (EMERENCIANO et al., 2013; MARTINEZ-CÓRDOVA et al, 2015). De igual manera los flocs pueden también liberar sustancias inmuno-estimulantes como los beta-1,3-glucanos, lipopolisacáridos y peptidoglucanos como parte de sus componentes estructurales (EKASARI et al., 2014) promoviendo la resistencia al estrés de los organismos cultivados.

Sin embargo, a pesar de los numerosos beneficios de la tecnología biofloc, pocas especies han sido cultivadas exitosamente en este tipo de sistemas debido a que “no todas las especies son candidatas al cultivo biofloc” (EMERENCIANO et al., 2013). En este tipo de sistema es necesario que los organismos presenten alta tolerancia a varios estresores como concentraciones medias o bajas de oxígeno disuelto (Emerenciano et al., 2013), altas densidades de cultivo (LIMA et al., 2019), variaciones en los compuestos nitrogenados (WIDANARNI et al., 2012), y en las concentraciones de sólidos sedimentables o sólidos suspendidos totales (BROWDY et al., 2012), siendo necesario evaluar el desempeño productivo y el bienestar de los organismos, en tanto se definen los parámetros adecuados para el cultivo de la especie en este tipo de sistema.

El paco (*P. brachypomus*) ha demostrado alta tolerancia a diferentes sistemas de cultivo y condiciones de calidad de agua, incluyendo los de tecnología de biofloc, con buenos resultados productivos en la etapas de pre cría (juveniles) y de crecimiento (BRÚ-CORDERO et al., 2017; CHAVERRA y PARDO, 2017; LASSO et al., 2021; POLEO et al., 2011). Los trabajos publicados sobre el cultivo de esta especie en sistemas biofloc muestran ganancias diarias de peso entre 0,4 ± 0,4 y 2,34 ± 0,05 g.día^{-1} y tasas específicas de crecimiento entre 2,1 ± 0,4 y 7,3 ± 0 %.día^{-1}, alcanzando biomasas finales entre los 3,9 y 12,9 kg.m^{-3} (BRÚ-CORDERO et al., 2017; CHAVERRA y PARDO, 2017; POLEO et al., 2011). Aún queda pendiente evaluar los valores máximos de tolerancia a variaciones en la calidad del agua, mejores proporciones de carbono:nitrógeno, manejo más eficiente de la alimentación, así como diferentes densidades de cultivo, entre otros, que permitan ajustar el manejo de esta especie en sistemas biofloc.

1.1.3 El estrés en sistemas de cultivo intensivo

Se denomina estrés a la respuesta biológica provocada cuando un animal se enfrenta a un agente "estresante" o una amenaza percibida, ya sea ambiental, social o de otro tipo, la cual interfiere con la dinámica de equilibrio del animal (conocida como "homeostasis") (CHROUSOS, 1992; MOBERG, 2000). La respuesta del organismo al agente estresor involucra respuestas fisiológicas a diferentes niveles de organización (células, tejidos, órganos y todo el organismo) (WENDELAAR BONGA, 1997; ALFONSO et al., 2020), siendo que el grado y tiempo de exposición a dicho agente estresante puede afectar al organismo y su comportamiento en el corto, mediano y largo plazo, llegando a causar la muerte. Sin embargo, como lo indican Barreto et al. (2020) y Schrek y Tort (2016), el estrés puede ser dividido en “eustress” o estrés beneficioso y “distress” o estrés negativo; en el primer caso la exposición es útil para la sobrevivencia (permitiendo, por ejemplo, “el escape a predadores o la búsqueda de alimento”) en tanto en el segundo caso se experimenta como una condición negativa.

La respuesta al estrés tiene diferentes niveles que dependen del reconocimiento de la fuente de estrés y de la activación de rutas neuroendocrinas, afectando la fisiología de los organismos (CONDE-SIEIRA, 2018). La respuesta primaria es iniciada y controlada por dos sistemas hormonales, que llevan a la producción de corticoesteoroides (principalmente cortisol) y catecolaminas (como adrenalina, noradrenalina y dopamina) (FAUGHT et al., 2016). La secundaria altera la distribución de fuentes de energía y oxigena áreas del cuerpo, así como compromete el balance hidromineral, y las funciones respiratoria, cardiovascular e inmune (BARTON, 2002; SHRECK et al., 2016) e involucra órganos y tejidos como resultado de la acción neuroendocrina (BARTON E IWAMA, 1991). En la terciaria se involucra a todo el organismo viéndose reflejada en la reducción del crecimiento, la respuesta a las enfermedades y el comportamiento (ROTTMAN et al., 1992) y se caracteriza por la pérdida de la capacidad adaptativa que puede llevar al agotamiento y muerte del individuo (CRISCUOLO et al., 2020).

En un sistema de cultivo comercial los organismos están expuestos de forma repetitiva a diversos estresores a lo largo del ciclo productivo, algunos de los estresores pueden presentarse en forma simultánea incrementando el efecto, siendo difícil su identificación y medición de forma aislada (AFONSO, 2020). De esta manera las condiciones de confinamiento aunadas a las condiciones de estrés pueden debilitar el sistema inmunológico (YADA Y TORT, 20016) en los organismos en cultivo haciéndolos susceptibles a contraer enfermedades (ROTTMAN et al., 1992). Un problema intrínseco de los sistemas de producción intensivos es la rápida acumulación de residuos de alimentos, materia orgánica y compuestos nitrogenados (AVNIMELECH, 2006) generados por la alta densidad poblacional o biomasa de organismos en cultivo. Dado que la sangre se encuentra en contacto con todos los órganos y tejidos del cuerpo, realizando diferentes funciones, y es de fácil acceso, se emplea como un indicador de la salud en vertebrados (THEML et al., 2004). Los valores de referencia de los analitos en sangre son herramientas importantes en el diagnóstico de la condición de los individuos permitiendo identificar cambios del estado nutricional, calidad de agua o de la presencia de enfermedades (FAZIO, 2019) y permiten la toma rápida de decisiones clínicas por lo que han sido documentados para numerosas especies (WRIGHT et al., 2021) siendo necesaria su correcta interpretación dado que sus valores pueden ser influenciados por diversos factores. De esta manera la evaluación del crecimiento y los parámetros productivos aunado a la evaluación hematológica, permitirían identificar el grado de bienestar o estrés de los organismos cultivados en sistemas intensivos como el de biofloc.

1.2- Objetivos do trabalho (geral e específicos)

Objetivo general

Evaluar el uso de sistemas biofloc como alternativa para la mejora en el crecimiento, sobrevivencia y resistencia al estrés de post-larvas y alevinos de paco (*P. brachypomus*).

Objetivos específicos

1. Evaluar el crecimiento, los eritrocitos y las alteraciones branquiales de alevines de paco rojo (*Piaractus brachypomus*) expuestos de forma crónica a diferentes concentraciones de sólidos suspendidos totales en biofloc
2. Evaluar el efecto de la densidad poblacional sobre el crecimiento, la producción y los parámetros hematológicos de alevinos de paco rojo (*Piaractus brachypomus*) cultivados en sistemas biofloc como una alternativa de producción para mejorar la tolerancia al estrés.

1.3- Hipóteses

Los alevines de paco (*P. brachypomus*) presentan mayor crecimiento, sobrevivencia y resistencia al estrés durante su crianza en la etapa de alevinaje si son mantenidos en sistemas biofloc.

1- Artículo Científico 1: Growth, red blood cells and gill alterations of red pacu (Piaractus brachypomus) fingerlings by chronic exposure to different total suspended solids.

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Growth, red blood cells, and gill alterations of red pacu *(Piaractus brachypomus)* fingerlings by chronic exposure to different total suspended solids in biofloc

Beatriz Elena Angeles-Escobar[1] | Suzianny Maria Bezerra Cabral da Silva[2] | William Severi[2]

[1]Facultad de Pesquería, Universidad Nacional Agraria La Molina, Lima, Peru

[2]Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil

Correspondence
Beatriz Elena Angeles-Escobar, Facultad de Pesquería, Universidad Nacional Agraria La Molina, Avenida La Molina s/n, La Molina, P.O. 12-056, Lima, Peru.
Email: bangeles@lamolina.edu.pe

Abstract

Chronic exposure to high biofloc total suspended solids (TSS) could affect growth, red blood cells, and generate gill alterations in fish. Red pacu, *Piaractus brachypomus*, fry (2.7 ± 0.5 g weight) were exposed to different biofloc TSS concentrations with low (T1: 200–300), and medium (T2: 400–600 mg/L) levels and compared to a treatment control (TC) without solids during a 45-day period in 50 L experimental units (in quadruplicate). Water quality, productive parameters, red blood cell values, and gill histopathological alteration index (HAI) were assessed. Red pacu reached higher final biomass (11.34 ± 0.73 kg/m^3), better growth (DWG = 0.30 ± 0.03 g/day) and food conversion rate (1.05 ± 0.02) in TC than in biofloc treatments ($p < .05$). T2 had higher RBC count, and lower hemoglobin, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration ($p < .05$) than T1 and TC. The histological sections of TC gills showed monogenean parasites in low quantity; thus, the HAI value was similar among treatments and corresponded to low to medium gill damage. Growth, red blood cell values, and gill morphology of red pacu fingerlings were affected by moderate (400–600 mg/L) chronic exposure to TSS concentration in biofloc.

KEYWORDS
freshwater fish, gill histopathology, hematology, pirapitinga, white cachama

1 | INTRODUCTION

The red pacu, *Piaractus brachypomus* (Cuvier 1818), also known as pirapitinga, white cachama, or red-bellied pacu, is a native species of the Orinoco and Amazon basins, cultivated in South America and Asia with an annual production of 210 thousand tons in 2019 (Fisheries and Aquaculture Software, 2019), and is a promising aquaculture species for Peru. This characid grows at temperatures of 23 to 30°C (Vásquez-Torres, Pereira-Filho, & Arias-Castellanos, 2011), reproduces in the wild during the rainy season but requires hormonal induction in captivity (Arias & Vásquez, 1988; Barrera-Bailón, Caldas, & Hurtado-Giraldo, 2008), and has an omnivorous feeding habit (plankton, leaves, seeds, and fruits) (Vásquez & Zacarías, 1996) that may change depending on food availability (Correa, Betancur-, de Mérona, & Armbruster, 2014). This species has shown high tolerance to various growing systems and water quality conditions, including those of biofloc technology (BFT) with good productive results in the juvenile and growing stages (Brú-Cordero, Pertúz-Buelvas, Ayazo-Genes, Atencio-García, & Pardo-Carrasco, 2017; Chaverra, García, & Pardo, 2017; Poleo, Aranbarrio, Mendoza, & Romero, 2011).

BFT has been used by many researchers and producers for its potential benefits and applications to fish and crustaceans, because this technology promotes nitrogen uptake through the production of microbial proteins by the addition of carbohydrates to the ponds (Avnimelech, 1999; Crab, Defoirdt, Bossier, & Verstraete, 2012), thus increasing the number of heterotrophic bacteria, which have higher growth rate than nitrifying bacteria (Hargreaves, 2013). In this way, BFT allows the conversion of waste from the culture medium (mainly feces, noncon-sumed feed, and ammonia) into microbial biomass with proper management of the C/N ratio, which can be used byculture organisms as a food source. This technology improves the immunological system of organisms cultivated, while maintaining water quality parameters within the appropriate ranges for the species, reducing the need of water addition (Avnimelech, 1999; Bossier & Ekasari, 2017; Ekasari et al., 2015; Emerenciano, Cuzon, Goguenheim, Gaxiola,, & AQUACOP, 2013; Samocha et al., 2017).

