

# Productive and physiological behavior of the palometa *Mylossoma duriventre* farmed in a floating cage system

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**ABSTRACT.** The palometa, *Mylossoma duriventre*, is a species widely distributed in South America in the Amazon, Orinoco, and Paraguay-Paraná basins, with commercial and aquaculture potential. However, its hematology in culture systems has not been described. Therefore, the objective of this study was to evaluate the hematological parameters of the palometa farmed in a floating cage system to constitute a tool for assessing the physiological state, health, and presence of productive alterations that affect the species in a production system facing different storage densities and protein levels in the feed. The experimental design was based on three population density factors (50, 100, and 150 fish m<sup>-3</sup>) and three percentages of crude protein (25, 30, and 34%), thus constituting nine treatments (n=3). The fish were housed in 1m<sup>3</sup> floating cages, with an experimental period of 210 days. Productive, hematological, and parasitological parameters were evaluated. Standard deviation was performed, analyzed using Tukey's test and a principal component analysis (PCA) was performed. Ultimately, the treatment with 25% crude protein demonstrated greater final weight. For their part, the numbers of red blood cells, thrombocytes, and leukocytes were within the ranges shown in the literature, while hemoglobin and hematocrit were increased. This is the first report of productive and hematological responses to *M. duriventre* in captivity.

**Keywords:** pacu; white blood cells; parasites; fish; blood.

Received on June 30, 2024.  
 Accepted on September 06, 2024.

## Introduction

The success of intensive farms of different aquaculture species depends on several factors to reach their maximum productive potential. Population density plays an important role in these intensive systems, as many individuals can either promote growth or reduce it depending on the species (Salazar & Vázquez, 2017). Likewise, nutritional requirements vary by species depending on their natural diet before being adapted to a production system. The correct adaptation of animals to farming conditions is essential since the diet and nutritional status of fish determine their energy status and resistance to stress (Baradica, 2010). It is vital to know the status of the farmed population, a process that is known as health management, and understood as the prevention of the primary diseases affecting the population (Alvis, 2006). These diseases manifest themselves in fish through the appearance of behavioral abnormalities (signs) or physical damage (lesions), leading to a reduction in yield and often to the death of the affected specimens, which are economic losses (Kinkelin et al., 1991). In order to try to avoid these losses, it is necessary to study the possible pathologies present in fish with due anticipation; for this purpose, it is appropriate to develop a permanent monitoring of individuals, a situation that is achieved through an accurate and timely hematological diagnosis (Rozas, 2020). Blood is par excellence the point of confluence of abnormal situations caused by different pathologies. Therefore, it is possible to anticipate the clinical manifestations of diseases through the permanent monitoring of the physiological, nutritional, and sanitary state of fish, both in inland and marine waters (Conroy & Armas de Conroy, 1987).

Fish coexist or are colonized by numerous pathogens without causing diseases. This situation is established by a balance between the resistance of the host (fish) and the virulence of the pathogen (harmfulness), a condition that is broken when there are stress factors that are sufficiently important to

cause the fish to become ill (Balbuena, 2011; Maria, 2009). Stress of the first degree, or acute stress, is indicated by the presence of cortisol in the blood, whereas stress of the second degree adds up hematological changes that reduce the functionality of the immune system, and stress of the third degree, or chronic stress, involves changes in resistance to disease (Barandica & Tort, 2008; Barandica, 2010).

The palometa *Mylossoma duriventre* (Cuvier, 1817) is a species widely distributed in South America, in the Amazon, Orinoco, and Paraguay-Paraná basins, inhabiting aquatic environments in Argentina, Bolivia, Brazil, Ecuador, Paraguay, Peru, Colombia, and Venezuela. It is mainly herbivorous and feeds on seeds, fruits, plant remains, and insect larvae (García et al., 2016). The palometa varies in size, with a minimum standard length of 15 cm and a maximum total length of 31 cm in the Colombian Orinoquia, according to the Instituto Nacional de Pesca y Acuicultura (INPA, 2001). It is one of the important species of scaly fish in consumer fishing in the Colombian Amazon and Orinoquia (Lasso et al., 2011). However, it is caught at a lower mean catch size (MCL) than that allowed in the Orinoquia region, which threatens the sustainability of the population and could lead to a collapse of the fisheries (Organisation for Economic Co-operation and Development [OECD], 2016).

Floating cage aquaculture is an alternative to obtaining fish from wild fisheries and using available water resources for fish production. It is presented as the most suitable production method to minimize the use of land and water resources and to provide large quantities of food to the population in record time (Aïzonou et al., 2021). Aquaculture presents several farming systems, among which floating cages, land, and concrete ponds stand out, with fish farming in floating cages being characterized as closed enclosures with holes through which water flows freely and with the ability to float or be suspended in the water (Bocek, 2020). These growth units can be mobile or semi-mobile and can be installed in large reservoirs, lakes, lagoons, and dams (Useche et al., 2001).

As the hematic profile is a necessary monitoring parameter for the health of aquaculture production, and has been little described in this species, with only two reports from populations in natural environments in the Amazon River, in Brazil (Chamy et al., 2015; Oliveira et al., 2021), these studies conclude that the results obtained can be used for comparison in future studies of this species in other environments. Nevertheless, studies should be carried out to better understand its blood profile, and establish reference values for *M. duriventre*, thus contributing to the development of the fisheries sector in the region. Therefore, the objective of this study was to evaluate the hematological parameters of the palometa farmed in a floating cage system.

## Material and methods

### Study population and setting

The trial was carried out in the La Venturosa lagoon (4°06'08.2" N and 72°57'2.1" W), located in the municipality of Puerto López, Meta. This area is characterized by an average temperature of 27.1°C, with a minimum of 22°C at dawn and a maximum of 35°C, and a total annual precipitation of 2133 mm. The fingerlings were obtained by artificial reproduction in the La Bohemia fish farm, in the municipality of Acacias, Meta, Colombia.

### Experimental design

A completely randomized experimental design was proposed, evaluating two factors: a first stocking density factor (50, 100, and 150 fish m<sup>3</sup>) and a second factor involving the percent inclusion of crude protein (CP) (25, 30, and 34%), forming a 3X3 factorial design, constituting nine treatments, with three replicates, and a total of 27 experimental units (1m<sup>3</sup> floating cages) (Table 1). The feed was commercial and had different percentages of protein. All fish were fed twice a day (8 am and 4 pm) *ad libitum*. The trial had a duration of 210 days.

**Table 1.** Experimental design of palometa *Mylossoma duriventre*, farmed in a floating cage system for 210 days, with three replicates per treatment (n=3), constituting 27 experimental units.

|   |    | Crude protein (CP%)           |    |                               |    |                               |
|---|----|-------------------------------|----|-------------------------------|----|-------------------------------|
| Stocking density<br>Fish m <sup>3</sup> | T1 | 50 fish m <sup>3</sup> - 30%  | T2 | 50 fish m <sup>3</sup> - 34%  | T3 | 50 fish m <sup>3</sup> - 25%  |
|   | T4 | 100 fish m <sup>3</sup> - 30% | T5 | 100 fish m <sup>3</sup> - 34% | T6 | 100 fish m <sup>3</sup> - 25% |
|   | T7 | 150 fish m <sup>3</sup> - 30% | T8 | 150 fish m <sup>3</sup> - 34% | T9 | 150 fish m <sup>3</sup> - 25% |

### Productive performance

Biometric assessments were made every 30 days. A sample of 20% of the total planting density was taken from each experimental unit. Variables such as total length (cm) and standard length (cm) were recorded to the nearest millimeter using an ichthyometer. Similarly, weight (g) was recorded using a scale. At the end of the experimental period, the following growth parameters were determined:

Final weight (FW) (g)

Standard length (SL) (cm)

Final total length (TL) (cm)

Weight gain (WG) = final body weight (g) – initial body weight (g)

### Physiological blood analysis

Individuals (n=5 per replicate) were anesthetized according to the technique proposed by Chen et al. (2014) and Wilson et al. (2009), using the method of hypothermia with ice slush (ice and water admixture) at a temperature of approximately 4°C. Individuals were subjected to this method for less than 30 seconds or until the loss of swimming axis was verified (Vargas, 2017). A 1 mL syringe was used to venipuncture the caudal vein, inserting the needle 1 cm below the lateral midline until the vertebral bodies were located. Vacutainer tubes of 0.5 mL with heparin anticoagulant were used for storage; samples were acclimatized for approximately 3 minutes and then cooled in a container with gel packs at a temperature between 4-8°C for transport to the laboratory (Rozas, 2020).

