BLOOD VARIABLES OF HYBRID TAMBACU FARMED IN AMAPÁ STATE, NORTHERN BRAZIL

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ABSTRACT

This study compared the blood characteristics of hybrid tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*) from three fish farms in the Macapá, State of Amapa (Brazil), which has a similar management. There were differences in the levels of dissolved oxygen in the water and pH in the ponds of the fish farms, but condition factor showed good body condition of the fish. In blood of tambacu were identified thrombocytes, lymphocytes, monocytes, neutrophils, PAS-positive granular leukocytes (PAS-GL) and eosinophils. Plasma glucose and protein levels, hematocrit, total leucocyte count, total thrombocytes count, monocytes, neutrophils and eosinophils count were similar among the three fish farms. However, hemoglobin concentration, total erythrocytes count, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), number of lymphocytes and PAS-GLshowed differences among fish of these fish farms. Although the results showed a relative similarity between the blood parameters in the fish of the different fish farms, but the management conditions regarding the water quality in the ponds and food should be improved to increase the productivity in these fish farms.

Keywords:fish farm, freshwater fish, management, white blood cells.

VARIÁVEIS SANGUÍNEAS DO HÍBRIDO TAMBACU CULTIVADO NO ESTADO DO AMAPÁ, NORTE DO BRASIL

RESUMO

Este estudo comparou as características sanguíneas do híbrido tambacu(*Colossoma macropomum* x *Piaractus mesopotamicus*)de três pisciculturas de Macapá, Estado do Amapá (Brasil), com manejos semelhantes. Diferenças nos níveis de oxigênio dissolvido e do pH da água dos viveiros foram observadas, mas o fator de condição mostrou a boa condição dos peixes. No sangue dos tambacus foram identificados trombócitos, linfócitos, monócitos, neutrófilos, leucócitos granulares PAS-positivo (LG-PAS) e eosinófilos. Os níveis de glicose e proteína, hematócrito, contagem total de leucócitos, contagem total de trombócitos, contagem de monócitos, neutrófilos e eosinófilos foram similares entre as três pisciculturas. Entretanto, a concentração de hemoglobina, contagem de eritrócitos, da concentração de hemoglobina corpuscular média (CHCM), volume corpuscular médio (VCM), número de linfócitos e LG-PAS mostraram diferenças entre os peixes destas pisciculturas. Embora, os resultados mostraram relativa similaridade entre os parâmetros sanguíneos dos peixes das pisciculturas, mas condições de manejo, relativo à qualidade da água dos viveiros e à alimentação fornecida, devem ser melhoradas para aumentar a produtividade nestas pisciculturas.

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Palavras-chave: piscicultura, peixes de água doce, manejo, leucócitos.

VARIABLES DE LA SANGRE EN HYBRIDO TAMBACU CULTIVADO EN EL ESTADO DE AMAPÁ, NORTE DE BRASIL

RESUMEN

Este estudio comparó las características de la sangre de híbrido tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*) tres criaderos piscícolas en Macapá, Estado de Amapá (Brasil), con gestiones similares. No se observaron diferencias en los niveles de oxígeno y pH del agua de los estanques en los criaderos piscícolas, pero el factor de condición mostró buenas condiciones corporales en los peces. En la sangre de los tambacus fueron identificados trombocitos, linfocitos, monocitos, neutrofilos, leucocitos granulares PAS-positivos (LG-PAS) y eosinofilos. Los niveles de glucosa y proteínas, hematocrito, recuento de leucocitos totales, el recuento total de trombocitos, monocitos, neutrófilos y eosinofilos fueron similares entre peces de los criaderos piscícolas. Sin embargo, la concentración de hemoglobina, recuento de eritrocitos, concentración de hemoglobina corpuscular media (CHCM), volumen corpuscular medio (VCM), el número de linfocitos y LG-PAS mostraron diferencias entre los peces de loscriaderos piscícolas. Aunque los resultados mostraron relativa similitud entre los parámetros de lo sangre de peces de los criaderos piscícolas, pero o manejo de las condiciones de la calidad del agua del estanque y la comida siempre debe mejorarse para aumentar la productividad en estas criaderos piscícolas.

Palabras clave:criaderos piscícolas, pez de agua dulce, manipulación, leucocitos.

