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Performance and hematological variables of piavuçu whose diets were supplemented with phytobiotic and probiotic additives

Desempenho e variáveis hematológicas do piavuçu suplementado com aditivos fitogênicos e probiótico

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Abstract

The performance and hematologic responses of juvenile piavuçu fish (*Leporinus macrocephalus)* that were fed with feeds containing garlic, cinnamon, and yeast before being submitted to stress from capture were evaluated. The experiment was conducted in the Fish Nutrition Laboratory (*Laboratório de Alimentação e Nutrição de Peixes*), where 192 piavuçu juveniles (3.09 ± 0.8 g) were distributed among 16 tanks (170 L), with a density of 12 fish per tank. The experiment consisted of four treatments and four repetitions. Treatments included the addition of 10 g Kg^{-1} garlic, 10 g Kg^{-1} cinnamon, and $9x10^5$ UFC g^{-1} of *Saccharomyces cerevisiae* yeast, in addition to a control diet that included no additives. The performance, body indices, and survival of the fish were evaluated. After the performance test, the fish were submitted to stress from capture, and the effect of the additives as a stress reducer was evaluated through hematologic analyses. Better weight gain and feed conversion ratios ($P \leq 0.05$) were observed in the fish fed with diets containing garlic and cinnamon; however, no alterations were noticed in the nutritional composition of fish carcasses, regardless of the treatment they were submitted to. A reduction in the number of leukocytes of the fish submitted to stress in all treatments was verified. It is possible to conclude that diets supplemented with garlic and cinnamon at the level of 10 g Kg^{-1} led to a better performance, while not influencing the hematologic standards after the stress from capture.

Key words: Aquafarming, garlic, cinnamon, *Leporinus macroencephalus*, nutrition, *Saccharomyces cerevisae*

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Resumo

Verificou-se o desempenho e a resposta hematológica de juvenis de piavuçu (*Leporinus macrocephalus)*, alimentados com rações contendo alho, canela e levedura submetidos a estresse de captura O experimento foi realizado no Laboratório de Alimentação e Nutrição de Peixes, onde 192 juvenis de piavuçu (3,09 ± 0,8 g) foram distribuídos em 16 tanques (170 L), com densidade de 12 peixes/tanque, com quatro tratamentos e quatro repetições. Os tratamentos consistiam na adição de 10 g Kg^{-1} de alho, 10 g Kg^{-1} canela e $9x10^5$ UFC g^{-1} de levedura *Saccharomyces cerevisiae* e uma dieta controle, sem a inclusão de aditivo. Foram avaliados o desempenho, índices corporais e sobrevivência dos peixes. Após o ensaio de desempenho os peixes foram submetidos ao estresse de captura e o efeito dos aditivos como mitigador do estresse foi avaliado por meio de análises hematológicas. Observou-se melhor ganho de peso e conversão alimentar ($P \leq 0,05$) nos peixes alimentados com as dietas contendo alho e canela, porém não foram observadas alterações na composição bromatológica da carcaça em nenhum dos tratamentos. Verificou-se redução nos números de leucócitos dos peixes submetidos ao estresse em todos os tratamentos. Conclui-se que dietas suplementadas com alho e canela ao nível de 10 g Kg^{-1} apresentaram melhor desempenho, não influenciando nos parâmetros hematológicos após o estresse de captura.

Palavras-chave: Aquicultura, alho, canela, *Leporinus macrocephalus*, nutrição, *Saccharomyces cerevisiae*

Introduction

With new efforts being made towards increased productivity and the more intense fish production, current farming systems have begun to use high storage densities, resulting in a risk of stress to fish. This stress makes fish more prone to diseases, resulting in economic losses. To mitigate this effect, the use of antibiotics became a promising practice, both as immunostimulants and as growth promoters. However, some restrictions were imposed regarding the use of those compounds, and they are admissible only for therapeutic purposes (SERRANO, 2005).