Two blood smears were performed per individual and stained with Giemsa-Wright stain for cell differentiation (Retamales & Manzo, 2017). They were evaluated under a Zeiss™ LED Primo Star optical microscope to assess the presence of blood parasites, total thrombocytes count, total leukocyte count (WBC), morphology, and cell differentiation according to the cited literature (Correa et al., 2009; Rozas, 2020). Total leukocyte count was performed in 10 random observation fields (40X), according to the methodology of Salgado and Ramirez (2017). Differential counts (%) of thrombocytes and leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, and PAS-positive granular leukocytes (PAS-GL)) were performed at 100X with oil immersion in the spread in zigzag (Ranzani et al., 2013; Witeska et al., 2022).

Hematocrit was determined using microhematocrit tubes (12.000 rpm, 5 minutes). Hemoglobin was assessed using the cyanmethemoglobin method with Drabkin's solution, and the total erythrocyte count (RBC) was measured by diluting the blood in physiological saline solution at a ratio of 1:200 (10 µL in 2 mL) and counted using a Neubauer (Copete-Sierra, 2013; Rozas, 2020). Red cell indices (MCV, MCH, and MCHC) were calculated and evaluated according to Wintrobe (1990).

### Parasitological analysis

Mucus, gill, and gut samples were collected and preserved individually in 4% formalin, and all samples were evaluated under a stereoscope (Conroy & Armas de Conroy, 1987; Morey, 2019). Parasite identification was performed using the second edition of the book "Aquatic Biodiversity in Latin America - Amazon Fish Parasites" (Thatcher, 2006). The quantitative analysis of the parasites was carried out through the determination of prevalence and average intensity (Hidalgo & García, 2018).

### Statistical analyses

All data were shown as mean ± standard deviation (SD). To evaluate the influence of the variables stocking density and % of crude protein (CP) on the hematological variables, 2-way ANOVA and the Tukey Test were performed. Principal component analysis (PCA) was performed to verify the influence between each parameter studied. In all cases, a value of  $P < 0.05$  was used as the statistical criterion to reveal significant differences. All statistical procedures were performed in the GraphPad Prism 9® (GraphPad Software, 2021) and R version 4.2.1 software (R Core Team, 2022).

### Ethical statements

Law No. 611 of 2000, "by which standards are dictated for the sustainable management of species of Wild and Aquatic Fauna," and Decree No. 309 of 2000, "by which scientific research on biological diversity is

regulated” were considered. The bioethics committee of the General Directorate of Research of the University of Los Llanos-Colombia endorsed the combined project for the analysis of the sustainability of Limited Resource Aquaculture - AREL in Puerto Lopez, Meta-Colombia. Approval was recorded in minute No. 7 of September 16, 2020.

## Results

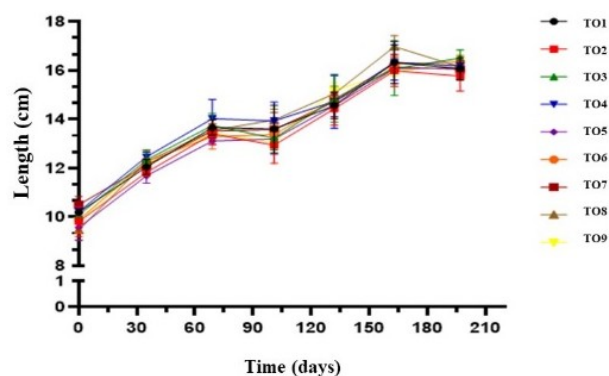
### Productive performance

In the present study, T3 (50 fish m<sup>-3</sup>- 25%CP) showed the highest value in total length of 16.0 ± 1.5 cm, while T2 (50 fish m<sup>-3</sup> - 34%CP) presented a lower length of 15.7 ± 1.4 cm, showing significant differences ( $p < 0.05$ ) with T3 and T6. However, the values were similar for the other treatments (Table 2). Similarly, the highest final weight of 113.4 ± 9.4 g was observed for T3 and the lowest final weight of 101.5 ± 17 g for T2, with no significant differences ( $p < 0.05$ ). Survival was over 96% in all cases, reflecting a favorable adaptation to the culture conditions. Figures 1 and 2 show the behavior of the length and total weight of palometa during the culture period, showing exponential growth in response to the density and percentage of protein in the diet.

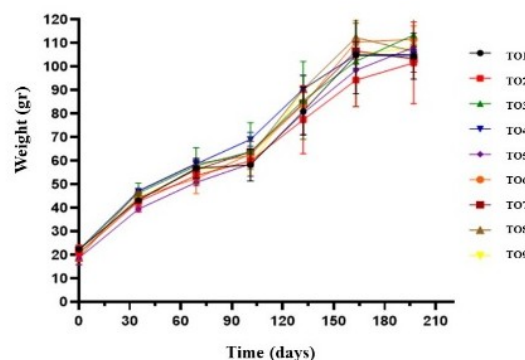
**Table 2.** Productive performance of palometa *Mylossoma duriventre*, farmed in a floating cage system for 210 days. Data are expressed as mean ± standard deviation (SD).

| Productive parameter      | Treatments             |                       |                        |                        |                        |                        |                        |                        |                       |
|---------------------------|------------------------|-----------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-----------------------|
|                           | T1                     | T2                    | T3                     | T4                     | T5                     | T6                     | T7                     | T8                     | T9                    |
| Stocking density          | 50                     | 50                    | 50                     | 100                    | 100                    | 100                    | 150                    | 150                    | 150                   |
| Crude protein (%)         | 30                     | 34                    | 25                     | 30                     | 34                     | 25                     | 30                     | 34                     | 25                    |
| Initial weight (g)        | 22.33±1.9              | 20.83±1.5             | 21.99±1.7              | 22.26±1.6              | 18.70±1.8              | 20.72±1.6              | 22.25±1.5              | 18.84±1.6              | 20.45±1.6             |
| Initial total length (cm) | 10.19±0.5              | 9.81±0.5              | 10.08±0.4              | 10.22±0.3              | 9.57±0.4               | 9.88±0.4               | 10.49±0.4              | 9.46±0.4               | 9.83±0.4              |
| Final weight (g)          | 104.4±9.8 <sup>a</sup> | 101.5±17 <sup>a</sup> | 113.4±9.4 <sup>a</sup> | 105.2±2.0 <sup>a</sup> | 108.1±1.4 <sup>a</sup> | 111.5±5.7 <sup>a</sup> | 103.1±5.5 <sup>a</sup> | 106.4±1.8 <sup>a</sup> | 108±2.7 <sup>a</sup>  |
| Total final length (cm)   | 16.0±1.5 <sup>ab</sup> | 15.7±1.4 <sup>a</sup> | 16.4±1.5 <sup>b</sup>  | 16.1±1.7 <sup>ab</sup> | 16.1±1.6 <sup>ab</sup> | 16.3±1.7 <sup>b</sup>  | 16.0±1.6 <sup>ab</sup> | 16.2±1.7 <sup>ab</sup> | 16.3±1.6 <sup>b</sup> |
| Total weight gain (g)     | 82±10.1 <sup>a</sup>   | 81±20.4 <sup>a</sup>  | 91±9.1 <sup>a</sup>    | 82.9±2.0 <sup>a</sup>  | 89±3.7 <sup>a</sup>    | 90.8±9.7 <sup>a</sup>  | 80.9±5.6 <sup>a</sup>  | 87.6±4.7 <sup>a</sup>  | 87.6±2.8 <sup>a</sup> |
| Daily weight gain (g)     | 0.5±0.1 <sup>a</sup>   | 0.44±0.1 <sup>a</sup> | 0.5±0.1 <sup>a</sup>   | 0.45±0.2 <sup>a</sup>  | 0.55±0.1 <sup>a</sup>  | 0.5±0.2 <sup>a</sup>   | 0.4±0.1 <sup>a</sup>   | 0.45±0.2 <sup>a</sup>  | 0.45±0.1 <sup>a</sup> |
| Final biomass (g)         | 5.221                  | 5.080                 | 5.496                  | 10.524                 | 10.813                 | 11.155                 | 15.478                 | 15.973                 | 16.208                |
| Survival (%)              | 100.0                  | 98.0                  | 98.0                   | 96.0                   | 99.0                   | 100.0                  | 99.3                   | 98.0                   | 96.6                  |