INTRODUCTION

Tambacu were first obtained in the 1980s, through crossing female tambaqui (*Colossoma macropomum*) with male pacu (*Piaractus mesopotamicus*), in an attempt to obtain a more robust fish, with the rapid growth of tambaqui, but more resistant to low temperatures, like pacu, during the winter in the southern and southeastern region (1,2). These hybrids still have great importance within Brazilian fishfarms, because of these zootechnical characteristics, omnivorous habits, resistance to stress and parasitic diseases and great ease of adaptation (3,4,5). Brazil produced 49,818 tons of tambacu in 2011, which was 120% more than in 2010 (6).

In fish farms in the state of Amapá, tambacu are the third most commonly farmed fish, after *C. macropomum*and the hybridtambatinga (*C. macropomum* x *Piaractus brachypomus*) (7). On fish farms in this region, tambacu present moderate parasitism due to*Ichthyophthirius multifiliis*,*Piscinoodinium pillulare* (Protozoa), *Anacanthorus spatulatus*,*Notozothecium janauachensis* and*Mymarothecium viatorum* (Monogenea), *Perulernaea gamitanae* (Crustacea), *Cucullanus colossomi* (Nematoda), *Neoechinorhynchus buttnerae* (Acanthocephala) and cestoides Proteocephalidae (5). The blood parameters of tambacu have been characterized in fish farmed in São Paulo state (8,9). However, there are little information onhematology offarmedtambacu in Amapá state.

Hematological and biochemical parameters can be used as physiological and immunological indicators of the conditions of a fish population (10,11,12),since the blood tissue presents properties relating to respiration, protection and transportation of a variety of substances such as nutrients. Therefore, hematology can be used as a tool in studies on the health of fish populations (13,14). Hence, the present study had the objective of comparing the blood variables of tambacu on three fish farms in Macapá, state of Amapá,Brazil.

MATERIAL AND METHODS

This study was conducted in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA). Between October 2009 and March 2010, healthy specimens of tambacu (*C. macropomum* x *P. brachypomus*) were collected from three fish farms in Macapá, state of Amapá, with the aid of cast or trawl nets. These fish, aged 4 to 10 months, were cultivated in dugout tanks of volume 1,000 to 9,000 m^3, at stocking densities ranging from 0.5 to 2.8 fish/m^3. Fish were fed with extruded commercial feed suitable for omnivorous fish, containing crude protein levels of 28 to 32%.

Immediately after each fish had been caught, a blood sample was collected from it (1.0 mL) by means of caudal vein puncture, using syringes and needles containing EDTA (10%). These samples were conserved in ice. In accordance with the methodology described by Ranzani-Paiva et al. (13), the following were determined: hematocrit, using the microhematocrit method; red blood cells (RBC) count, in a Neubauer chamber; hemoglobin concentration, using Drabkin's reagent; and absorbance, through readings on a spectrophotometer at 540 nm. From these data, the Wintrobe hematimetric indices were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

One part of the blood was used to produce blood extensions, which were stained panchromatically using a combined May-Grünwald-Giemsa-Wright stain, in order to perform differential white blood cell counts on up to 200 cells of interest, in each extension. The white blood cellswere identified as lymphocytes, monocytes, neutrophils, eosinophils and PAS-GL, following the nomenclature recommended by Tavares-Dias et al. (8). The determinations of total white blood cells (WBC) and thrombocyte counts followed the recommendations of Ranzani-Paiva et al. (13). After centrifugation of the blood, the plasma was separated in order to determine the glucose and total protein levels, using commercial colorimetric kits (Doles, Goiânia, GO).

After the blood samples had been collected, the fish were examined in relation to the mouth, opercula, gills and gastrointestinal tract, to ascertain whether any parasites were present. Only the specimens that were free from parasites and macroscopic lesions were used. The methodology used for the procedures of collection, fixation and parasitological analysis were as described by Eiras et al. (15). The fish were weight (g) and measured (cm), and these data were used to calculate the relative condition factor (16).

On each occasion when fish were collected, the dissolved oxygen level, pH and temperature of the water in the fish ponds of the three fish farms were determined, using digital devices specific for each of the parameters measured.

All the data were initially evaluated with regard to the assumptions of normal distribution and homocedasticity, using the Shapiro-Wilk and Bartlett tests, respectively. The data that presented normal distribution were evaluated using analysis of variance (ANOVA) followed by Tukey's test, to compare between the means. The data that did not present normal distribution were evaluated using the Kruskal-Wallis test to compare between the medians using the Tukey's test.Differences were considered significant at the 5% probability level (17).

RESULTS AND DISCUSSION

In fish farming, constant monitoring of the physical and chemical parameters of the water in the ponds is recommended (1,2), given that maintenance of good water quality is vital to good health and growth among the fish, because of its influence on their metabolic processes (18,19).