Due to this premise, there has been a recent intensification of studies of environmentally correct functional additives that leave no residue and cause no danger to the health of humans or fish. It is expected that those additives have beneficial effects on the use of food, improving the zootechnical indices, and contributing to an intestinal balance. It is also expected that they contain substances that soften the effects of on fish physiology (ROMERO et al., 2012). Among the additives used in fish nutrition, probiotics, such as *Saccharomyces cerevisiae* yeast, stand out. The benefits of these microorganisms in animal performance are related to the better use of food, to the stimulation of the immune response, and to the selective colonization of the gastrointestinal tract, thus decreasing the burden of the disease (MEURER et. al., 2006).

Another important option in the search for productive improvements is the use of natural compounds that originate from plants, called phytogenic additives. According to Windisch et al. (2007), these compounds are defined as products extracted from plants that are used to improve development. Studies have demonstrated the benefits of adding different herbal extracts in fish nutrition and health, such as *Echinacea purpurea* (GUZ et al., 2011), *Allium sativum* (TALPUR; IKHWANUDDIN, 2012) and *Cinnamomum zeylanicum* (AHMAD et al., 2011), and *Camellia sinensis* (ABDEL-TAWWAB et al., 2010). However, the information found in the literature is still scarce. Garlic and cinnamon are being used in aquafarming because they improve diet palatability, stimulate the discharge of enzymes, regulate the intestinal microbiota, and kill microbes, while also providing positive results as immunomodulators (AHMAD et al., 2011; TALPUR; IKHWANUDDIN, 2012).

There are hundreds of endemic fish species that have aquafarming potential. However, there is a lack of studies regarding their physiology and nutrition.

Piavuçu (*Leporinus macrocephalus*) is a reophilic fish that is omnivorous, feeding off of a wide range of foods, including when they are supplied as feed. Studies on the nutrition and hematology of this species were developed due to the species' great production potential (TAVARES-DIAS et al., 1999; MARTINS et al., 2004a). However, there is no information regarding the effects of phytogenic additives on that species. Because of this, the goal of this study was to assess the effect of diet supplementation with garlic, cinnamon, and the *Saccharomyces cerevisiae* yeast in the nutrition of *Leporinus Macrocephalus* piavuçu juveniles, as well as the supplementation's potential benefits on the zootechnical parameters and their effect on reducing the stress from capture.

Materials and Methods

The experiment was conducted at the Laboratório de Nutrição e Alimentação de Peixes (Aquanut), Ilhéus, Bahia State, Brazil (14º 47' 20" S 39º 02' 58" W), for 35 days between December 8, 2012 and January 12, 2013. In this study, 500 piavuçu juveniles were used (2.0 ± 1.2 g), which came from the Canta Galo Farm (Ibirataia, Bahia State, Brazil). In the laboratory, the fish were immersed in a saltwater solution at 10 g L^{-1} for 15 minutes as a prophylactic measure. The fish were then brought to the laboratory temperature conditions and fed in a controlled way for two weeks, being housed in fiberglass tanks equipped with constant ventilation and biological filters.

Following this acclimation period, 192 piavuçu juveniles were selected, with an average initial weight of 3.09 g ± 0.8, and were distributed among 16 randomly aligned fiberglass tanks (170 L), with a density of 12 fish per tank, using four treatments and four repetitions. The tanks were filled with a continuous flow (0.084 m^3 h^{-1}) of water in a closed loop circulation system using a water pump (Dancor®, RJ, Brazil - ¾ hp) with biological filtering and constant ventilation through a blower (a 1-hp WEG). Each day, the tanks were siphoned to take off possible leftovers from the diets and feces. Each week, the physical and chemical variables of the water were measured in such a way that the temperature, oxygen dissolved (O_2D), oxygen saturation (O_2%), and hydrogen potential (pH) stayed at 29.3°C, 7.4 mg L^{-1}, 98%, and 7.2, respectively.