<sup>a,b</sup> Different letters between rows show statistically significant differences ( $p < 0.05$ ).



**Figure 1.** Total length (cm) of palometa *Mylossoma duriventre*, farmed in a floating cage system for 210 days.



**Figure 2.** Final weight (g) of palometa *Mylossoma duriventre*, farmed in a floating cage system for 210 days.

### Water quality variables

The pH was maintained at  $5.72 \pm 0.70$ , temperature was  $29.21 \pm 0.83$  °C, conductivity was  $11 \pm 1.48$   $\mu\text{S cm}^{-1}$ , transparency remained greater than 30 cm, solids dissolved at  $6 \pm 0.52$  ppm and the oxygen dissolved mean was  $2.3 \pm 1.11$   $\text{mg L}^{-1}$  throughout the trial.

### Physiological blood analysis

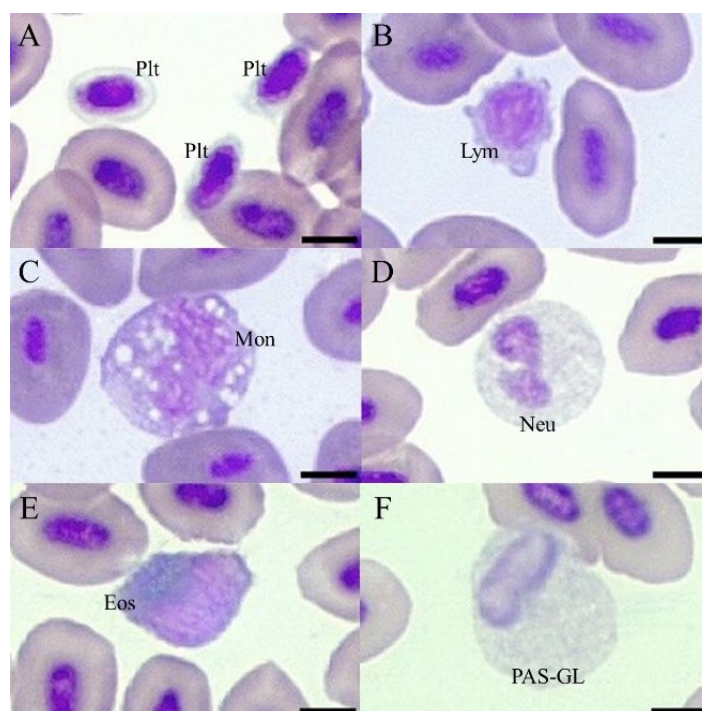
As observed, T8 (150 fish  $\text{m}^3$  - 34%CP) and T6 (100 fish  $\text{m}^3$  - 25%CP) showed the highest and lowest RBC,  $1.73 \pm 0.81 \times 10^6 \mu\text{L}^{-1}$  and  $0.78 \pm 0.52 \times 10^6 \mu\text{L}^{-1}$ , respectively. Furthermore, there was a significant difference ( $p > 0.05$ ) between T8 and T9 (150 fish  $\text{m}^3$  - 25%CP)  $0.99 \pm 0.58 \times 10^6 \mu\text{L}^{-1}$  (Table 3). Hematocrit (Hct) was the erythrocyte parameter with the lowest coefficient of variation (CV) in *M. duriventre*, whereas mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and RBC showed the highest CV (Table 3).

**Table 3.** Erythrocyte parameters of palometa *Mylossoma duriventre*, farmed in a floating cage system for 210 days. Data are expressed as mean  $\pm$  standard deviation (SD).

| Variables                              | Treatments        |                   |                   |                  |                    |                   |                    |                    |                   | CV (%) |
|--|-------------------|-------------------|-------------------|------------------|--------------------|-------------------|--------------------|--------------------|-------------------|--------|
|  | T1                | T2                | T3                | T4               | T5                 | T6                | T7                 | T8                 | T9                |        |
| RBC ( $\times 10^6 \mu\text{L}^{-1}$ ) | 1.2 $\pm$ 0.6     | 1.3 $\pm$ 0.6     | 0.9 $\pm$ 0.4     | 0.9 $\pm$ 0.6    | 1.1 $\pm$ 0.5      | 0.7 $\pm$ 0.5     | 1.1 $\pm$ 0.8      | 1.7 $\pm$ 0.8*     | 0.9 $\pm$ 0.5*    | 24.6   |
| Hb (g $\text{dL}^{-1}$ )               | 19.5 $\pm$ 5.5    | 15.7 $\pm$ 1.5    | 16.2 $\pm$ 2.2    | 17.1 $\pm$ 4.1   | 16.7 $\pm$ 1.9     | 16.5 $\pm$ 2.3    | 17.0 $\pm$ 3.0     | 17.2 $\pm$ 2.3     | 17.0 $\pm$ 2.1    | 5.2    |
| Hm (%)                                 | 54.0 $\pm$ 6.6    | 52.1 $\pm$ 9.0    | 54.1 $\pm$ 8.4    | 59.2 $\pm$ 9.9   | 55.6 $\pm$ 6.6     | 54.0 $\pm$ 7.5    | 60.0 $\pm$ 8.3     | 54.5 $\pm$ 7.1     | 60.9 $\pm$ 8.0    | 6.1    |
| MCV (fL)                               | 641.4 $\pm$ 610.8 | 571.0 $\pm$ 595.5 | 886.7 $\pm$ 771.6 | 2130 $\pm$ 4211  | 612.7 $\pm$ 308.4* | 1025 $\pm$ 669.4* | 891.8 $\pm$ 811.6* | 430.9 $\pm$ 308.3* | 913.2 $\pm$ 695.1 | 55.6   |
| MCH (pg)                               | 226.9 $\pm$ 188.8 | 179.8 $\pm$ 139.5 | 263.3 $\pm$ 263.4 | 816.4 $\pm$ 1765 | 173.1 $\pm$ 86.11  | 320.9 $\pm$ 223.8 | 248.3 $\pm$ 225.9  | 134.9 $\pm$ 100.8  | 251.4 $\pm$ 172.9 | 70.2   |
| MCHC (g $\text{dL}^{-1}$ )             | 36.7 $\pm$ 12.0   | 30.9 $\pm$ 6.0    | 30.3 $\pm$ 4.8    | 29.5 $\pm$ 6.9   | 30.6 $\pm$ 5.7     | 30.9 $\pm$ 4.1    | 28.5 $\pm$ 4.2     | 31.9 $\pm$ 4.4*    | 28.3 $\pm$ 4.7*   | 8.0    |

RBC: red blood cell count; Hb: hemoglobin; Hm: Hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; CV: coefficient of variation; \* significant differences ( $p < 0.05$ ).