Despite the proposed benefits of this technology, few species have been successfully cultivated in this type of system to date because "not all the species are candidates to BFT" (Emerenciano, Gaxiola, & Cuzon, 2013). Some biological characteristics of the organisms are required to enable acclimatization and tolerance to various stressors such as medium levels of dissolved oxygen (DO; Emerenciano, Gaxiola, & Cuzon, 2013) or high cultivation densities (Lima et al., 2019). They should also tolerate variations of nitrogenous compounds (Widanarni, Ekasari, &Maryam, 2012), settleable solid (SS) or total suspended solid (TSS) concentrations (Browdy, Ray, Leffler, & Avnimelech, 2012).

Hematological parameters such as hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration (MCHC) can be used to assess physiological condition in fry, juveniles, and adult fish, because they can be considered as highly reliable characteristics for the condition of blood quality (Centeno et al., 2007). Recent research showed that the hematological parameters are important indicators of fish health status (Fazio, 2019) and their variability depends on endogenous and exogenous factors (Ahmed, Reshi, & Fazio, 2020). These parameters can be used to determine the effects of stress reflecting the momentary physiological status of fishes (Sado, de Bicudo, & Cyrino, 2014). Also, this is the fastest way to identify and control stress situations or the presence of diseases in cul-tivation (Tavares-Dias & Moraes, 2003, 2006; Tavares-Dias, Sandrim, Moraes, & Carneiro, 2001; Tavares-Dias, Schalch, & Moraes, 2003). Red pacu, *P. brachypomus*, hematological parameters in semi-intensive production systems were reported by Garay and Paredes (2011) and Tocidlowsky, Lewbart, and Stoskopf (1997). Other hematological parameters as the morphology of red pacu blood cells (De Oliveira, Oliveira, & dos Santos Souto, 2018), blood chemistry values (Sakamoto, Lewbart, & Smith, 2001), and nonspecific immune response (Lochmann et al., 2009) have been reported.

In culture systems, morphological variations in gills can also be evaluated and used as early indicators of stress (Strzyżewska-Worotyńska, Szarek, Babińska, & Gulda, 2017). Chronic exposure of the gills to high concen- tration of suspended or settling solids can cause sublethal stress compromising fish health (Au et al., 2004), affecting gill morphology (Hatem, Abdelhay, Alayafi, & Suloma, 2013), and blood parameters (Schumann & Brinker, 2020; Witeska, 2013). The histopathological alteration index (HAI) can be used as a tool to assess the damage in gills caused by parasites or bad water quality and can enable a comparison between culture systems (Bernet, Schmidt, Meier, Burkhardt-Holm, & Wahli, 1999; Flores-Lopes & Thomaz, 2011; Strzyzewska, Szarek, & Babińska, 2016).

Reports indicate that *P. brachypomus* has a great tolerance to TSS concentration, enduring values above 500 mg/L in the fingerling stage (0.8–65.0 g) (Chaverra et al., 2017; Ueno-Fukura, Corredor-Ruiz, Jiménez-Ojeda, & Collazos-Lasso, 2019) and settling solids above 118 mL/L in fattening stage (30–200 g) (Brú-Cordero et al., 2017) without presenting high mortalities. The objective of this work was to determine if chronic exposure to low and moderate suspended solids on biofloc systems can also affect growth, hematologic values, or affect the functionality of the gills in the red pacu, *P. brachypomus*, at fingerling stage.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Fish fingerlings were exposed to different biofloc TSS concentrations with 200–300 (T1) and 400–600 mg/L (T2) and compared to a treatment control (TC) without solids for a 45-day period. Such concentration were chosen based on previous reports of Gaona, de Almeida, Viau, Poersch, and Wasielesky Jr (2017) who tested ranges of biofloc TSS concentrations of 100–300 (low) and 300–600 mg/L (medium) on shrimp, and Poli, Schveitzer, and de Nuñer (2015) who tested ranges of 0–200 (low) and 200–400 mg/L (medium) on *Rhamdia quelen* larvae. Each treatment consisted of four 50 L experimental units that drained into a mixing tank (30 L) containing a submerged pump (2,000 L/hr) which returned water to the experimental units, working as a recirculation system (Figure 1). This allowed for the regulation of suspended solids and the addition of sucrose (adjusted to C:N ratio of 15:1) and sodium bicarbonate for biofloc management. TC had additionally mechanical filtration media cleaned daily, with a 50 to 100% water change weekly. Every experimental unit had an adjustable heater (50 W) and constant aeration by diffuser stones; the mixing tank had an additional air diffuser to avoid anoxic areas. All units were filled with aged tap water before the introduction of the fish. Water was added to treatments with biofloc to compensate evaporation losses, routine measurements, and disposal of settling solids (around 2% daily).

2.2 | Water quality parameters

The temperature, DO concentration, and percent saturation were measured daily using a DO meter model YSI 550 A (Yellow Springs, OH), also pH was measured daily in the experimental units using a portable pH meter (Oakton pHtestr30). Electrical conductivity (EC) was measured once a week with a multiparameter model HACH HQ40d (Hach Company, CO). Total ammonia nitrogen, nitrite, and nitrate (NO_3-N) were assessed once a week using Hach methods 8155, 8507, and 8039, respectively, and were read in a spectrophotometer model HACH DR-3900 (Hach

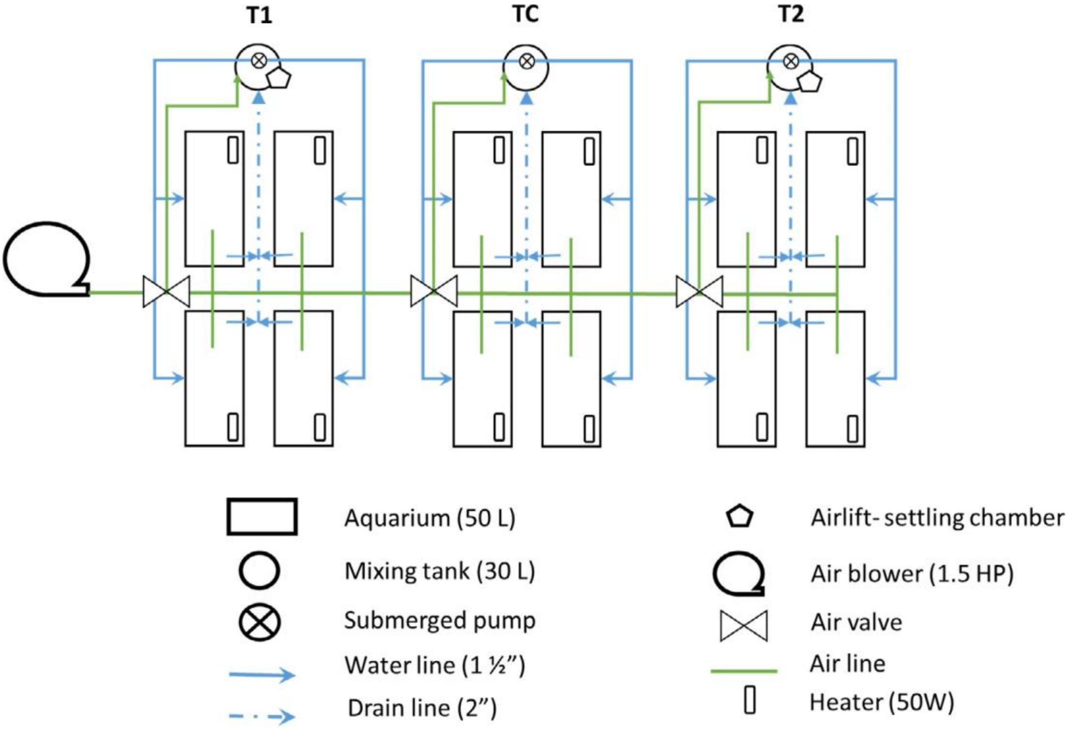

FIGURE 1 Schematic diagram of experimental treatment components and distribution for the evaluation of chronic exposure (45 days) of red pacu, *Piaractus brachypomus*, fingerlings to biofloc total suspended solids (TSS) of 200–300 (T1) and 400–600 mg/L (T2), and treatment control (TC)

Company). Alkalinity ($CaCO_3$) and TSS were assessed every 3 days, while SS volume, daily. These measurements were taken following the methodology of the Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

2.3 | Fish, feeding, and growth parameters

Red pacu, *P. brachypomus*, fingerlings (1–2 g) were obtained from a fry aquaculture center in Tarapoto (Genetika, San Martin, Peru) and transported by a commercial flight to Lima, adapted to aged tap water (temp. = 27 ± 1°C, pH = 8.4 ± 0.3, EC = 640 μS/cm) in 300-L tanks with aeration and laboratory photoperiod of 10 hr light:14 hr dark. They were fed ad libitum with commercial starter extruded feed (Aquatech tilapia, Lima, Peru) with 45% protein for 20 days before the beginning of the experiments.

Red pacu fingerlings (2.7 ± 0.5 g weight) were randomly distributed into 12 experimental units previously filled with tap water at a density of 40 fish per unit with a total of 160 fishes per treatment, as observed in Figure 1. All the fish were considered healthy on the basis of an external examination for any signs of abnormalities or infestation (Morey, 2019). Fish were fed with commercial tilapia starter extruded feed (Aquatech tilapia) with 42.72% protein and 6.19% fat, three times daily (8:00, 12:00, and 16:00) with a ratio of 4 to 8% of body weight to apparent satiation for 45 days. Sampling of 40 fish per treatment was performed weekly to adjust the feed ratio and to evaluate growth. All the fish were weighed at the beginning and at the end of the experiment for the evaluation of initial and final biomass, as well as, survival rates (SRs). Daily weight gain (DWG = weight gain by fish [g] / culture days), specific growth rate (SGR [%/day] = 100 × [ln final weight (g) — ln initial weight (g) / experimental period]), condition factor (K = 100 × [final weight / final length^3]), feed conversion ratio (FCR = feed intake [g] / weight gain [g]), and SR% = 100 × (final number of fish / initial number of fish). Feed intake was based on feed dry weight.

2.4 | Hematological parameters

During the experiment, desensitization and euthanasia procedures were followed according to the ethical protocols for animal use (Use of Fishes in Research Committee (Joint Committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists), 2014). At the end of the experiment, 12 fish per treatment were randomly chosen and anesthe- tized (75 mg/L Eugenol immersion bath) and blood samples were drawn from the caudal vein using a 25 5/8^{00} gauge micro syringe previously treated with 4% EDTA solution. The blood was immediately transferred to a test tube (Vacutainer™ tubes) with adjustment of the relation of anticoagulant and drawn blood volumes (Marín-Mendez, Torres-Cortes, Naranjo-Suarez, Chacón-Novoa, & Rondón−Barragan, 2012). Hematocrit (Hct; %) was determined by the microhematocrit method using capillary tubes with sodium heparin (80 IU/mL, Marienfeld, Lauda, Königshofen, Germany), and blood was centrifuged for 5 min at 12,000 g in a microcentrifuge model HC240 (BOECO Germany, Hamburg, Germany). Hemoglobin (Hb; g/dl) was determined by the cyanmethemoglobin method using a commercial kit (Valtec Diagnostics, Santiago de Chile, Chile), where 10 μL of blood was diluted in 2.5 mL of Drabkin solution for 3 min before a spectrophotometric reading at 540 nm. Hematocrit and hemoglobin were analyzed the same day of the collection, while red blood cell (RBC; $\times 10^6/\mu L$) count was performed the following day in a Neubauer chamber using a formaldehyde−citrate solution modified by Oliveira-Junior, Tavares-Dias, and Marcon (2008). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCHC were also calculated.