The water temperature in the ponds of fish farms 2 and 3 was higher, while the oxygen levels and pH were lower than those of fish farm 1 (Table 1). Moreover, in the ponds of fish farms 2 and 3, the dissolved oxygen levels were less than 4.0 $mg.L^{-1}$, since renewal of the pond water and water aeration were generally not done. For optimum growth and performance of round fish, the oxygen levels in fish-farm ponds ought to be higher than this level (20). Although round fish are able to survive for hours in waters with low oxygen levels (0.5 $mg.L^{-1}$), using an emergency respiratory strategy consisting of expansion of the lower lip (aquatic surface respiration) (1,2,21,22), the health of these fish becomes compromised, given that they cease to feed. For fish farms, the water pH should be within a band between 6.5 and 7.5 (19,23,24).

Table 1. Water quality parameters (mean ± standard deviation) of three fish farm of tambacu in Macapá, Amapá State.

Fish farms	1	2	3
Oxygen ($mg.L^{-1}$)	$4,8 \pm 0,4^{b}$	$3,3 \pm 1,6^{a}$	$3,9 \pm 1,6^{a}$
pH	$7,0 \pm 0,3^{b}$	$5,9 \pm 0,8^{a}$	$5,9 \pm 0,8^{a}$
Temperature (°C)	$29,2 \pm 0,8^{a}$	$30,2 \pm 0,8^{ab}$	$30,3 \pm 0,6^{b}$

Small different letters in the same row indicate statistically significant differences ($p<0.05$) between fish farms by Tukey's test.

The condition factor (Kn) is a quantitative indicator of body and dietary conditions and the influence of population density and other environmental conditions (16,25). Thus, the Kn of intensively farmed tambacu indicates that they present good body conditions. The weight, length and condition factor of the tambacu on fish farm 2 were greater than those of the fish on the other two farms (Table 2), because of differences in the fish ages.

In the tambacu of the three fish farms investigated, the plasma glucose and protein levels were similar (Table 2), despite management differences between these farms. In general, the fish on farms 1 and 3 did not receive balanced feed continually. Moreover, the fish on these three farms were sometimes not fed with balanced feed but, rather, with leftover products from supermarkets or with feed that presented inappropriate daily protein levels and feed quantity (7,26). The glucose and plasma protein levels in the tambacu of the present study were also similar to those that have been reported for tambaqui (27) and tambacu (12). However, they were higher than those reported for farmed tambacu in the state of Paulo (9). Nonetheless, when food restrictions are present, high levels of plasma proteins may occur in farmed fish (28). These high protein levels may indicate states of hemolysis (29), but such conditions were not observed among the fish in this study. Glucose and plasma protein levels not only vary between species, but also may be influenced by the fish ecophysiology and by their sex, gonadal development, diseases, seasonality, management, diet and other factors(4,9,30).

Among farmed fish, erythrocyte parameters may help in evaluating the effects of the diet administered, management stress and presence of diseases (4,9,12,30). The hemoglobin concentration, RBC counts and MCHC were lower among the tambacu on farm 2, while the MCV was higher. However, the hematocrit of the tambacu was similar on the three fish farms investigated (Table 2). These results can be attributed to the fact that the fish on farm 2 were older than the others. Furthermore, higher MCV levels are a mean of compensating for these low hemoglobin levels and RBC counts. Nonetheless, the values for these parameters were similar to those described for this same hybrid cultivated in the state of São Paulo (8). Therefore, it seems that the lower dissolved oxygen levels and lower pH in the water of the ponds of fish farms 2 and 3 did not have any significant effect on the erythrogram of the fish, thus confirming the hardiness of tambacu.

Table 2. Body and blood parameters (mean ± standard deviation) of tambacu from three fish farms from Macapá, Amapá State. MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, relative condition factor de (Kn).RBC: Red blood cells.