In *M. duriventre* blood smears, erythrocytes, thrombocytes, lymphocytes, monocytes, neutrophils, eosinophils, and PAS-GL were identified (Figure 3). As a result of this investigation, thrombocytes, leukocytes, and absolute lymphocytes ( $\mu\text{L}$ ) were considered to be the cells with the lowest CV, while absolute eosinophils had the highest CV. For white blood cells, thrombocytes had the highest presentation ( $22.398 \pm 5.650 \mu\text{L}$ ). On the other hand, lymphocytes were the most frequent leukocytes ( $12.183 \pm 2.220 \mu\text{L}$ ), while eosinophils had the lowest absolute frequency ( $68 \mu\text{L}$ ) (Table 4).



**Figure 3.** Blood cells of *Mylossoma duriventre*, farmed in a floating cage system for 210 days. Cells stained by the Giemsa-Wright staining method. a. thrombocytes (Plt), b. lymphocyte (Lym), c. monocyte (Mon), d. neutrophil (Neu), e. eosinophil (Eos), and f. PAS-positive granular leukocyte (PAS-GL). 100X objective. Scale bar: 5.0  $\mu\text{m}$ .

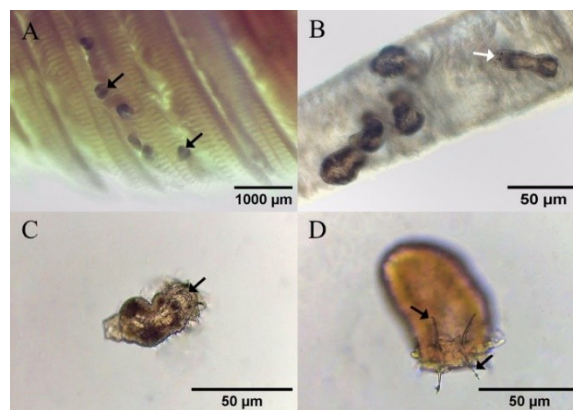
**Table 4.** Thrombocytes and leukogram of palometa *Mylossoma duriventre*, farmed in a floating cage system for 210 days. Data are expressed as mean  $\pm$  standard deviation (SD).

| Blood Cell Group                                | Treatments     |                |                |                |                |                |                 |                 |                | CV (%) |
|---|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|-----------------|----------------|--------|
|   | T1             | T2             | T3             | T4             | T5             | T6             | T7              | T8              | T9             |        |
| Thrombocytes ( $\times 10^3 \mu\text{L}^{-1}$ ) | 18.0 $\pm$ 3.5 | 19.2 $\pm$ 6.6 | 17.6 $\pm$ 4.7 | 20.8 $\pm$ 5.2 | 20.6 $\pm$ 4.2 | 18.0 $\pm$ 4.7 | 22.3 $\pm$ 5.6  | 21.1 $\pm$ 2.9  | 19.4 $\pm$ 6.4 | 8.3    |
| Leukocytes ( $\times 10^3 \mu\text{L}^{-1}$ )   | 10.2 $\pm$ 3.1 | 10.0 $\pm$ 3.9 | 10.1 $\pm$ 3.2 | 10.9 $\pm$ 2.3 | 10.3 $\pm$ 2.1 | 8.4 $\pm$ 2.2* | 12.4 $\pm$ 2.1  | 10.3 $\pm$ 2.3* | 10.6 $\pm$ 3.0 | 10.0   |
| Lymphocytes ( $\times 10^3 \mu\text{L}^{-1}$ )  | 10.0 $\pm$ 3.0 | 9.7 $\pm$ 3.9  | 9.7 $\pm$ 3.0  | 10.7 $\pm$ 2.2 | 9.9 $\pm$ 2.0* | 7.8 $\pm$ 1.9  | 12.1 $\pm$ 2.2* | 10.0 $\pm$ 2.2  | 10.5 $\pm$ 3.0 | 11.0   |
| Monocytes ( $\mu\text{L}$ )                     | 116 $\pm$ 128  | 128 $\pm$ 82   | 152 $\pm$ 115  | 146 $\pm$ 124  | 117 $\pm$ 66   | 116 $\pm$ 76   | 126 $\pm$ 57    | 79 $\pm$ 55     | 109 $\pm$ 81   | 17.5   |
| Neutrophils ( $\mu\text{L}$ )                   | 203 $\pm$ 143  | 177 $\pm$ 81   | 298 $\pm$ 212  | 198 $\pm$ 131  | 319 $\pm$ 192  | 153 $\pm$ 105  | 239 $\pm$ 77    | 238 $\pm$ 188   | 296 $\pm$ 182  | 24.7   |
| Eosinophils ( $\mu\text{L}$ )                   | 68.0 $\phi$    | 0.0            | 0.0            | 0.0            | 0.0            | 0.0            | 54.0            | 0.0             | 0.0            | 200.1  |
| PAS-GL ( $\mu\text{L}$ )                        | 0.0            | 85.0           | 75.0           | 0.0            | 0.0            | 0.0            | 0.0             | 0.0             | 0.0            | 198.9  |

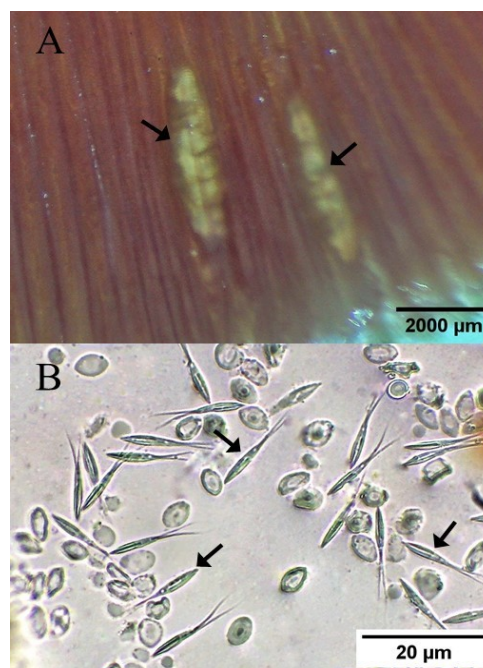
CV: coefficient of variation; \* significant differences ( $p < 0.05$ ).

### Parasitological analysis

*M. duriventre* individuals presented three types of parasites located only in gill tissue for the different treatments. With a prevalence greater than 60% in all treatments, parasites of the genus *Apedunculata* sp. (Monogenea class) were found (Figure 4). Similarly, cysts with spores of parasites of the genus *Henneguya* sp. (family Myxosporidae) were found, with a prevalence of more than 26% in all treatments (Figure 5).



**Figure 4.** Parasitism in the palometa *Mylossoma duriventre*, by monogeneans of the genus *Apedunculata* sp. A. view of monogeneans (black arrows) on the lamellae of a gill arch (stereoscope); B. monogeneans in gill lamella, view of a pair of ocelli (white arrows) in the Prohaptor of a parasite (10X); C. view of the ventral bar (black arrow) in the Haptor of the parasite (10X); D. view of some hooks (black arrows) on the haptor of the parasite (20X).



**Figure 5.** Parasitism in the palometa *Mylossoma duriventre*, by myxosporidian cysts of the genus *Henneguya* sp. A. cysts of *Henneguya* sp. (black arrows) in the lamellae of a gill arch (Stereoscope); B. mature bi-prolonged myxospores (orange arrows) from a ruptured cyst of *Henneguya* sp. in gills (40X).