2.5 | Gill morphology evaluation

The second gill arch of the left side was extracted and placed in 4% buffered formalin (pH = 7.04). Gill samples were prepared for histologic analysis using a routine technique of dehydration in ethanol and then impregnated and embedded in paraffin. Sections of 3 μm were made with a microtome and stained with hematoxylin and eosin for general visualization of the affected tissues (Flores-Lopes & Thomaz, 2011).

The presence of histopathological alterations of the gills was semiquantitatively determined by the degree of tissue alteration (HAI) observed under a light microscope (Nikon Eclipse E200, Melville, NY) and classified into progressive states of tissue damage (Table 1), based on the severity of the lesions (Flores-Lopes & Thomaz, 2011; Strzyżewska-Worotyńska et al., 2017). The HAI was calculated for each fish with the formula HAI = 1 × DI + 10 × DII + 100 × DIII, where DI, DII, and DIII corresponded to the number of lesions classified as stages 1 to 3 degrees of severity, respectively, according to the score scale developed by Poleksic and Mitrovic- Tutundzic (1994).

2.6 | Statistical analysis

Homoscedasticity and normality of the data were assessed using the Fligner-Killeen test and Anderson−Darling test, respectively. A one-way analysis of variance (ANOVA) was subsequently performed and mean values were compared using the Tukey method. Parameters that did not meet the requirements for normality were evaluated using non-parametric Kruskal−Wallis test followed by a Mann−Whitney *U* test for mean comparison. All the data were evaluated using the R software version 4.0.0 (2020-04-24) (R Development Core Team, 2020) ($p < .05$).

TABLE 1 Classification of microscopic lesions in the gills of rainbow trout modified by Strzyżewska-Worotyńska et al. (2017) and used in the evaluation of gill alteration of red pacu, *Piaractus brachypomus*, exposed to 200–300 and 400–600 mg/L biofloc total suspended solids for 45 days. Degrees I, II, and III corresponded to the severity of the lesions

Groups of microscopic lesions and types of lesion	Degree
G1: Hypertrophy and hyperplasia of gill epithelia	
1. Hyperplasia of the gill filament epithelium	I
2. Hyperplasia of the lamellar epithelia	I
3. Decrease in interlamellar space	I
4. Epithelial lifting of gill filament epithelium	I
5. Epithelial lifting of lamellae	I
6. Intercellular edema	I
7. Incomplete fusion of several lamellae	I
8. Complete fusion of several lamellae	I
9. Rupture and peeling of gill filament epithelium	II
10. Rupture of the lamellar epithelium	II
G2: Changes in mucous and chloride cells	
1. Hypertrophy and hyperplasia of mucous cells	I
2. Empty mucous cells or their disappearance	I
3. Hypertrophy and hyperplasia of chloride cells	I
G3: Blood vessel changes	
1. Filament blood vessel enlargement	I
2. Lamellar telangiectasis	I
3. Hemorrhages with an epithelium rupture	II
4. Aneurysms	II
G4: Terminal stages	
Fibrosis	III
Necrosis	III

3 | RESULTS

3.1 | Water quality parameters

Water quality parameters are shown in Table 2 where pH, $CaCO_3$, SS, TSS, NO_3-N, and conductivity values were similar between biofloc treatments but differed significantly from TC ($p < .05$).

3.2 | Fish growth parameters

Red pacu fingerlings showed lower growth in biofloc than in TC treatment, with TC reaching the highest weight gain ($p < .05$) (Table 3). Initial biomass was 2.0 kg/m^3 in all treatments and had increments of 6.21, 7.29, and 9.27 kg/m^3 in T2, T1, and TC, respectively, with significant differences among treatments ($p < .05$). SR, SGR, and K were similar among treatments; however, DWG (0.30 ± 0.3 g/day) and FCR (1.05 ± 0.02) showed better growth and lower food conversion rate in TC with significant differences from T1 and T2 ($p < .05$).

TABLE 2 Water quality parameters of temperature, dissolved oxygen (DO) concentration, DO saturation, pH, alkalinity, settleable solids (SS), total suspended solids (TSS), total ammonia nitrogen (TAN), N-nitrite (NO_2-N), N-nitrate (NO_3-N), and electrical conductivity (EC) after 45 days of red pacu, *Piaractus brachypomus*, exposure to 200–300 (T1) and 400–600 mg/L (T2) biofloc TSS, and treatment control (TC) without solids

Parameters	Treatments			
	TC	T1	T2	*p* value
Temperature (°C)	26.01 ± 1.17	26.10 ± 1.00	25.95 ± 1.02	.7195
DO concentration (mg/L)	6.82 ± 0.38	6.76 ± 0.64	6.90 ± 0.64	.0726
DO saturation (%)	83.92 ± 3.69	82.66 ± 6.59	84.53 ± 6.26	.0744
pH	7.74 ± 0.23a	8.00 ± 0.15b	8.00 ± 0.13b	6.3×10^{-9}
Alkalinity (mg $CaCO_3$/L)	58.37 ± 21.10a	103.27 ± 22.23b	96.21 ± 20.60b	0.28×10^{-9}
SS (mL/L)	0.15 ± 0.19a	13.00 ± 8.35b	17.21 ± 11.43b	0.22×10^{-15}
TSS (mg/L)	7.20 ± 16.95a	278.50 ± 76.07b	363.3 ± 159.24b	0.36×10^{-6}
TAN (mg/L)	0.33 ± 0.24	0.25 ± 0.19	0.26 ± 0.26	.6960
NO_2-N (mg/L)	0.31 ± 0.21	3.33 ± 2.81	0.27 ± 0.16	.3094
NO_3–N (mg/L)	21.73 ± 12.36a	59.63 ± 27.77b	48.13 ± 22.84b	.0030
EC (μS/cm)	711.90 ± 82.61a	1,192.40 ± 312.32b	1,165.7 ± 281.04b	.0002

Notes: Values are expressed by mean ± *SD* (range) of four replicates. Different letters in the same line indicate significant difference between treatments ($p < .05$).

TABLE 3 Initial and final weight, initial and final biomass, survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), condition factor (K), and feed conversion rate (FCR) of red pacu, *Piaractus brachypomus*, exposure to 200–300 (T1) and 400–600 mg/L (T2) biofloc TSS, and treatment control (TC) without solids for 45 days

Parameters	Treatments			
	TC	T1	T2	*p* value
Initial weight (g)	2.73 ± 0.49	2.55 ± 0.36	2.80 ± 0.59	
Final weight (g)	16.33 ± 3.06a	14.30 ± 2.99b	13.13 ± 2.16b	4.67×10^{-6}
Initial biomass (kg/m^3)	2.07 ± 0.06	1.98 ± 0.07	2.06 ± 0.06	
Final biomass (kg/m^3)	11.34 ± 0.73a	9.27 ± 0.71b	8.27 ± 0.69b	.0097
SR (%)	93.13 ± 5.15	88.75 ± 8.54	81.25 ± 9.24	.1699
DWG (g/day)	0.30 ± 0.03a	0.26 ± 0.02ab	0.23 ± 0.02b	.0292
SGR (%/day)	3.97 ± 0.19	3.83 ± 0.13	3.44 ± 0.27	.0627
K	1.57 ± 0.03	1.65 ± 0.10	1.64 ± 0.08	.1672
FCR	1.05 ± 0.03a	1.17 ± 0.09a	1.36 ± 0.09b	.0125

Notes: Values are expressed by mean ± *SD* (range) of four replicates. Different letters in the same line indicate significant difference between treatments ($p < .05$).

3.3 | Hematological parameters

Most hematological parameters followed the same pattern, where TC had higher values and is different from T2 but not T1 (Table 4).

TABLE 4 Hematocrit (Hct), hemoglobin (Hb), red blood cell (RBC) count, mean corpuscular value (MCV), mean corpuscular hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) of red pacu, *Piaractus brachypomus*, exposed to 200–300 (T1) and 400–600 mg/L (T2) biofloc TSS, and control treatment (TC) without solids for 45 days

	Treatments			
Parameters	TC	T1	T2	*p* value
Hct (%)	39.00 ± 3.13	36.75 ± 4.60	37.17 ± 2.92	.1610
Hb (g/dL)	15.49 ± 1.76a	14.79 ± 1.52a	12.48 ± 1.67b	.0002
RBC (cell $\times 10^6$/μL)	2.16 ± 0.19a	2.36 ± 0.37ab	2.83 ± 0.80b	.0138
MCV (fl)	181.47 ± 14.72a	158.70 ± 26.32b	137.86 ± 31.51b	.0014
MCH (pg)	72.58 ± 12.58a	64.66 ± 14.60a	46.47 ± 12.5b	2×10^{-13}
MCHC (g/dL)	39.97 ± 5.79a	40.41 ± 4.89a	33.66 ± 4.57b	.0040

Notes: Values are expressed by mean ± *SD* (range) of 12 fish per treatment. As RBC count, MCV and MCH had a nonparametric statistical evaluation. Different letters in the same line indicate significant difference between treatments ($p < .05$).

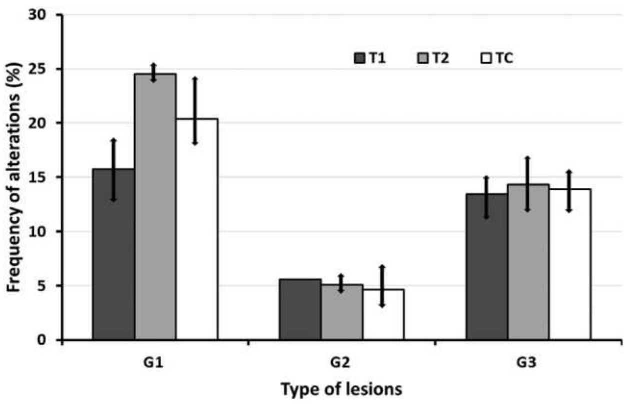

FIGURE 2 Type of gill alterations observed in red pacu, *Piaractus brachypomus*, fingerlings exposed for 45 days to 200–300 (T1) and 400–600 mg/L (T2) biofloc total suspended solids, and treatment control (TC) without solids. Type of lesions observed: G1, hypertrophy and hyperplasia of gill epithelia; G2, changes in mucous and chloride cells; G3, blood vessel changes

3.4 | Gill morphology evaluation

The main alterations observed in treatments were hyperplasia of gill filament epithelium, followed by blood vessel changes and mucous and chloride cell changes (Figure 2). Gill epithelial lesions observed in TC were hyperplasia of branchial filament, complete fusion of several lamellae, thickening of the blood vessels of filaments and lamellae, and the presence of intense bleeding in 58.3% (7/12) of the samples. Biofloc treatments showed mild to severe hyperpla-sia of the branchial epithelium, partial and complete fusion of several lamellae, thickening of the filament and lamellablood vessels (lamellar congestion), and in some cases hemorrhage and aneurysm formation (Figure 3).