A base diet was formulated as the control (Table 1) with the help of the SUPERCRAC® computer software, based on the nutritional needs of *Leporinus macrocephalus*. The base diet was used to prepare the diets in the remaining treatments, through the addition of garlic, cinnamon, and yeast. To prepare the diets, ingredients were individually processed in a knife mill with a 0.5 mm-sieve, and then the additives were added, according to each treatment, being pelletized in a grinder with a 1.7-mm matrix. The granules were dried in a over drying at 55°C for 24 hours, then ground and stored in a refrigerator (12 °C) during the whole experiment period. In all treatments, the control formula was used; however, the diets varied according to the additive added. The control treatment had no additives, and the other ones received the addition of: powder garlic (10 g Kg^{-1}) (KITANO; YOKI ALIMENTOS S.A), powder cinnamon (10 g Kg^{-1}) (KITANO; YOKI ALIMENTOS S.A) and DS-5 *Saccharomyces cerevisiae* yeast (9 x 10^5 UFC g^{-1}) (CORNÉLIO et al., 2013), partially replacing the cellulose. During the experimental period, the fish were fed four times a day (9:00 am; 12:00 pm; 2:00 pm; 4:00 pm).

The *Saccharomices cerevisae*was isolated from the fermentation vats used to produce artisan sugar cane brandy. A test was conducted to verify cell purity, viability, and growth. In the laboratory, the yeasts were incubated for 30 hours in 200 mL of malt extract medium (2,0 g glucose; 2,0 g malt extract; 0,1 g peptone; 200 mL distilled water) in a shaker with a temperature of 30°C and a rotation of 150 rpm. Furthermore, they were transferred to 1000 mL of malt extract medium (20 g glucose; 20 g malt extract; 1.0 g peptone; 1000 mL distilled

water), and then incubated for 14 hours in a shaker with a temperature of 30°C and a rotation of 150 rpm. The purity was verified again, with the cells kept in contact with a methylene blue solution for 10 minutes. Following this, two centrifugations were performed in an ultracentrifuge. In the first one, the supernate was discarded and distilled water was added. During the second centrifugation, the distilled water was taken off and the yeast mass was collected from the bottom of the containers.

Table 1. Composition (g Kg^{-1}) of experimental diets.

Ingredient	Treatment			
	Control	Garlic	Cinnamon	Yeast
Soybean meal - 45%	306.90	306.90	306.90	306.90
Wheat bran	39.60	39.60	39.60	39.60
Cornmeal	49.50	49.50	49.50	49.50
Corn starch	129.88	129.88	129.88	129.88
Fish meal - 55%	306.90	306.90	306.90	306.90
Gluten meal - 22%	95.24	95.24	95.24	95.24
Powder cellulose	46.93	36.93	36.93	16.65
Mineral and Vitamin Premix[1]	10.00	10.00	10.00	10.00
Salt (NaCl)	5.00	5.00	5.00	5.00
BHT	0.02	0.02	0.02	0.02
Yeast (UFC.g^{-1})	-	-	-	$9x10^5$
Cinnamon	-	-	10.00	-
Garlic	-	10.00	-	-
Nutritional Composition (g Kg^{-1})				
Dry matter	954.52	950.32	945.74	945.47
Crude protein	36.95	36.94	35.30	36.00
Gross energy (Kcal Kg^{-1})	4129	4004	3968	4130
Ether extract	97.86	96.57	100.23	93.71

1Mineral and vitamin premix: Composition per Kg of product: Mg - 2,600 mg; Zn - 14,000 mg; Fe - 10,000 mg; Cu - 1,400 mg; Co - 20 mg; I - 60 mg; Se - 60 mg; Vit. A - 1,000,000 UI; Vit. D3 - 400,00 UI; Vit. E - 10,000 mg; Vit. K3 - 500 mg; Vit. B1 - 2,500 mg; Vit. B2 - 2,500 mg; Vit. B6 - 2,500 mg; Vit. B12 - 3,000 mcg; Vit. C - 35,000 mg; Folic Acid - 500 mg; Pantothenic Acid - 5,000 mg; Niacin - 10,000 mg; Biotin - 80,000 mcg; Choline - 200,000 mg; Methionine - 130 g; Inositol - 5,000 mg; Etoxiquin - 15,000 mg, BHT (Butylhydroxytoluene).