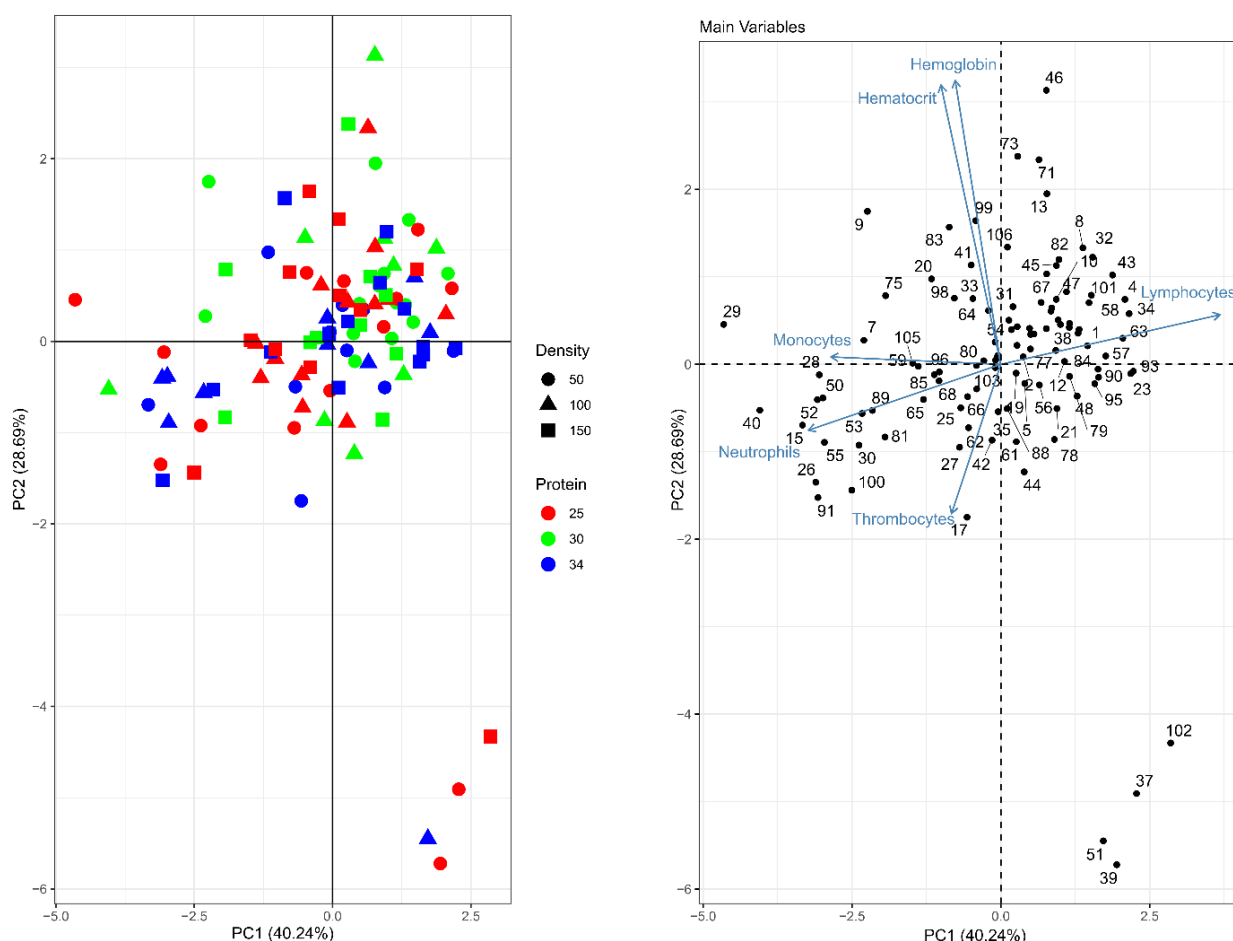
In treatment 9, only one individual was found parasitized on the gills by an unidentified parasite. The parasite *Apedunculata* sp. presented the highest mean abundance per host at T3 (50 fish m<sup>-3</sup> - 25%CP), and *Henneguya* sp. cysts presented the highest mean abundance per host at T4 (100 fish m<sup>-3</sup> - 30%CP) (Table 5).

**Table 5.** Prevalence and mean abundance per host of gill protozoan parasites in palometa *Mylossoma duriventre* farmed in floating cage systems in La Venturosa Lagoon, Puerto López, Meta, Colombia.

| Taxon/Parasite                              | Treatments |          |           |        |          |          |          |        |          |
|---|------------|----------|-----------|--------|----------|----------|----------|--------|----------|
|   | T1         | T2       | T3        | T4     | T5       | T6       | T7       | T8     | T9       |
| Myxosporea/<br><i>Henneguya</i> sp. (cysts) | 40/0.5     | 26.7/0.6 | 40/0.7    | 80/3.7 | 26.7/0.7 | 33.3/0.7 | 33.3/0.5 | 40/1.1 | 46.7/1.1 |
| Monogenea/<br><i>Apedunculata</i> sp.       | 93.3/5.9   | 60/5.0   | 86.7/10.8 | 60/4.5 | 93.3/7.9 | 66.7/5.2 | 73.3/7.3 | 80/6.2 | 80/8.9   |
| Unidentified                                | 0          | 0        | 0         | 0      | 0        | 0        | 0        | 0      | 6.7/0.1  |

Data presented in % prevalence and mean abundance per host.

The PCA did not show the effect of CP% (25, 30, and 34%) on the erythrogram, leukogram, and thrombocytes studied in *M. duriventre* individuals of the present study. The colors and shapes representing the crude protein and seeding density variables respectively (Figure 6), are in disorder resulting in a significantly low estimate to derive a correlation.



**Figure 6.** Principal Component Analysis (PCA), crude protein and seeding density on hematological, and productive parameters.

## Discussion

The farming of fish in floating cages allows a better use of space and the growth of individuals (Beveridge, 1986). During approximately 210 days of farming in floating cages, palometa individuals reached a weight of more than 100 g and a total weight gain of more than 80 g. According to the results, the factor of rearing density did not influence growth, while the diet with a CP content of 25% showed the best results at the end of the study.

The effect of rearing density on productive performance was similar to the study on the white cachama, *Piaractus brachypomus*, in floating cages, which showed no significant differences between densities of 20, 40, and 60 fish m<sup>3</sup> (Reátegui et al., 2018). However, in another similar study, the white cachama, *Piaractus orinoquensis*, was farmed in floating cages at densities of 150, 200, and 250 fish m<sup>3</sup>, with the highest growth at a density of 250 fish m<sup>3</sup> (Cuan et al., 2021). It is important to note that the present study is the first report on the rearing of *M. duriventre* species in floating cage systems.

Our results suggest that the palometa, as a gregarious species, would increase its growth or productive yield when confined in a cage with a high population density due to the adaptation to a minimum displacement. This provides energy saving and an increase in water circulation given the massive movement of the fins, with a consequent improvement in water quality, mainly in terms of dissolved oxygen content (Salazar & Vásquez, 2017).

The diet based on 25% CP showed the highest productive performance due to the omnivorous tendency of *M. duriventre* (Santos, 2009). Therefore, the best diets for this species are those with a lower protein content than those of carnivorous species. In addition, the productivity of the rearing site must be taken into account so that the species can feed on phytoplankton, zooplankton, and seeds from the same area (Tratado de Cooperacion Amazonica, 1999).

Water quality variables were in the acceptable range for fish survival and growth; however, dissolved oxygen concentrations ( $2.3 \pm 1.11$  mg L<sup>-1</sup>) were below the limit considered necessary for good productivity. On the other hand, the preferred and most productive dissolved oxygen is between 3 and 8 mg L<sup>-1</sup> for adult fish according to the Food and Agriculture Organization (FAO, 2016). It should be noted that this experiment was carried out in the La Venturosa lagoon located in the municipality of Puerto López, Meta, which is characterized by its low mobility and oxygen exchange by air currents and rain (Cuan et al., 2021). In the present study, a survival rate higher than 96% was observed for all treatments, confirming the adaptation of the species to low oxygen culture conditions, as fish can show an adaptive response with a hypometabolic state (Naya et al., 2021). However, aerators are recommended, as they aim to meet oxygen demands and obtain better production rates and health conditions for the fish.