The HAI values in the gills of red pacu fingerlings exposed to biofloc TSS are presented in Figure 4. HAI mean in T1 (11.50 ± 5.44) was lower than that in TC (HAI = 13.33 ± 7.36) and T2 (16.92 ± 9.54), but without significant differences ($p = .051$), and all corresponded to low to medium damage of the gills according to the scale proposed by Poleksic and Mitrovic-Tutundzic (1994).

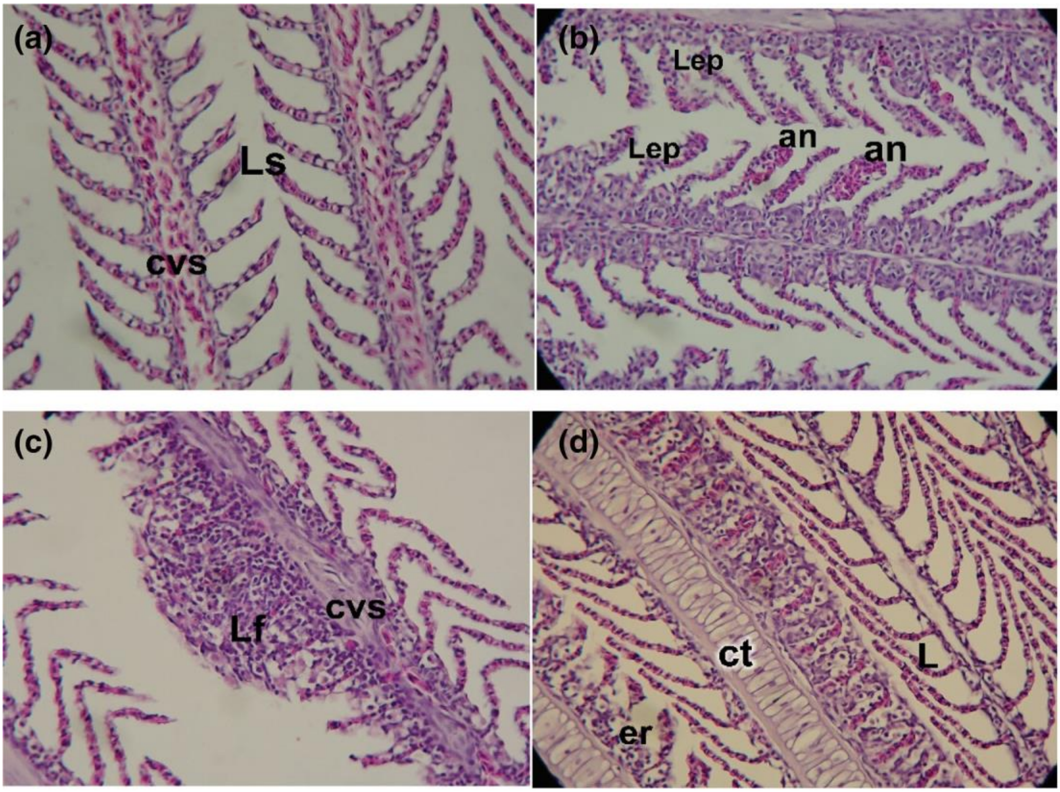

FIGURE 3 Morphological gill lesions in red pacu, *Piaractus brachypomus*, fingerlings cultivated in biofloc with total suspended solids for 45 days. (a) Lamellar congestion and shortening (Ls). (b) Aneurysms (an) in the branchial lamellae and proliferation of the lamellar epithelium (Lep). (c) Hyperplasia of epithelial tissue with partial fusion of lamellae (Lf). (d) Normal filament and lamella (L) and rupture of epithelium (er) in the lamella of another filament. Ct, cartilaginous tissue, cvs, central venous sinus. H-E staining (400×)

Elongated parasites were found in 83.3% (10/12 samples) of TC, 8.3% (1/12 samples) of T1, and none in T2. Parasites had low incidence per gill in TC (1–4 specimens) and only one in T1; additionally, they were observed in the middle part of the gill with hooks attached to the branchial lamellae, corresponding to the characteristics of monogenetic trematodes (Figure 5).

4 | DISCUSSION

4.1 | Water quality parameters

Most water quality parameters were kept within the appropriate range for the species and BFT treatments (Arias & Vásquez, 1988; Collazos-Lasso & Arias-Castellanos, 2015; Emerenciano, Gaxiola, & Cuzon, 2013) as shown in Table 2. Mean nitrite values in T1 (Table 2) was considered high, exceeding the recommended concentration for tropical freshwater species (0.5–2.5 ppm) (Boyd, 2014), although no mortality was observed in experimental units. Ochoa, Peña, and González (2002) showed the great tolerance of red pacu, *P. brachypomus*, fingerlings (mean weight of 5 g) to high concentrations of nitrites, evaluating its physiological response and survival at concentrations of 35, 50, and 65 ppm for 96 hr. At 35 ppm in a 96-hr-exposure mortality was 6.6%, methemoglobin formation was

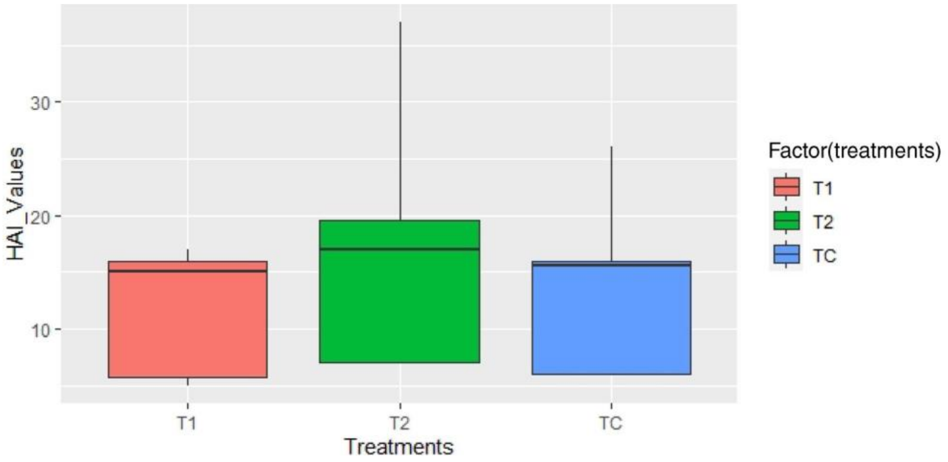

FIGURE 4 Gill histopathological alteration index (HAI) of red pacu, *Piaractus brachypomus*, fingerlings exposed to 200–300 (T1) and 400–600 mg/L (T2) biofloc total suspended solids and treatment control (TC) without solids for 45 days. Degree of gill HAI (0–10 = normal functioning; 11–20 = low damage, 21–50 = moderate damage), according to the scale proposed by Poleksic and Mitrovic-Tutundzic (1994)

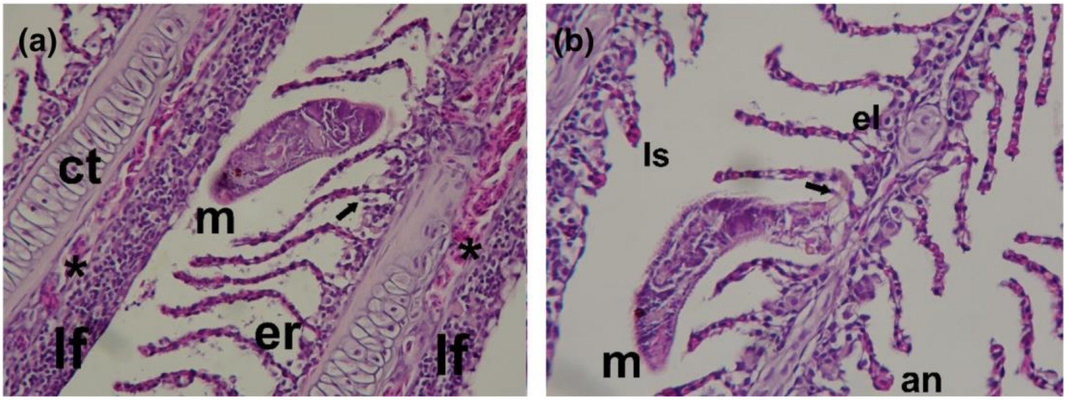

FIGURE 5 Gill histopathological changes in red pacu, *Piaractus brachypomus*, fingerlings in control treatment parasites with monogenean trematode (m). (a) Lamellar fusion (lf), lifting of the epithelium (arrow), and epithelial rupture (er). (b) Monogenean trematode (m) hooked on the lamella (arrow), aneurysms (an), and lamella shortening (ls) in the filament adjacent. ct, cartilage tissue, el, epithelial lifting, * arterial venous sinus. H-E staining (400×)

38.5 ± 24.2% and N-NO_2 accumulation in the liver and blood were 12.8 ± 10.8 and 32.8 ± 14.2 ppm, respectively, indicating that this species could activate methemoglobin reductase system in red blood cells at 72 hr, reducing the toxicity of nitrite exposure. However, exposure to nitrites could influence growth and the hematological characteristics of fish (Kroupová, Máchová, & Svobodová, 2005; Svobodová et al., 2005).

In BFT systems, bacterial flocs are formed from feces, uneaten food remains, and the development of bacteria that adhere to these surfaces in order to degrade the organic material present in the system (Collazos-Lasso & Arias-Castellanos, 2015; Hargreaves, 2013). The volume of solids, their composition, and rate of increase will depend on the quantity and quality of the food delivered (Crab et al., 2012), inorganic and organic carbon source (Ekasari, Crab, & Verstraete, 2010; Ueno-Fukura et al., 2019), and C/N ratio (Ebeling, Timmons, & Bisogni, 2006; Silva, Falcon, Pessôa, & Correia, 2017). Excess should be removed depending on the species' tolerance, since high suspended solids concentrations have been shown to have a negative effect on the culture, reducing oxygen availability (Avnimelech, 2012) and growth (Gaona et al., 2017), and affecting gill morphology to a lesser or greater extent depending on the type, concentration, and time of exposure to these solids (Hatem et al., 2013). Hargreaves (2013) mentioned that concentrations of 200 to 500 mg/L are appropriate for the proper function of biofloc system, without having a high bacterial oxygen demand in the tank, while Avnimelech (2012) recommended concentrations between 200 and 400 mg/L, and Hatem et al. (2013) recommend maximum values of 220 to 250 mg/L for tilapia culture in zero exchange systems, avoiding or reducing gill alteration. Red pacu showed great tolerance to solid concentration standing SS values of 56.6 ± 20.7 mL/L in juveniles (weights 33.5–182.6 g) (Brú-Cordero et al., 2017) and TSS between 384 and 645 mg/L in fingerling stage (weights 5.7–10.2 g) (Ueno-Fukura et al., 2019), with survivals between 84 and 99% in BFT systems. The results of the present experiment confirmed that red pacu fry can tolerate different levels of SS and TSS in biofloc systems, without presenting mortality in experimental units, which would indicate its ability to tolerate a range between 200 and 600 mg/L TSS in water, nevertheless they showed a better performance at levels of 200 to 300 mg/L TSS.