To analyze the yeast viability, after the diet was pelletized, 25 g was added to a 225 mL saline solution (8.5 g L^{-1}). Afterwards, 1 mL of that solution was transferred to a test tube containing 9 mL of saline solution, with serial dilutions then being performed. After that, 200 µL of each dilution was spread onto three plates containing PDA (Agar 1.5%; dextrose 1.0%; potato broth 2.0%) with the help of a Drigalsky spatula. Following that, the plates were incubated at 25ºC for three days. Afterwards, the cell viability count was performed. The same procedure was carried out at the end of the experiment for all of the diets used.

At the end of the experiment, all juveniles from each experiment unit were counted, weighed, and measured individually to determine the survival parameters, weight gain (WG), apparent feed consumption (FC), apparent feed conversion ratio (FCR), total length (TL, standard length (SL), and height (H).From each experiment unit,

two individuals were randomly collected and euthanized with benzocaine (3 mg L^{-1}), so that their gastrointestinal tract and liver could be taken out in order to determine digestive somatic and hepatosomatic indices.

The analyses for body composition were performed using 12 fish per treatment. At the end of the experiment, the fish fasted for 24 hours so that their digestive tract could be emptied. Then, they were euthanized following the same protocol used when some fish were gutted. The euthanized individuals were dried in a over drying at 55ºC for 72 hours, and ground whole for dry matter, crude protein, crude fat, and crude mineral analyses, according to AOAC's (2000) methodology.

In order to assess the effect of the garlic, cinnamon, and yeast additives on stress, the fish were submitted to stress from capture with a blood test before, right after, and 24 hours past the stressing event. At the end of the performance analysis, the fish were taken out the tanks with a dip net, and kept off the water for 30 s. This procedure was repeated four times, as per the Martins et al. (2004b) methodology. Two fish from each repetition at each sampling time were used for blood collection (0.5 mL from each fish via cardiac puncture, using syringes washed with heparin).

Regarding the hematologic analyses, the blood and white cell counts were performed under the hemocytometer method, using a toluidine blue solution at 0.01% (diluted in a sodium chloride solution) in a Thoma pipette. The hematocrit was verified through the microhematocrit method, with a rotation of 5,000 rpm for five minutes, in addition to further capillary reading. The hemoglobin level was determined using the cyanmethemoglobin method, conducted with a commercial kit (Analisa Diagnóstica®). Leukocyte differentiation was performed in a blood smear dyed via the fast panoptic method. The data obtained in the experiments were submitted to an analysis of variance (ANOVA) at a probability level of 5%, and, in the case of statistical difference, the Tukey Test was applied, using the statistical software R Core Team (2011).

Results and Discussion

During the experimental period, no mortality was observed in any of the treatments, thus demonstrating that the physical and chemical parameters of the water were inside of the comfort range, and that the food management was appropriate for the species. Differences were observed ($p \leq 0.05$) in the weight gain, feed consumption, and feed conversion parameters between treatments. The highest weight gain and lowest apparent feed conversion ratio were detected in the treatments containing the addition of garlic and cinnamon. The daily feed consumption of the fish in the garlic and yeast treatments were different between one another, but there were no differences in the cinnamon and control treatments (Table 2). There were also no effects from the additives tested ($p>0.05$) on the total length, standard length, height, or the digestive somatic and hepatosomatic indices of the fish. Alterations in the hepatosomatic index may indicate a possible noxious effect due to the removal of toxic substances from the body (TENG et al., 2013).