The blood of teleosts is made up of three major groups of cells: erythrocytes, leukocytes, and thrombocytes (Tavares, 2006). Erythrocytes are the most abundant cells in the blood circulation of fish, and their main function is to transport oxygen and gaseous carbon dioxide through tissue hemoglobin. This internal protein of the erythrocyte, together with the hematocrit, is mainly used to identify anemic processes and/or respiratory disorders that may affect the metabolism of animals (Ranzani et al., 2013).

Reports of physiological responses at the blood level in fish native to Colombia are scarce, although they are very useful for physiological understanding and diagnosis of populations in aquaculture or natural systems (Arias et al., 2003). RBC values of *M. duriventre* in the present study were lower than those described by Chamy et al. (2015) ( $2.21 \pm 0.47 \times 10^6$  µL<sup>-1</sup>). However, they are close to those reported by Fries et al. (2016) for the pacu *Piaractus mesopotamicus* ( $1.99 \pm 0.33 \times 10^6$  µL<sup>-1</sup>), as well as to those observed in the tambaqui *Colossoma macropomum* in the study by Minaya (2018), with similar values for RBC ( $0.96 \pm 0.13 \times 10^6$  µL<sup>-1</sup>), hemoglobin ( $10.77 \pm 2.15$  g dL<sup>-1</sup>, min:7.6 max:17.1), MCH ( $112.95 \pm 19.76$  pg, min:85.74 max:157.32) and MCHC ( $36.18 \pm 6.66$  g dL<sup>-1</sup>). In this study, the hematocrit did not differ between treatments ( $p > 0.05$ ) and the values remained within the normal range when compared with the reference values described in Juruá River, Amazonas, Brazil ( $45.2 \pm 56\%$ ) (Oliveira et al., 2021).

The increased values of erythrocyte parameters in *M. duriventre* in the present study, such as hemoglobin and hematocrit, compared to other studies, indicate a possible chronic adaptation caused by the low oxygen levels in the lagoon where the experiment was carried out. This is not only due to the degree of hypoxia, but also to the length of time that the environmental parameter was at a low concentration, generating the release of sufficiently mature erythrocytes (with sufficient hemoglobin) to maintain an adequate level of oxygen transport in the circulating blood, through a homeostatic control (Valenzuela et al., 2002).

In this study, the hematological profile suggests that the system or rearing conditions did not induce anemic processes that could affect the metabolism or health status of the fish. Interspecific and intraspecific variations in hematological parameters have been reported in fish and have been attributed to various factors such as genetic variation, capture stress, handling, and the handling of the fish for blood sampling (Kori, 1985; Tavares et al., 2003).

Regarding leukogram and thrombogram, no significant differences ( $p > 0.05$ ) were observed between the different treatments, indicating that the farmed and environmental conditions did not affect the number of cells. In *M. duriventre*, the number of thrombocytes was similar to that of the pacu *P. mesopotamicus*, even in the

aquaculture environment (Fries et al., 2016), but it was different in the same species in the wild, although within the range of the minimum and maximum considered in the Brazilian study (Chamy et al., 2015).

Such variations are to be expected, as thrombocytes are mainly involved in blood coagulation and contribute to the fish defense, being in constant movement between the hematopoietic organs (spleen and kidneys) and the circulation (Tavares et al., 2008). When comparing the leukocytes of the present study with other Characiformes, a high score was found as compared to the yamú *Brycon amazonicus*, but within the ranges of this reference culture (Gonzales et al., 2019). Differently, a low score was reported in the farmed tambaqui *C. macropomum*, but then again, our results were between the expected ranges (Minaya, 2018).

Leukocyte differentiation (lymphocytes, monocytes, neutrophils, eosinophils, and PAS-positive granular leukocytes) in the blood of *M. duriventre* was similar to that reported in wild fish of the same species and *B. amazonicus* (Chamy et al., 2015; Gonzales et al., 2019). The predominance of lymphocytes, followed by neutrophils in *M. duriventre*, suggests that these granulocytes constitute the main defense barrier in this fish (Ranzani et al., 2013). However, the composition of blood granulocytes is highly variable among fish, as only some species, such as *M. duriventre*, are PAS-GL positive, while eosinophils and basophils are rare.

Neutrophils and monocytes are responsible for the defense of the organism and are the main cells activated in fish exposed to stress and/or parasitic infections (Ranzani et al., 2013). In a study on *P. mesopotamicus*, where the hematological parameters of the fish were evaluated under conditions of parasitic infestation by *Lernaea* spp., there was an increase in neutrophils, followed by lymphocytes and monocytes (Fries et al., 2016). Thus, it has been shown that several stress factors in fish cause a decrease in lymphocytes, while an increase in neutrophils occurs, a tendency not shown in the present study despite the presence of two parasites (Mario et al., 2017). The number of circulating leukocytes varies between fish species depending on age, sex, season, nutritional status, diseases, as well as the method of analysis (Ranzani et al., 2013). However, the coefficient of variation between treatments for thrombocytes and leukocytes of *M. duriventre* from the present study was low, suggesting normal data in this apparently healthy species.

In the palometa *M. duriventre* of the present study, gill parasitism was observed with the presence of a Monogenean of the genus *Apedunculata* sp., reported for the first time in this species. This Monogenean has already been reported in the literature in gills of the streaked prochilod *Prochilodus lineatus* and the black prochilod *Prochilodus nigricans* (Cuglianna et al., 2009; Hidalgo & García, 2018). The encystment of myxospores of the genus *Henneguya* sp. in the gills of *M. duriventre* of the present study had already been reported in the literature for the same species (Thatcher, 2006).

As mentioned above, it is possible that these parasites did not generate an immunological response such as an increase in leukocytes because their increase at the blood level is the result of a disease process triggered by the need to produce more antibodies, a process that was not evident in this study because no differences were found between highly parasitized treatments and those with low parasitization (Fries et al., 2016; Olabuenaga, 2000). In addition, it has been evidenced that the presence of parasitism that generates disease tends to increase the number of eosinophils as an antigenic response to parasitic infections, which did not occur in this study since eosinophils were the cell group that appeared less frequently (Clauss et al., 2008).

## Conclusion

This is the first study with *M. duriventre* on its production in floating cages, resulting in low mortality in production. The diet with 25% crude protein had the best productive behavior, showing a greater average weight gain compared to other treatments. The high hemoglobin and hematocrit levels in this study could be due to the low oxygen concentrations in the lagoon, as it is a lentic body of water that is oxygenated by rain and surface aeration. The hematological results of this study in this species studied in the Colombian Orinoquia, can be used for comparison with future studies.

## Data availability

The data resulting from the study are included in the body of the article in the Results section.

## Acknowledgments

To the Asociación de Pescadores de Puerto López, Meta (ASOPESPOL) for its work in the experimental and farmed part, to the Grupo de Investigación Sobre Reproducción y Toxicología de Organismos Acuáticos (GRITOX)

for its support throughout the investigation, to the Instituto de Acuicultura y Pesca de los Llanos (IALL) for the provision of laboratories, to the Kotsala research group and corporation for laboratory reinforcement, and to the Universidad de Los Llanos (Unillanos) for the logistical and institutional support provided.