4.2 | Fish growth parameters

There are few experiences published of red pacu in BFT systems (Brú-Cordero et al., 2017; Chaverra et al., 2017; Poleo et al., 2011). The results showed a DWG of 0.27 ± 0.2 g/day (Ueno-Fukura et al., 2019) similar to our results in TC and T1 treatments for the same size range (5.65–10.17 g). Red pacu fingerlings and juveniles that reached 65, 183, and 450 g final weight had higher DWG with values of 0.4 ± 0.4, 1.2 ± 0.4, and 2.34 ± 0.05 g/day, and depended of the final weight (Brú-Cordero et al., 2017; Chaverra et al., 2017; Poleo et al., 2011). The final biomass reached in our treatments in 45 days was similar to those obtained by Brú-Cordero et al. (2017) and Poleo et al. (2011) (7.3 ± 2.0 and 12.96 ± 0.53 kg/m^3, respectively) in 4 to 6.5 months of growth in BFT systems. Survival was high (>80%) in all the treatments, with mortalities that could be attributed to jump of animals outside the experimental units, as reported by Poleo et al. (2011), confirming the high tolerance of red pacu to medium TSS (Ueno-Fukura et al., 2019). Food conversion rate in red pacu was around 1 to 1.5 showing high efficiency in food conversion in intensive culture systems, as indicated by Chaverra et al. (2017) and Brú-Cordero et al. (2017) in biofloc systems. However, the control treatment with clear-water presented lower food conversion efficiency, higher final biomass, and better growth performance than biofloc treatments. Similar results were obtained by Sgnaulin et al. (2018) for piracanjuba, *Brycon orbignyanus*, and Vinatea et al. (2018) for mullet, *Mugil cephalus*, where BFT sys- tem did not improve growth performance as compared to recirculation clear-water system.

4.3 | Hematological parameters

Red pacu, *P. brachypomus*, hemoglobin concentration and hematocrit were high in biofloc and control treatment compared to those reported for the same species under semi-intensive culture conditions (Garay & Paredes, 2011; Tocidlowsky et al., 1997) or for other freshwater fish on intensive culture condition, such as *Piaractus mesopotamicus* (Copatti, Baldisserotto, De Freitas Souza, & Garcia, 2019; Sado et al., 2014), *Labeo rohita* (Ahmad et al., 2019), or *Oreochromis niloticus* (Hisano, Barbosa, Hayd, & Mattioli, 2019) in fingerling stage. Similar, or even higher values, were reported for *Colossoma macropomum* (Tavares-Dias et al., 2001) and *P. mesopotamicus* (Klein et al., 2014) juveniles, and *Mugil cephalus*, *Odonthestes regia*, and *Sciaena deliciosa* adults (Sáez et al., 2018). As mentioned by Witeska (2013), under aquaculture conditions hematological parameters may be affected by the rearing system, stressors, or nutritional factors.

Red blood cell counts obtained for red pacu fingerlings in our experiment were within the range for culture species (1.0–4.0 × 10^6 cel/mm^3) (Dal'Bó et al., 2015; Tavares-Dias et al., 2007) and for *P. brachypomus* juveniles

(22–30 g of weight) maintained in recirculation systems (range, 0.98–2.95 × 10^6 cel/mm^3) (Tocidlowskyet al., 1997), but they were high for red pacu fingerlings (9.35 g) in semi-intensive systems (Garay & Paredes, 2011). High RBC counts could be attributed to feeding behavior, life style and adaptation to the habitat (Dal'Bó et al., 2015; Fazio, Marafioti, Arfuso, Piccione, & Faggio, 2013a), feed protein intake and culture system (Abduljabbar et al., 2015), salinity (Fazio, Marafioti, Arfuso, Piccione, & Faggio, 2013b; Parrino et al., 2019), or $CaCO_3$ levels (Copatti et al., 2019).

In our study, there was no sign of hematological alteration in control and biofloc (200–300 mg/L SST) treatment due low level of parasite presence. Similar results were found in *P. mesopotamicus*, hybrid tambacu, *Brycon amazonicus*, *Leporinus macrocephalus* (Tavares-Dias, Moraes, & Martins, 2008), *Schizodon borelli*, *Prochilodus lineatus* (Ranzani-Paiva, Silva-Souza, Pavanelli, & Takemoto, 2000), and *Mugil platanus* (Ranzani-Paiva & Tavares-Dias, 2002). The results of these authors suggest that the parasite and host were adapted to survival in these conditions, without significant loss to the host health.

In fish there is a relationship among hematocrit, hemoglobin, red blood cell concentration, and gas transport and oxygen exchange capacity at gill level. Tests of acute hypoxic stress in black cachama, *C. macropomum*, and the presence of parasites in gills resulted in a reduction of these values (Tavares-Dias et al., 2001), while exposure to substances such as nitrites in RAS systems (Kroupová et al., 2005), high concentrations of suspended solids (Schumann & Brinker, 2020; Svobodová et al., 2005), or inflammation (Dotta et al., 2011) can lead to an increase in hematocrit, hemoglobin concentration, or red blood cell count as a mean of fish compensation to maintain adequate oxygen transport. In 400 to 600 mg/L TSS biofloc treatment, hematological alterations, as reduction in hemoglobin concentration and cell volume or increase in red blood cell count, could be attributed to the exposition to medium TSS concentration that generated an inflammation response on the gill surface that may reduce the oxygen transport capacity.

4.4 | Gill morphology evaluation

Because gills are structures in direct and constant contact with water for the processes of respiration, osmoregulation, and excretion, they are the first to show the effects generated by inadequate environmental conditions or by the presence of pathogens (Negreiros & Tavares-Dias, 2019; Poleksic & Mitrovic-Tutundzic, 1994). The response to stressors in the water can be observed in gill morphological changes that depend on the level and type of exposure to the stressor (Kjelland, Woodley, Swannack, & Smith, 2015; Schumann & Brinker, 2020). Secondary stress responses in fish could increase blood circulation on gills, brain, muscles, and gill permeability, also increasing red blood cell count with higher oxygen affinity to increase tissue oxygenation (Urbinati, Zanuzzo, & Biller- Takahashi, 2014). Moreover, alteration of lamellar tissue can increase diffusion distance or mucus production and secretion, reducing xenobiotic absorption, although generating a reduction in respiratory gases and internal hypoxia(Fernandes & Moron, 2014). Studies carried out on the effect of settling solids in sublethal concentrations on estua-rine and marine fishes indicate effects at the hematological and histological level related to a reduction of the oxygendiffusion distance in lamellae (Au et al., 2004; Hess et al., 2017). At the hematological level, an increase in micro- hematocrit, Hb, and RBC count, and histologically, an increase in the number of mucous cells in the anterior marginof the branchial filament, thickening of the branchial lamellae, separation of the pillar cells from the epithelium, enlargement of the epithelial cells (forming a thick covering), and occasional rupture of pillar cells have been reported (Appleby & Scarratt, 1989; Au et al., 2004). Similar results were found by Hatem et al. (2013) with tilapia in biofloc systems, who mentioned that edema caused by the presence of suspended solids would reduce the interlamellar space, generating respiratory stress, with the consequent dilatation of the blood vessels (Strzyzewska et al., 2016).These responses are essential to facilitate gas exchange by shortening the diffusion distance and increasing the resi- dence time of blood on the respiratory surface. The mucous, produced by mucous cells, cover the skin, the gills andthe gastrointestinal tract and is involved in nonspecific immune defensive mechanisms, as it contains several antimicrobial and possibly antiparasitic compounds (Rubio-Godoy, 2010), giving protection against abrasive injuries by solid material suspended in water, pathogenic bacteria, and parasites (Dezfuli, Giari, Konecny, Jaeger, & Manera, 2003; Moron, de Andrade, & Fernandes, 2009; Moron, Matos, Ramos, & Gomes, 2018). The continuous presence of suspended solids in biofloc systems could activate the innate immune response in fish producing an inflammatory response in gill mucous cells increasing mucous production and reducing the presence of monogeneans in biofloc treatments.

5 | CONCLUSIONS

Red pacu, *P. brachypomus*, fingerlings can be cultivated in biofloc systems with a TSS concentration between 200 and 300 mg/L without visible health loss, at higher values (400–600 mg/L), growth is reduced, red blood cell values are altered, and gill morphology is affected. The continuous presence of suspended solids at low levels in biofloc systems could activate the innate nonspecific immune response in gills reducing the presence of parasites.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Beatriz Elena Angeles-Escobar is responsible for the conception, design, acquisition, analysis, and interpretation of data, also drafting of the manuscript. Suzianny Maria Bezerra Cabral da Silva and William Severi participated in the design and interpretation of data, also revised, and approved the manuscript.

ORCID

Beatriz Elena Angeles-Escobar https://orcid.org/0000-0003-0463-9349

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3- Artículo Científico 2. High tolerance to stocking density effects on growth, production and blood hematology of red pacu (*Piaractus brachypomus*) fingerlings reared in a biofloc (BFT) system

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High tolerance to stocking density effects on growth, production and blood hematology of red pacu (*Piaractus brachypomus*) fingerlings reared in a biofloc (BFT) system

Red pacu high stocking density in biofloc

Beatriz Elena Angeles Escobar[1]
Elsa Victoria Vega Galarza[1]

Suzianny Maria Bezerra Cabral da Silva[2]
William Severi[2]

1 Facultad de Pesquería, Universidad Nacional Agraria La Molina, Lima, Peru
2 Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil

Correspondence
Beatriz Elena Angeles Escobar,
Facultad de Pesquería,
Universidad Nacional Agraria La Molina,
Avenida La Molina s/n, La Molina,
P.O. 12-056, Lima, Peru.
Email: bangeles@lamolina.edu.pe

ABSTRACT

The aim of this work was to evaluate the tolerance of red pacu (*P. brachypomus*) fingerlings reared in a biofloc system to high stocking density on growth, production and blood hematology. Fingerlings (2.76 ± 0.74 g) were randomly distributed into four (50 L) experimental units per treatment in biofloc treatments, at densities of 400 (D400), 800 (D800) and 1600 (D1600) fish.m^{-3}, and 400 fish.m^{-3} in control treatment (recirculation system) They were fed with commercial feed (427 g.kg^{-1} protein) at 5% body weight for 50 days. Water quality, growth and productive parameters were evaluated and blood samples were extracted for a complete cell blood count. Stocking density had an important effect on water quality. Density treatments showed statistical differences ($p < 0.05$) in final weight, with the highest value on the biofloc treatment at the lowest density (D400 = 28.37± 6.78g), and the highest yield on the highest density (D1600 = 27.87 ± 1.40 kg.m^{-3}), while feed conversion ratio were kept between 0.97 (D400) and 1.3 (D1600). Higher values of red blood cell counts and hematocrit levels ($p < 0.05$) were found in biofloc treatments without significant differences for any other blood parameters examined. Red pacu demostrated good growth and high tolerance to stocking density in biofloc systems.

Keywords: High densities, growth parameters, hematology, intensive systems, white cachama.