Fish farms	1	2	3
Body weight (g)	$715,7 \pm 103,6^{ab}$	$1.011,2 \pm 450,0^{b}$	$523,7 \pm 116,7^{a}$
Body length (cm)	$36,7 \pm 2,0^{b}$	$35,7 \pm 5,5^{ab}$	$31,2 \pm 2,5^{a}$
Kn	$0,99 \pm 0,02^{a}$	$1,04 \pm 0,05^{b}$	$1,00 \pm 0,01^{a}$
Glucose ($mg.dL^{-1}$)	$93,9 \pm 27,5^{a}$	$128,4 \pm 32,6^{a}$	$100,3 \pm 24,1^{a}$
Total protein ($g.dL^{-1}$)	$3,0 \pm 0,4^{a}$	$3,3 \pm 0,2^{a}$	$3,3 \pm 0,3^{a}$
Hematocrit (%)	$28,5 \pm 2,2^{a}$	$31,7 \pm 4,4^{a}$	$30,3 \pm 2,4^{a}$
Hemoglobin ($g.dL^{-1}$)	$10,9 \pm 0,8^{b}$	$9,0 \pm 2,3^{a}$	$10,6 \pm 3,5^{ab}$
RBC ($x\ 10^{6}.\mu L^{-1}$)	$1,6 \pm 0,3^{b}$	$1,2 \pm 0,1^{a}$	$1,6 \pm 0,3^{b}$
MCV (fL)	$186,8 \pm 33,1^{a}$	$264,9 \pm 32,0^{b}$	$202,4 \pm 38,8^{a}$
MCH ($g.dL^{-1}$)	$71,3 \pm 12,2^{a}$	$71,3 \pm 8,6^{a}$	$63,8 \pm 10,1^{a}$
MCHC ($g.dL^{-1}$)	$38,4 \pm 3,5^{b}$	$26,9 \pm 4,2^{a}$	$29,0 \pm 4,6^{a}$

Different letters in the same row indicate statistically significant differences ($p<0.05$) between fish farms by Tukey's test.

In the blood of the tambacu of this study, thrombocytes, lymphocytes, monocytes, neutrophils, PAS-GLs and eosinophils were observed (Table 3), with similar characteristics to those described by Tavares-Dias et al. (8), for the same fish. In fish, thrombocytes are cells that are mainly involved in hemostasis, but may also remove damaged cells from the organism and may migrate to the induced inflammatory focus (13,31). In the tambacu of these three fish farms, the thrombocyte counts were similar to each other and lower than what was reported by Tavares-Dias et al. (4) for tambacu, but within the reference limits for tambacu described by Tavares-Dias (12).

Table 3. Thrombocyte and white blood cell counts (mean ± standard deviation) of tambacu from three fish farms from Macapá, Amapá State. PAS-GL: PAS-positive granular leukocytes. WBC: White blood cells.

Fish farms	1	2	3
Thrombocytes (µL)	30.425 ± 14.638^{b}	54.624 ± 20.026^{a}	27.888 ± 20.142^{a}
WBC (µL)	12.520 ± 5.582^{a}	5.630 ± 3.368^{a}	11.070 ± 8.737^{a}
Lymphocytes (µL)	10.626 ± 4.642^{a}	4.845 ± 2.331^{bc}	11.727 ± 9.7129^{ac}
Monocytes (µL)	723 ± 526^{a}	488 ± 575^{a}	176 ± 310^{a}
Neutrophils (µL)	414 ± 382^{a}	454 ± 382^{a}	125 ± 578^{a}
Eosinophils (µL)	291 ± 344^{a}	229 ± 411^{a}	67 ± 98^{a}
PAS-GL (µL)	560 ± 510^{a}	8 ± 16^{b}	39 ± 102^{b}

Different letters in the same row indicate statistically significant differences ($p<0.05$) between fish farms by Tukey's test.

Piscine white blood cells (WBC) may indicate the status of the immunological system, given that these cells perform a wide range of functions (13,31). Lymphocytes are involved in production of immunoglobulins and modulation of defenses. Monocytes are phagocytes that undergo migration to the inflammatory focus during infectious processes. Neutrophils are the first phagocytes to respond to infections, and in tambacu, they present alkaline phosphatase, peroxidase, esterase and glycogen. PAS-GLs are white blood cells with a strong reaction to Schiff's periodic acidand with little-known function in fish, but they may present elevated quantities in parasitized fish (13,31).

The tambacu hybrids on farm 2 had the lowest lymphocyte and PAS-GL counts, while the WBC, monocyte, neutrophil and eosinophil counts were similar among the fish on the three farms (Table 3). Moreover, the WBC, monocyte, neutrophil, PAS-GL and eosinophil counts were within the reference limits described by Tavares-Dias (12), for this same hybrid. However, neutrophils and monocytes are cells in constant movement between the circulation and tissues, because of their important participation in inflammatory processes together with other granulocytes (13,31). There may thus be greater variation between fish in different places.

CONCLUSIONS

This contribution on the blood parameters of tambacu hybrids cultivated on fish farms in the state of Amapá showed results than can possibly be used for comparisons in future studies on this fish and in other localities. However, other studies should be conducted in order to attain greater comprehension of the blood profile of tambacu at different stages of the development of this hybrid.

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