## References

- Aïzonou, R., Achoh, M. E., Hountcheme, I. A. C., Agadjihouèdé, H., Ahouanssou-Montcho, S., & Montchowui, E. (2021). Zootechnical Knowledge of floating cage aquaculture in freshwaters ecosystems and load capacity determination: Review. *Egyptian Journal of Aquatic Research*, 47(1), 81-86. <https://doi.org/10.1016/j.ejar.2020.10.013>
- Alvis, G. (2006). La Hematología como herramienta indicadora de la salud en los peces. *Revista Electrónica De Ingeniera En Producción Acuícola*, 2(2), 4. <https://revistas.udenar.edu.co/index.php/reipa/article/view/1613>
- Arias, C. J. A., Benavides, B. M., Hernandez, A. G., & Eslava, M. P. R. (2003). Valoración hematológica y química sanguínea del yamú *Brycon siebenthalae*, en tres etapas de cultivo. *Revista Orinoquia*, 7(1), 34-41. <https://orinoquia.unillanos.edu.co/index.php/orinoquia/article/view/260>
- Balbuena, R. E. (2011). *Manual Básico de Sanidad Piscícola*. FAO Paraguay. <https://openknowledge.fao.org/server/api/core/bitstreams/09396359-2d51-4145-853e-cd6bca3a2c45/content>
- Barandica, C., & Tort, B. (2008). Neuroendocrinología e inmunología de la respuesta al estrés en peces. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 32(123), 267-284. [https://doi.org/10.18257/raccefyn.32\(123\).2008.2290](https://doi.org/10.18257/raccefyn.32(123).2008.2290)
- Barandica, C. L. (2010). *Efectos de las dietas experimentales en la respuesta inmune de los peces*. Universidad Autónoma de Barcelona. <https://www.tdx.cat/handle/10803/3627#page=1>
- Beveridge, M. C. M. (1986). *Piscicultura en jaulas y corrales. Modelos para calcular la capacidad de carga y las repercusiones en el ambiente* (Documento Técnico de Pesca, v. 255). FAO. <https://www.fao.org/4/ad021s/ad021s00.htm>
- Bocek, A. (2020). *El cultivo de peces en estanques artificiales*. Auburn University. <https://cales.arizona.edu/azaqua/AquacultureTIES/publications/Spanish%20WHAP/GT9%20Jaulas.pdf>
- Chamy, M. N. C. L., Souza, R. P., Costa, A. G., & Tavares, D. M. T. (2015). Hematologia do *Mylossoma duriventre* (serrasalminidae) da bacia do rio Solimões, Amazônia central (Brasil). *Veterinária e Zootecnia*, 22(4), 597-606. <https://rvz.emnuvens.com.br/rvz/article/view/873>
- Chen, K., Wang, C., Fan, Y., Xie, Y., Yin, Z., Xu, Z., Zhang, H., Cao, J., Han, Z., Wang, Y., & Song, D. (2014). The evaluation of rapid cooling as an anesthetic method for the zebrafish. *Zebrafish*, 11(1), 71-75. <https://doi.org/10.1089/zeb.2012.0858>
- Clauss, T., Dove, A., & Arnold, J. (2008). Hematologic Disorders of Fish. *Veterinary Clinics of North America - Exotic Animal Practice*, 11(3), 445-462. <https://doi.org/10.1016/j.cvex.2008.03.007>
- Conroy, D., & Armas de conroy, G. (1987). *Manual de métodos de diagnóstico en ictiopatología, con especial referencia a los salmónidos* (v. 4). FAO. <https://www.fao.org/4/ab469s/ab469s00.htm>
- Copete-Sierra, M. (2013). Aspectos Generales de la evaluación hematológica en fauna silvestre y no convencional. *Memorias de la Conferencia Interna en Medicina y Aprovechamiento de Fauna Silvestre, Exótica y no Convencional*, 9(1), 17-55. <https://www.veterinariosvs.org/memorias-de-la-cima-2013-09-01/>
- Correa, N. J., Garrido, C. A., Prieto, G. M., Atencio, G. V., & Pardo, C. S. (2009). Caracterización de células sanguíneas y parámetros hematológicos en blanquillo *Sorubim cuspicaudus*. *Zootecnia Tropical*, 27(4), 393-405. [https://ve.scielo.org/scielo.php?script=sci\\_arttext&pid=S0798-72692009000400005](https://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0798-72692009000400005)
- Cuan, B. J. A., Parada, G. S. L., Murillo, P. R., & Ramirez, M. J. A. (2021). Productive parameters of white cachama culture, *Piaractus orinoquensis*, in floating cages. *Revista U.D.C.A Actualidad and Divulgacion Científica*, 24(2), e2068. <https://doi.org/10.31910/rudca.v24.n2.2021.2068>
- Cuglianna, A., Cordeiro, N., & Luque, J. (2009). *Apedunculata discoidea* gen. n., sp. n. (Monogenea: Dactylogyridae) parasitic on *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae) from southeastern Brazil. *Brazilian Journal of Biology*, 69(3), 895-898. <https://doi.org/10.1590/S1519-69842009000400018>

- Food and Agriculture Organization. (2016). Mejora de la calidad de agua en los estanques. En *Gestión de la piscicultura de agua dulce: estanques y prácticas acuícolas* (Colección FAO Capacitación, n° 21/1, p. 1-65). [https://www.fao.org/fishery/docs/CDrom/FAO\\_Training/FAO\\_Training/General/x6709s/x6709s02.htm#4a](https://www.fao.org/fishery/docs/CDrom/FAO_Training/FAO_Training/General/x6709s/x6709s02.htm#4a)
- Fries, E. M., Hassemer, M. Z., Ferraezi, M. L., & Feiden, A. (2016). Avaliação dos parâmetros hematológicos do pacu *Piaractus mesopotamicus* infectado por *Lernaea* spp. *Revista Cultivando o Saber*, 9(4), 479-48. <https://cultivandosaber.fag.edu.br/index.php/cultivando/article/view/739>
- García, V. A., Duponchelle, F., & Alcantara, F. (2016). *Evaluación de los parámetros reproductivos de palometa Mylossoma duriventre como base para el manejo sostenible de su pesquería en la región Loreto - Perú*. Universidad Nacional de la Amazonía Peruana. <https://repositorio.unapiquitos.edu.pe/handle/20.500.12737/4412>
- Gonzales, A., Curto, G., & Fernández, M. C. (2019). Hematological parameters of *Brycon amazonicus* (Bryconidae) broodstock in captivity. *Revista de Investigaciones Veterinarias del Peru*, 30(1), 133-142. <https://doi.org/10.15381/rivep.v30i1.14935>
- GraphPad Software. (2021). *GraphPad Prism (Version 9)* [Computer software]. GraphPad Software Inc. <https://www.graphpad.com>
- Hidalgo, P. L., & García, P. G. (2018). *Fauna parasitaria en alevinos y juveniles de "Boquichico" Prochilodus nigricans (Agassiz, 1829) provenientes del río Amazonas (Padre isla) y de estanque de cultivo del Ciec Piscigranja u.n.a.p, Loreto – Perú*. 2016. Universidad Nacional de la Amazonía Peruana. <https://repositorio.unapiquitos.edu.pe/handle/20.500.12737/5431>
- Instituto Nacional de Pesca y Acuicultura. (2001). *La pesca en la baja Orinoquia Colombiana: una visión integral* (87-90). INPA. <https://repository.agrosavia.co/handle/20.500.12324/18526>
- Kinkelin, P., Michel, C. D., & Ghittino, P. (1991). *Tratado de las enfermedades de los peces*. Acribia S. A.
- Kori, S. O. (1985). Haematological characteristics of *Clarias isheriensis* Sydenham. *Journal of Fish Biology*, 27(3), 259-263. <https://doi.org/10.1111/j.1095-8649.1985.tb04026.x>
- Lasso, C., Agudelo, C., Jiménez, S. L., Ramírez, G. H., Morales, B. M., Ajiaco, M. R., Gutiérrez, F. P., Oviedo, J. S. U., Torres, S. E. M., & Sanabria, O. I. (2011). Catálogo de los recursos continentales pesqueros de Colombia. In: *Serie recursos hidrobiológicos y pesqueros continentales de Colombia* (p. 37-39). Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. <http://hdl.handle.net/20.500.11761/32542>
- Maria, P. B. (2009). Farmed fish welfare-suffering assessment and impact on product quality. *Italian Journal of Animal Science*, 8(1), 139-160. <https://doi.org/10.4081/ijas.2009.s1.139>
- Mario, C., Baquero, R., Julián, A., Caviedes, P., & Pérez, A. P. (2017). Respuestas hematológicas, hepáticas y esplénicas al estrés de tilapias en jaulas y libres en el embalse de Betania, Colombia. *Revista AquaTIC*, 49, 8-20. <https://www.redalyc.org/journal/494/49463406002/html/>
- Minaya I. A. P. (2018): *Evaluación del perfil hematológico y bioquímico en Gamitana (Colossoma macropomum) de La Amazonía*. Universidad Peruana Cayetano Heredia. <https://hdl.handle.net/20.500.12390/1624>
- Morey, G. A. M. (2019). *Parasitología en peces de la Amazonía. Fundamentos y Técnicas parasitológicas, Profilaxis, Diagnóstico y Tratamiento*. Instituto de Investigaciones de la Amazonía Peruana. <https://repositorio.iiap.gob.pe/items/8275b5d2-7654-4592-a52e-c1e3e81e5e76/full>
- Naya, C. F., Martos, S. J. A., De las heras, V., Simó, M. P., Caldusch, G. J., & Pérez, S. J. (2021). Targeting the mild-hypoxia driving force for metabolic and muscle transcriptional reprogramming of gilthead sea bream (*Sparus aurata*) juveniles. *Biology*, 10(5), 416. <https://doi.org/10.3390/biology10050416>
- Olabuenaga, S. (2000). E. Sistema inmune en peces. *Gayana (Concepción)*, 64(2), 205-215. <http://dx.doi.org/10.4067/S0717-65382000000200010>
- Oliveira, A., Sadalla, P., Silva, L., Pantoja, L. J., Vitor de paiva, A., & Rocha, A. (2021). Fisiologia sanguínea do Pacu *Mylossoma duriventre* e da pescada *Plagioscion squamosissimus*. Em: *Aquicultura na Amazônia: Estudos Técnico-científicos e Difusão de Tecnologias* (p. 269-276). Atena Editora. <https://atenaeditora.com.br/catalogo/ebook/aquicultura-na-amazonia-estudos-tecnico-cientificos-e-difusao-de-tecnologias>
- Organisation for Economic Co-operation and Development. (2016). *Fisheries and Aquaculture in Colombia*. OECD. <https://cdi.mecon.gob.ar/bases/docelec/az3289.pdf>