1. INTRODUCTION

Red pacu (*Piaractus brachypomus*, Cuvier 1818), also known as pirapitinga or white cachama, is a native species of the Orinoco and Amazon basins, cultivated in South America and Asia with an annual production of 122 thousand tons in 2020 (FAO, 2023). The main producer in 2020 was China (59449 t), followed by Colombia (33073 t), Vietnam (23663 t), Peru (2165 t) and Brazil (1820 t) (FAO, 2023). Red pacu has high tolerance to a wide range of water quality and temperature (16.5 – 35°C), disease resistance and high feed conversion (Di Santo et al. 2018, Mesa-Granda & Botero-Aguirre 2007, Ochoa, Peña & González 2002, Vasquez-Torres & Arias-Castellanos 2012). In Peru this is an important specie with a 24.4% (529.6 t) yield increase between 2020 and 2021 and the third continental aquaculture specie after rainbow trout and tilapia (PRODUCE, 2023). The producers (1143 farms, 120.63 Ha total) are mainly farmers in the limited resource aquaculture (AREL) and micro and small business (AMyPE) groups with limited access to seeds (available only on the rainy season), funding or technological improvements, producing for self-consumption or local market (PRODUCE, 2021, FAO, 2013). Extensive and semi-intensive production systems are the most used for the farming of this specie, however, due to the importance of red pacu and black cachama (*Colossoma macropomum*) in the food security of Amazonic population, the peruvian government, through the National Program for Innovation in Fishing and Aquaculture (PNIPA), invested 6.7 million US$ dollars between 2017 and 2020 on projects to improve farming systems, feeding and nutrition, seed production, quality, health and production safety of these species (PNIPA, 2021). Among the best projects selected by the PNIPA for innovation, execution and projection to the community, there are three for the cultivation of red pacu and black cachama in biofloc systems (PNIPA, 2022). The main reasons for Amazonian farmers to cultivate red pacu in biofloc systems were the variation of environmental conditions, scarcity of water resources and low levels of productivity (PNIPA, 2022). Biofloc technology (BFT) reduces the use of water, optimizes the use of feed and space, allows to intensify production, and reduces the presence of pathogens. It has been used in the production of several aquaculture organisms in the early and growing stages, showing good results and improving larval growth, survival and resistance to various stressors and to some bacteria, increasing fry quality and production performance (Crab, Bossier, Verstraete & Defoirdt 2012, Ekasari et al. 2015, Ekasari et al. 2016, Lasso, Castellanos, Fukura, Carrasco & Arana 2021). Therefore, it can be used as an alternative to increase stocking density without affecting the well-being of the cultivated organisms in amazonic fishes. The aim of this work was to evaluate the tolerance of red pacu (*P. brachypomus*) fingerlings reared in a biofloc system to high stocking density on growth, production and blood hematology.

2. MATERIAL AND METHODS

2.1 Experimental design

Biofloc (adjusted to C:N ratio of 10:1) was prepared three weeks before the beginning of the experiment on a 500 L tank filled with 400 L aerated tap water, water temperature was kept at 26-28 °C with 300W heaters. Eighteen tilapia (*O. niloticus*) juveniles (68 g mean weight) were added to the tank (at 3.4 $kg.m^{-3}$ biomass) and fed 3% bodyweight daily with commercial food (35% protein). The day before the beginning of the experiment, biofloc was diluted with dechlorinated tap water on a 1:3 proportion (1200 L total), and added to the experimental units. Each biofloc treatment consisted of four 50 L experimental units that drained into a mixing tank (30 L) containing a submerged pump (2,000 L/h), which returned water to the experimental units, working as a recirculation system (See Angeles-Escobar et al., 2021). The mixing tank allowed for the regulation of suspended solids, the addition of sucrose (adjusted to C:N ratio of 10:1) (Samocha et al., 2017) and sodium bicarbonate (added interdayly to keep alkalinity over 100 $mg.L^{-1}$), for biofloc management. Every experimental unit had an adjustable heater (50 W) and constant aeration by diffuser stones; the mixing tank had an additional air diffuser to avoid anoxic areas. A recirculation system (RAS) with the same characteristics as the biofloc treatments (four 50 L experimental units) was used as control treatment, except for the mixing tank, which worked as a biofilter with mechanical and biological filtration. For mechanical filtration sinthethic fiber (perlon) was used (changed daily), while the biofilter received 3 kg of biofilter pellets (Micromec – JBL) and 10 m of one inch flexible corrugated pipe (cut in one inch pieces), previously activated for a month on another RAS system. RAS was filled with aged tap water before the introduction of the fish.

2.2 **Water quality parameters**

Temperature, pH, oxygen dissolved concentration (DO) and saturation were monitored daily in the experimental units while settling solids (SS) were monitored in the mixing tank. Alkalinity and total suspended solids (TSS), total ammoniacal nitrogen (TAN), nitrite ($N\text{- }NO_2$), nitrate ($N\text{- }NO_3$) and electrical conductivity (EC) were evaluated twice a week in the mixing tank.

Temperature, DO and saturation were measured using a DO meter (YSI Pro -Yellow Springs, OH, USA), pH was measured using a portable pH meter (Oakton pHtestr30) and electrical conductivity was measured with a multiparameter (HACH HQ40d, Hach Company, CO, USA). Alkalinity, SS and TSS were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Nitrogen species were measured using Hach Methods 8155, 8507, and 8039, respectively. Dilutions (1 / 10) were made as necessary due to some high nitrogen concentrations and samples were read in a spectrophotometer (HACH DR-3900, Hach Company, CO, USA) at 655 nm, 507 nm, and 500 nm for total ammonia nitrogen (TAN), nitrite ($N\text{-}NO_2$), and nitrate ($N\text{- }NO_3$), respectively.

2.3 **Fish, feeding, and growth parameters**

Red pacu (*Piaractus brachypomus*) fry (1.5 ± 0.08 g weight) were obtained from an aquaculture center in Tarapoto (Peru), transported by commercial flight to Lima (Peru), and acclimated to aged tap water (temp.= 27.0 ± 1°C, pH = 8.4 ± 0.3, E.C. = 640 $\mu S.cm^{-1}$, 12 h light:12 h dark photoperiod) in 300 L tanks, with constant aeration. They were fed (5% body weight daily) with commercial starter extruded feed (Aquatech tilapia, Lima, Peru), containing 427 $g.kg^{-1}$ protein and 62 $g.kg^{-1}$ fat, until the beginning of the experiments. After 28 days, they were randomly distributed into experimental units at densities of 400 (D400), 800 (D800) and 1600 (D1600) $fish.m^{-3}$ (1.1, 2.1 and 4.7 $kg.m^{-3}$ initial biomass), and in the control (400 $fish.m^{-3}$, 1.1 $kg.m^{-3}$ initial biomass). Fish were fed using the same commercial food and feeding ratio used previously, three times daily (8:00, 12:00, and 16:00) for 50 days. All fish were weighed at the beginning and end of the experiment with weekly sampling (25% of fish per treatment) for growth and productive parameters evaluation, also for feed adjustment. Daily weight gain (DWG = weight gain by fish (g)/ culture days), specific growth rate (SGR (%/day) = 100 × [ln final weight (g) − ln initial weight (g)/experimental period]), feed conversion ratio [FCR = feed intake (g) / weight gain (g)] and survival rate [SR% = 100 × (initial number of fish/final number of fish)] were calculated using initial and final evaluations. Feed intake was based on feed dry weight.

2.4 **Hematological parameters**

During the experiment desensitization procedures were followed according to the ethical protocols for animal use (Use of Fishes in Research Committee 2014). At the end of the experiment, 12 fish per treatment (Witeska, Kondera, Ługowska & Bojarski 2022) were randomly chosen (four per experimental unit), anesthetized (50 $mg.L^{-1}$ eugenol immersion bath), and blood samples (0.3 mL) were drawn from the caudal vein (Angeles-Escobar et al. 2021). Blood samples were kept cold (4 °C) and transferred to the Veterinary Clinical Pathology Laboratory (Universidad Nacional Mayor de San Marcos, Peru) within 2 hours of collection, for the evaluation of hematocrit (Hct, %), total red (RBC, $cell.10^6.\mu L^{-1}$) and white (WBC, cell. μL^{-1}) blood cell counts, and differential leukocyte counts (DLC, %) by laboratory routine methodology. RBC and WBC counts were performed by diluting the blood samples with Natt-Herricks solution, making 1:200 dilutions in Thomas pipettes and counting 5 cells in a 0.0025 mm^2 Neubauer chamber, while DLC count was evaluated in blood smears with Wright staining (Weiss & Wardrop 2010). Hemoglobin (Hb, $g.dL^{-1}$) was evaluated by cyanmethemoglobin method with a commercial kit (VALTEK, Chile). The hematimetric indices mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCMH) and mean cell volume (MCV) were calculated using the equations proposed by Wintrobe (1934).

2.5 Statistical Analysis

Homoscedasticity (Fligner Killen test) and normality (Anderson-Darling test) of the data were assessed. One-way variance analysis (ANOVA) was performed for productive and hematological parameters (Hb, Hc, WBC). Treatment means were compared using the Tukey method. Parameters that did not meet the requirements for normality as water quality parameters, RBC, hematimetric indices, and DLC, were evaluated using a non-parametric Kruskal-Wallis test followed by a Mann-Whitney *U* test for mean comparison. All the data were evaluated using R software version 4.0.0 (2020-04-24) (R Development Core Team, 2020) ($p < 0.05$).

3. Results

3.1 Water quality

Biofloc treatments showed significant differences in water quality parameters within densities and with control treatment (Table 1), indicating an important effect of stocking density on water quality. Dissolved oxygen, oxygen saturation, pH and alkalinity values had a significative reduction within densities, but were kept among those requiered by the specie and biofloc system. Nitrogen compounds, electric conductivity and total suspended solids increased in time and with density showing significative differences among biofloc treatments ($p < 0.05$) and with the control ($p < 0.05$).

3.2 Fish growth parameters

Stocking density treatments of red pacu showed statistical differences ($p < 0.01$) in final weight, with the highest value on the biofloc treatment with the lowest density (D400 = 28.37± 6.78g), and the highest yield on the highest density (D1600 = 27.87 ± 1.40 $kg.m^{-3}$). Similar survival rates values were found among treatments (97.5 – 98.75%) while FCR presented significant differences among them, eventhough all the FCR values were between 0.97 (D400) and 1.3 (D1600) showing the high tolerance and efficiency on food conversion in this specie (Table 2).

3.3 Hematological parameters

Red blood parameters showed statistical differences and higher values of RBC count (1.64 ± 0.20 $x10^{6} \cdot \mu L^{-1}$) and hematocrit levels (34.17 ± 2.12 %) in D400 treatment compared with control treatment (RBC = 1.48 ± 0.08 $x10^{6} \cdot \mu L^{-1}$, Hc = 31.67 ± 1.83 %). Even when RBC count and hematocrit levels in D800 and D1600 treatments were also higher values than control, no statistical differences were found, so red blood cell differences did not showed relation with stocking density (Fig. 1). White blood cell values and differential leukocyte count did not differ among treatments (Fig. 2).