- Ranzani, P. M., Tavares, D. M., & Egami, M. (2013). *Métodos para análise hematológica em peixes*. Eduem. <https://doi.org/10.7476/9788576286530>
- Reátegui, A. C. R., Oliva, P. R., Villegas, P. P. P., & Vargas F. J. I. (2017). Efecto de la densidad de siembra en el desempeño productivo y parametros hematologicos de juveniles de *Piaractus brachipomus* “paco” cultivados en jaulas flotantes en la laguna Yarinacocha. *Repositorio de Revistas de la Universidad Privada de Pucallpa*, 2(2), 27-42. <https://doi.org/10.37292/riccva.v2i02.58>
- R Core Team. (2022). *R: A language and environment for statistical computing (Version 4.2.1)* [Computer software]. R Foundation for Statistical Computing. <https://www.r-project.org/>
- Retamales, C., & Manzo, G. (2017). *Recomendaciones para la tinción de frotis sanguíneos para la lectura del hemograma: serie blanca, roja y plaquetaria*. Instituto de Salud Pública de Chile. <https://www.ispch.cl/sites/default/files/RECOMENDACIONES%20PARA%20LA%20TINCI%C3%93N%20DEL%20FROTIS%20SANGU%C3%8DNEO.pdf>
- Rozas, S. M. A. (2020). *Patología clínica en salmónidos* (2a ed.). Laboratorio Pathovet. <https://pathovet.cl/wp-content/uploads/2021/08/Manual-de-Patologi%C3%81a-Cli%C3%81nica-de-Peces-Salmo%C3%81nidos.pdf>
- Santos, G. M. (2009). *Peixes comerciais de Manaus* (2a ed. Rev.). INPA. <https://repositorio.inpa.gov.br/items/54096335-83ad-4519-a41a-1198486eaacb>
- Salazar, C. E., & Vásquez, M. (2017). *Cultivo de Oreochromis spp (O.niloticus x O.aureus) “Tilapia Híbrida” a diferentes densidades de siembra de cultivo intensivo en jaulas flotantes*. Facultad de ciencias bilógicas, Universidad Nacional Pedro Ruiz Gallo. <https://repositorio.unprg.edu.pe/handle/20.500.12893/10946>
- Salgado, S. P., & Ramirez, K. A. (2017). *Cuenta de leucocitos en frotis sanguíneo como alternativa de campo al método del hemocitómetro en especímenes de trucha arcoíris (Oncorhynchus mykiss) clínicamente sanos*. Universidad de Chile. <https://repositorio.uchile.cl/handle/2250/145886>
- Tavares, D. M. (2006). A morphological and cytochemical study of erythrocytes, thrombocytes and leukocytes in four freshwater teleosts. *Journal of Fish Biology*, 68(6), 1822-1833. <https://doi.org/10.1111/j.1095-8649.2006.01089.x>
- Tavares, D. M., Affonso, E. G., Oliveira, S. R., Marcon, J. L., & Egami, M. I. (2008). Comparative study on hematological parameters of farmed matrinxã, *Brycon amazonicus* Spix and Agassiz, 1829 (Characidae: Bryconinae) with others Bryconinae species. *Acta Amazonica*, 38(4), 799-805. <https://doi.org/10.1590/S0044-59672008000400026>
- Tavares, D. M., Schalch, S. H. C., & de Morales, F. (2003). *Hematological Characteristics of Brazilian Teleosts . Vii. Parameters of Seven Species Collected*. *Boletim do Instituto de Pesca*, 29(2), 109-115. <https://institutodepesca.org/index.php/bip/article/view/Dias>
- Tratado de Cooperacion Amazonica. (1999). *Piscicultura amazónica con especies nativas*. Secretaria Pro Tempore. <http://www.iiap.org.pe/upload/Publicacion/CDinvestigacion/iiap/iiap1/TEXT001.htm>
- Thatcher, V. (2006). *Biodiversidad Acuática en América Latina. Parásitos de Peces Amazónicos* (2. ed.). PENSOFT.
- Useche, L. C., Aviles, B. M., & Dorado, L. M. (2001). Cultivo de peces en Jaulas. In H. Rodriguez, P. Daza, M. Carrillo (Ed.). *Fundamentos de Acuicultura Continental* (p. 367-388). Grafimpresos Quintero. [https://repository.agrosavia.co/bitstream/handle/20.500.12324/19718/65043\\_27481.pdf?s](https://repository.agrosavia.co/bitstream/handle/20.500.12324/19718/65043_27481.pdf?s)
- Valenzuela, A., Alveal, K., & Tarifeño, E. (2002). Respuestas hematologicas de truchas (*Oncorhynchus mykiss* walbaum 1792) a estres hipoxico agudo: serie roja. *Gayana (Concepción)*, 66(2), 255-261. <http://dx.doi.org/10.4067/S0717-65382002000200024>
- Vargas, V. R. (2017). *Pez cebra (Danio rerio) y anestesia. Un modelo animal alternativo para realizar investigación biomédica básica*. *Anestesia en México*, 29(1), 86-96. [https://www.scielo.org.mx/scielo.php?script=sci\\_arttext&pid=S2448-87712017000400086](https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2448-87712017000400086)
- Wilson, J., Bunte, R., & Carty, A. (2009). Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science*, 48(6), 785-789. <https://pmc.ncbi.nlm.nih.gov/articles/PMC2786934/>
- Wintrobe, M. (1990). The size and hemoglobin content of the erythrocyte. Methods of determination and clinical application. *The Journal of Laboratory and Clinical Medicine*, 115(3), 374-387.

Witeska, M., Kondera, E., Ługowska, K., & Bojarski, B. (2022). Hematological methods in fish – Not only for beginners. *Aquaculture*, 547. <https://doi.org/10.1016/j.aquaculture.2021.737498>