4. Discussion

4.1 Water Quality

Due to the high temperature, oxygen consumption was increased for the processes of respiration and decomposition of organic matter, as well as the use of alkalinity sources (Ebeling et al, 2006), reducing the pH values in treatments with higher density (D800 and D1600). In the same way, the excretion was increased and the values of NAT and N-nitrite were higher in D800 and D1600 treatments in the last weeks, however, the addition of sucrose and sodium bicarbonate, as part of the management of the biofloc system, allowed to maintain the values among those suitable for the species, considering the high tolerance of red pacu to nitrite concentrations (Avnimelech 2012, Collazos and Arias, 2015, Collazos-Lasso, Gutiérrez-Espinosa & Restrepo-Betancur 2014, Emerenciano, Gaxiola & Cuzon 2013, Emerenciano et al. 2013, IIAP 2000, Ochoa et al. 2002). In the control (RAS system) there was an increase in NAT and N-nitrites in the last weeks of the experiment due to a reduction in oxygen supply in the mixing tank and the increase of fine solids because of a higher feeding, which affected the biofilter efficiency, problems identified by Emparanza (2009) for commercial RAS for salmonids in Chile. Similarly SS and SST values increased over time, but values to be kept in the appropiate ranges for the growth of the specie (Angeles-Escobar et al. 2021, Poli, Schveitzer & Nuñer 2015).

4.2 Fish growth parameters

High-stocking density in aquaculture has shown negative effects on fish performance and welfare (Refaey et al. 2018, Da Costa et al. 2018), due to organism exposure to a combination of handling and/or stressful environmental conditions that can occur, simultaneously, throughout the production cycle (Afonso 2020). Body reactions to stressor agents involve physiological responses at different levels of organization affecting growth and welfare (Wendelaar-Bonga 1997, Alfonso, Gesto & Sadoul 2020) which depends on the degree and time of exposure, the stage of development and the culture system (Pottinger 2008, Relić, Hristov, Vučinić, Poleksić & Marković 2010). Red pacu *(P. brachypomus)* has been reared at fry, juvenile and growth stage in BFT systems at medium (3.9 kg.m^{-3}) to high densities (12.96 ± 0.53 kg.m^{-3}), showing high daily weight gain (0.4 to 1.2 g.day^{-1}), specific growth rate (1.4 to 7.3 %.day^{-1}), and good feed conversion ratio (1.1 to 1.3) (Brú-Cordero et al. 2017; Chaverra et al. 2017, Poleo et al. 2011, Ueno-Fukura et al. 2019). Our results, even at the highest density (1600 fish.m^{-3}), not reported before for the species, showed good growth (DWG = 0.3±0.19, SGR= 3.63 ± 2.2), productive parameters (yield = 27.87 ± 1.40) and survival (97.5 ± 2.5 %), in addition, feed conversion ratio in all biofloc treatments (0.93 – 1.3) indicate high efficiency in food conversion, confirming the productive and commercial potencial of red pacu in different rearing systems (Mesa-Granda and Botero-Aguirre, 2007).

Sandoval-Vargas et al. (2020) compared the zootechnical performance and water quality of juveniles of *P. brachypomus* cultivated at population densities of 20 and 40 fish. m^{-3} in biofloc systems and with daily water change for 13 weeks, finding better growth in treatments with daily water change, but also a water consumption 24 to 48 times higher than in BFT systems. The authors concluded that the cultivation of red pacu can be carried out with daily water changes or in bft systems at a density of 40 fish. m^{-3}, however, the BFT system would be more suitable for environments with water limitations, pending determination of the optimal biomass per unit volume. Another important aspect of BFT systems, in addition to saving water, is the ability to grow fish in a safe environment by reducing the possibility of pathogens entering from natural water sources to the rearing system (Hargreaves 2013). However, it is necessary to estimate the cost of adding sources of organic and inorganic carbon, as well as energy expenditure to maintain aeration and water movement, and compare it as a function of increased productivity of traditional production systems.

4.3 Hematological parameters

Research done on the effects of high stocking densities on culture organisms indicate that there is a reduction in growth and production parameters (Machado-Neto, Nordi, Pontin, Pampolini & Moreti 2018, Rafatnezhad, Falahatkar & Tolouei 2008; Refaey et al. 2018), as well as an alteration of their hematological parameters, with increase in hematocrit, hemoglobin, red blood cell counts and in the number of immune cells (Da Costa et al. 2019, Machado-Neto et al. 2018, Poli et al., 2021, Yarahmadi et al. 2015). Variation in erythocite values could be related to environmental conditions (temperature and dissolved oxygen level), rearing systems, stressors or nutricional factors (Witeska, 2013). For example, tilapia cultivated in BFT systems had a reduction of hemoglobin, hematocrit and RBC count at the highest density (60 fish.m^{-3}, 50.47 ±0.5 g) (Zaki et al. 2020), while Poli et al. (2021) found, in the same specie, higher values of hematocrit and leukocytes at 266 fish.m^{-3} (9.64 ± 0.14 g). Meanwhile, Da Silva et al. (2022) did not found significant diferences in total or differential leukocyte counts in nile tilapia juveniles reared in biofloc systems with different stocking densities between 200 and 800 fish.m^{-3} for 44 days. However, variation in Ht and RBC counts on *P. brachypomus* did not present differences that could be related to temperature, dissolved oxygen level, nutritional factors or stocking densities, but between biofloc and control (RAS) systems. The main differentiating element of biofloc systems is the presence of bacterial flocs that carry out the process of incorporation of the excreted ammonium into bacterial biomass, reducing the toxicity of nitrogen compounds in the rearing system. However, even when TSS in biofloc treatments were kept within values recomended for the specie (<300 mg.L^{-1}), flocs presence could cause an inflammation response on the gill surface reducing the oxygen transport capacity, affecting hematological variables, as Pellegrin et al. (2021) found in pacu (*Piaractus mesopotamicus*) juvenils. Also, red pacu hematological profile, in biofloc treatments, was similar to that reported in semi-intensive and intensive production systems (Garay & Paredes 2011, De

Oliveira, Oliveira & dos Santos 2018), without showing alterations in the proportion or type of red and white cells, except for monocyte cells. This cells had higher values (7-12 % in BFT treatments, 10% in control RAS system) than those cultivated in semi-intensive (1.1 ±0.73%) and intensive (5.58%) systems (Garay & Paredes 2011, De Oliveira, Oliveira & dos Santos 2018). Monocytes have a nonspecific phagocytic and cytotoxic activity, being considered transit cells in the blood. During the inflammatory process they migrate to the connective tissue where they become macrophages, destroying the microorganisms (Urbinati, Zanuzzo, & Biller-Takahashi 2014). Monocyte increase in fish blood may be related to high densities and load of pathogens (monogenea) in gills (Da Costa et al. 2019) as well as to natural environments with high aquatic pollution (Correa et al. 2017). On the contrary, Vicente et al (2020) and Da Silva et al (2022) did not find differences in the hematological variables of the red and white line in relation to stocking densities of tilapia reared in a BFT and a chemoautotrophic system, respectively. Thus, keeping BFT systems in the adecuate water quality values for the species did not compromise fish health at different stocking densities.

In conclusion, this work has demonstrated the high tolerance of red pacu to grow at high stocking densities in biofloc systems. The use of this technology in the nursing stage would reduce the amount of water used, the possibility of contamination with pathogens and increase the productivity and availability of fingerlings of this species in tropical environments.

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Statements and Declarations

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Ethic statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the guidelines of the Use of Fishes in Research Committee (2014) had been followed.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The data have not been shared yet in anyform.

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Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Beatriz Angeles Escobar and Elsa Vega Galarza. The first draft of the manuscript was written by Beatriz Angeles Escobar and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ORCID

Beatriz Elena Angeles Escobar iD https://orcid.org/0000-0003-0463-9349
Elsa Victoria Vega Galarza iD https://orcid.org/0000-0002-4295-2339
Suzianny Maria Bezerra Cabral da Silva iD https://orcid.org/0000-0002-7571-3465
William Severi iD https://orcid.org/0000-0001-8873-5965

TABLES

Table 1. Water quality parameters (mean ± sd) in fish stocking-density experiments with *P. brachypomus* fingerlings cultivated for 50 days at three stocking densities (D400 = 400 fish.m^{-3}, D800 = 800 fish.m^{-3} and D1600 = 1600 fish.m^{-3}) in biofloc systems and control recirculation system (400 fish.m^{-3}). Significant differences ($p < 0.05$) among pairwise comparison are denoted by different letters.

Water quality parameters	**Treatments**				
	D400	**D800**	**D1600**	**Control**	P
Temperature (°C)	27.63 ± 0.76b	27.87 ± 0.66a	27.76 ± 0.75a	27.42 ± 0.49c	**<2.2x10^{-16}**
D.O. (mg.L^{-1})	6.99 ± 0.51a	6.67 ± 0.51b	6.45 ± 0.65c	6.99 ± 0.42a	**<2.2x10^{-16}**
Saturation (%)	88.81 ± 5.97a	84.93 ± 6.25b	81.81 ±7.30c	88.40 ± 5.22a	**<2.2x10^{-16}**
pH	7.74 ± 0.36a	7.53 ± 0.36b	7.40 ± 0.38c	7.73 ± 0.27a	**<2.2x10^{-16}**
SS (mL.L^{-1})	12.55 ± 16.83a	12.64 ± 10.35a	23.60 ±19.38b		**0.00198**
TSS (mg.L^{-1})	92.06 ± 81.78a	176.60 ±115.31b	232.98 ± 90.53b		**0.00629**
Alkalinity (mg CaCO3.L^{-1})	95.65 ± 10.78a	85.33 ± 16.75b	76.96 ±21.95b	94.11 ± 14.41a	**0.00013**
E.C. (µS.cm^{-1})	1100.13 ± 338.60a	1389.63 ± 593.42b	1589.85 ± 674.04b	1097.03 ± 286.69a	**0.00018**
TAN (mg.L^{-1})	0.85 ± 0.35a	1.28 ± 0.59b	1.92 ±1.10c	5.07 ± 5.62c	**0.00044**
N-NO2 (mg.L^{-1})	1.53 ± 4.31a	3.97 ±7.00ab	5.74 ±10.55ab	4.35 ± 2.50b	**0.00832**
N-NO3 (mg.L^{-1})	64.38 ± 34.92a	103.69 ± 47.08b	138.46 ± 65.25b	65.31 ± 40.92a	**0.00226**

Note: D.O.= dissolved oxygen concentration, SS= settleable solids, TSS = total suspended solids, E.C.= electric conductivity, TAN= total ammonia nitrogen, N-NO$_2$= N-nitrite, N-NO$_3$= N-nitrate

Table 2. Growth and productive parameters (mean ± sd) of red pacu (*P. brachypomus*) fingerlings cultivated at three stocking densities (D400 = 400 fish.m^{-3}, D800 = 800 fish.m^{-3} and D1600 = 1600 fish.m^{-3}) for 50 days in biofloc systems and control recirculation system (400 fish.m^{-3}). Significant differences ($p < 0.05$) among pairwise comparison are denoted by different letters.

Growth parameters	**Treatments**				**P**
	D400	**D800**	**D1600**	**Control**	
Initial weight (g)	2.75 ± 0.70a	2.72 ± 0.86a	2.84 ± 0.69a	2.75 ± 0.68a	0.783
Final weight (g)	28.37 ± 6.78a	21.85 ±6.02b	17.86 ±4.94c	27.67 ±5.64a	$2.2x10^{-16}$
Yield (kg.m^{-3})	11.21 ± 0.57c	17.15 ± 1.24b	27.87 ± 1.40a	10.93 ± 0.31c	$4x10^{-5}$
DWG (g.day^{-1})	0.49 ± 0.29a	0.41 ± 0.23a	0.30 ± 0.19a	0.49 ± 0.44a	0.505
SGR (%.day^{-1})	4.56 ±1.20a	4.24 ± 1.15a	3.63 ± 2.22a	4.14 ± 1.30a	0.764
FCR	0.93 ± 0.02a	1.10 ± 0.01c	1.30 ± 0.05d	1.00 ± 0.03b	$2.4x10^{-4}$
SR (%)	98.75 ± 2.50a	98.13 ± 1.25a	97.50 ± 2.50a	98.75 ± 2.50a	0.913

Note: DWG = daily weight gain, SGR= specific growth rate, FCR= feed conversion ratio, SR= survival rate.

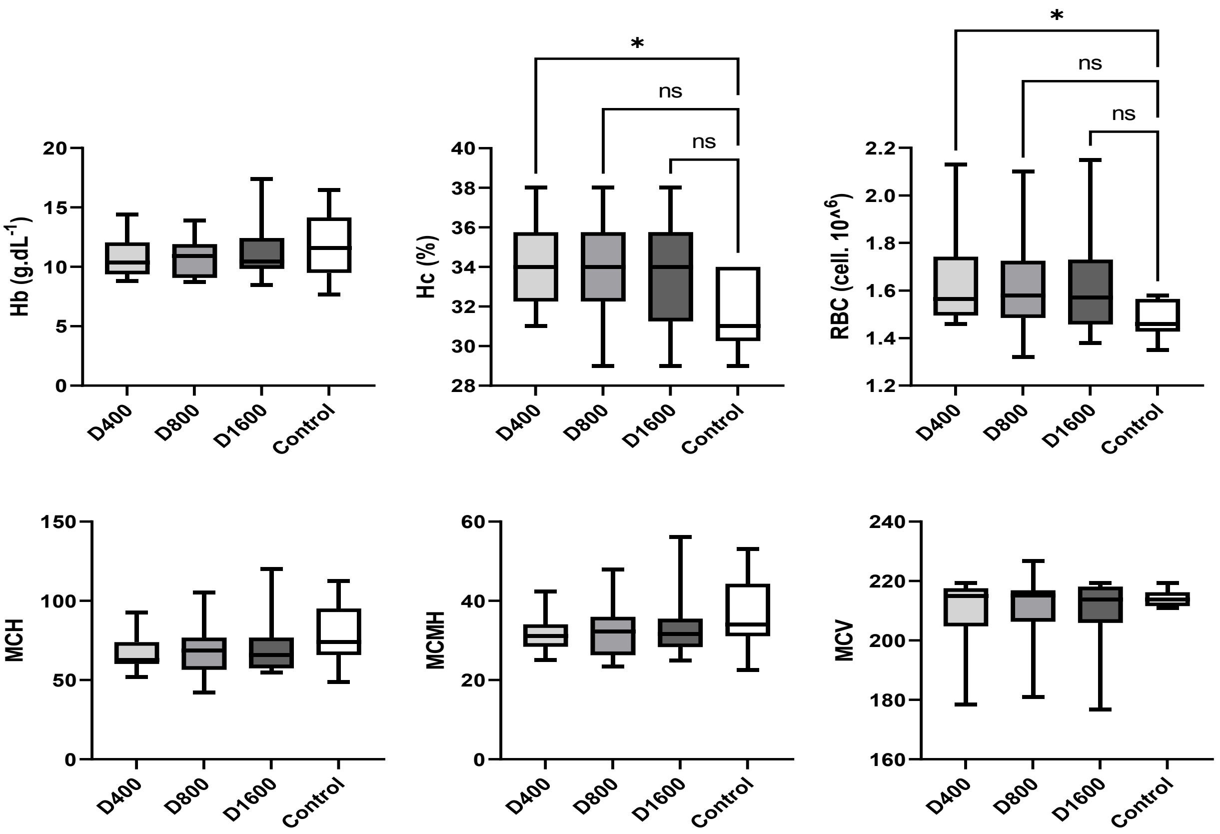

Fig. 1. Red blood cell evaluation of red pacu (*P. brachypomus*) fingerlings cultivated at three stocking densities (D400 = 400 fish.m^{-3}, D800 = 800 fish.m^{-3} and D1600 = 1600 fish.m^{-3}) for 50 days in biofloc systems and a recirculation system (control, 400 fish.m^{-3}). Significant differences ($p < 0.05$) among pairwise comparison are denoted by asterisk. Note: Hb= hemoglobin, Ht= hematocrit, RBC= red blood cells, MCH= mean corpuscular hemoglobin, MCMH= mean corpuscular hemoglobin concentration, MCV= mean cell volume.

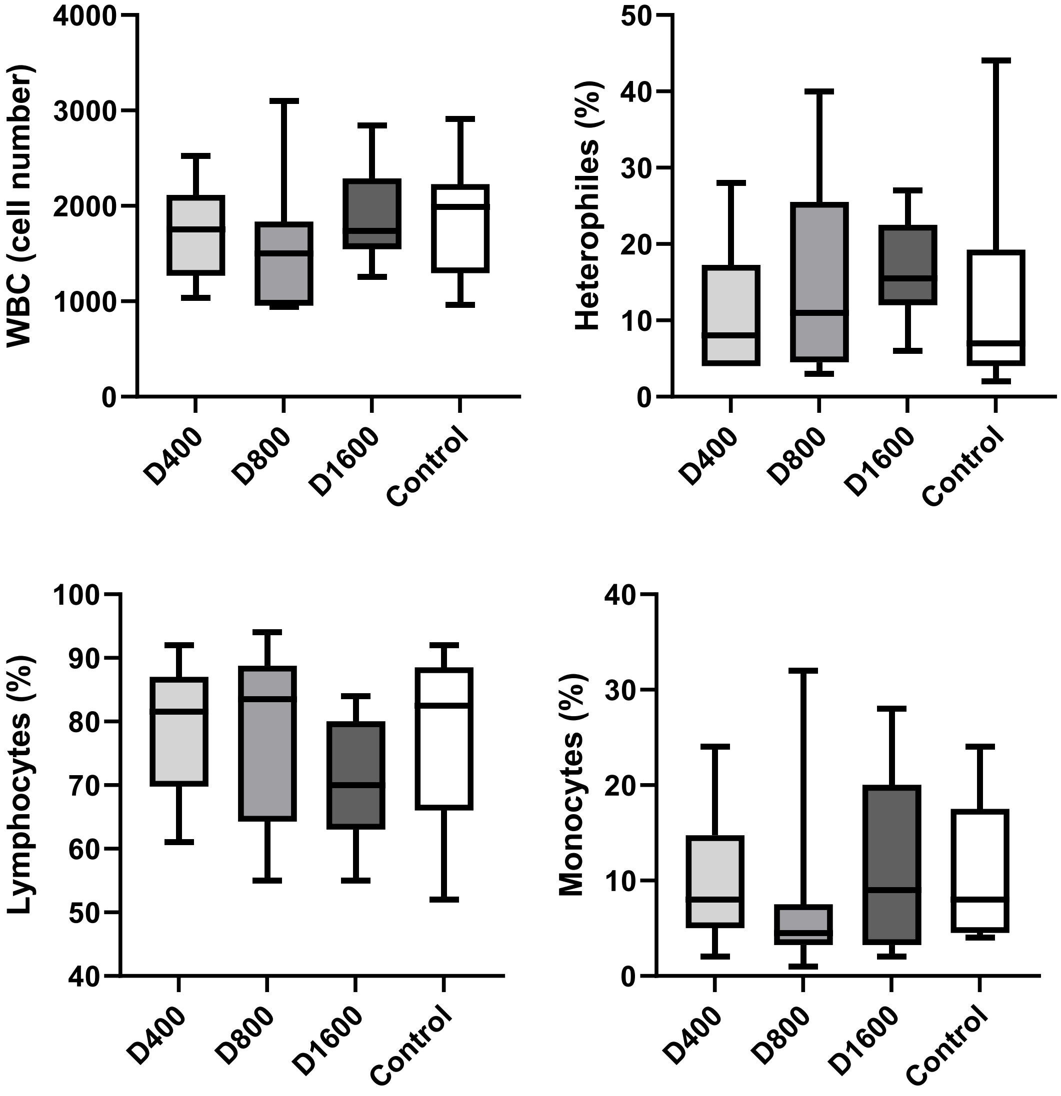

Fig. 2. White blood cell (WBC) and differential leukocyte count (%) of red pacu (*P. brachypomus*) fingerlings cultivated at three stocking densities (D400 = 400 fish.m^{-3}, D800 = 800 fish.m^{-3} and D1600 = 1600 fish.m^{-3}) for 50 days in biofloc systems and a recirculation system (control, 400 fish.m^{-3}).

4- Considerações finais

Los trabajos publicados sobre el cultivo de paco rojo (*P. brachypomus*) en sistemas biofloc muestran que es una especie resistente y capaz de adecuarse a este tipo de sistema presentando buen crecimiento, factor de conversión alimenticia y sobrevivencia. Sin embargo algunas características propias de los sistemas biofloc como las variaciones en la calidad del agua, la concentración de sólidos suspendidos totales o la densidad, pueden generar estrés en los peces cultivados, por lo que es necesario evaluar la respuesta de esta especie ante variaciones de dichos parámetros.

Los resultados obtenidos con las investigaciones de esta tesis muestran que esta especie puede ser cultivada en sistemas biofloc en concentraciones entre 200 y 300 mg.L^{-1} de sólidos suspendidos totales, sin afectar su salud, sin embargo, a concentraciones mayores (400 a 600 mg.L^{-1}) se reduce el crecimiento, se alteran los parámetros hematológicos y se afecta la morfología branquial. A pesar de lo mencionado, la presencia continua de los sólidos suspendidos en baja concentración en los sistemas biofloc pueden activar la respuesta inmune no específica en las branquias reduciendo la presencia de parásitos.

Por otra parte se ha demostrado que el paco rojo puede crecer en etapa de alevin hasta una densidad de 1600 peces.m^{-3}, estando sus parámetros de crecimiento entre los valores reportados por otros trabajos para esta especie en sistemas biofloc. En cuanto a los parámetros hematológicos evaluados se encontró que los valores en los sistemas biofloc eran similares a los reportados en otros sistemas semi-intensivos y extensivos, sin mostrar alteraciones en las proporciones o tipo de células blancas y rojas, indicando la alta tolerancia de esta especie a altas densidades en este tipo de sistema de cultivo.

Los resultados obtenidos en este trabajo pueden ser utilizados en la producción como valores límites de sólidos suspendidos totales y de densidad poblacional para el manejo y cultivo adecuado de esta especie durante la etapa de pre-cría en sistemas biofloc.

Si bien para determinar el estrés de esta especie en sistemas biofloc por periodos de 45-50 días, se han usado los parámetros hematológicos, por la facilidad de aplicación metodológica en centros de producción o laboratorios de patología animal, queda pendiente la evaluación de parámetros bioquímicos, inmunológicos así como de concentraciones de gases (oxígeno, dióxido de carbono) y electrolitos en sangre, permitiendo un reconocimiento más fino el grado del estrés de los organismos cultivados.

5- Referências

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