The highest weight gain observed, which occurred in treatments with the addition of garlic and cinnamon, might be related to the beneficial effect on food digestion, increasing the pancreatic discharges, reducing the passage rate, and promoting changes in the intestinal microbiota (PETROLLI et al., 2012). Talpur and Ikhwanuddin (2012) stated that the garlic increases antimicrobial, therapeutic, and immunostimulant activities, causing a better fish performance. Rafsanjani et al. (2006) studied the behavior of gastric acid and pepsin on rats provided with feeds supplemented with garlic, and observed an increase in the discharge of those substances, possibly resulting in better digestion and performance for the animal. Guo et al. (2012) demonstrated the applicability of garlic to fish nutrition by supplementing the diet of groupers

(*Epinephelus coioides*) with garlic (13 g Kg^{-1}) for 14 days, observing an increased weight gain, better food efficiency, and longer survival of those fish when challenged by *Aeromonas hydrophila*. The authors stated that these effects occurred because garlic is bactericide. Shalaby et al. (2006), when using garlic and chloramphenicol as additives in the diet of Nile tilapias, found a better performance and protein efficiency rate as the level of garlic increased, thus recommending its use as a growth promoter and antibiotic. Nevertheless, the excessive addition may be harmful due to allyl sulfide concentrations, which interfere in metabolism, resulting in low growth (LEE; GAO, 2012).

Table 2. Mean performance values of piavuçu juveniles fed with diets containing garlic, cinnamon and yeast.

Variable	Treatment				CV (%)	(*p*)
	Control	Garlic	Cinnamon	Yeast		
WG[1]	6.54[B]	8.44[A]	8.53[A]	5.72[B]	9.61	0.002
DFC[2]	0.19[AB]	0.24[A]	0.20[AB]	0.19[B]	8.12	0.025
DWG[3]	0.23	0.23	0.21	0.18	26.46	0.687
FCR[4]	0.98[B]	0.96[AB]	0.81[A]	1.11[B]	7.23	0.004
TL[5]	87.17	93.32	92.03	85.83	3.40	0.054
SL[6]	73.29	8.59	75.12	73.00	5.68	0.345
H[7]	21.52	3.23	22.88	21.02	4.78	0.097
HSI[8]	0.82	0.78	0.73	0.90	19.03	0.618
DSI[9]	3.04	3.02	2.87	3.27	7.65	0,286

Mean values followed by different letters on the lines are different among one another, at a 5% probability level, as per the Tukey test ($P<0.05$).
[1]WG: Weight gain (g); [2]DFC: Daily feed consumption; [3]DWG: Daily weight gain (g Kg^{-1}); [4]FCR feed conversion ratio; [5]TL: Total length; [6]SL: Standard length;[7] H: Height; [8]HSI: Hepatosomatic index; [9]DSI: Digestive somatic index.

The beneficial effects on health of cinnamon are related to the chemical compounds that compose it, such as the active ingredient cinnamaldehyde, which is responsible for the smell, as well as the antioxidant, antimicrobial, and antifungal activities (SINGH et al., 2007). Boudry and Perrier (2008) stated that cinnamaldehyde induced the discharge of HCO_3^- and CL^- by intestinal epithelium cells, also having an effect in the adhesion of bacteria to the intestinal microbiota. The reduced bacterial load resulted in less competition for nutrients, as well as an increased area of mucosal absorption (SANDERS, 2003). Rattanachaikunsopon and Phumkhachorn (2010) observed the antimicrobial activity of cinnamon when challenging Nile tilapias fed with cinnamon essential oil with *Streptococcus iniae*. This phenomenon originated from hydrofobicity, making the cell membrane more permeable. Ahmad et al. (2011) also observed positive effects from including cinnamon in the diet. These researchers found a better performance and resistance in tilapias infected by *Aeromonas hydrophila* when provided feeds containing 10 g Kg^{-1} cinnamon. They posited that this additive would lead to improvements in health and performance. Abdel-Wahab et al. (2007) assured that supplementation with only 5 g Kg^{-1} cinnamon would be enough to achieve better performances.

The supplementation of piavuçu juveniles with *Saccharomyces cerevisiae* yeast has not yet resulted in improvements in zootechnical performance parameters. This result is similar to the ones obtained by Meurer et al. (2006), who fed Nile tilapias with a commercial product containing yeast in a

concentration of $1x10^5$ UFC kg^{-1}. However, Li and Gatlin (2003) stated that yeast positively influenced growth and the use of foods in a hybrid (*Morone saxatilis* × *M. chrysops)*. The lesser expressiveness in the zootechnical indices of fish whose diets were supplemented with yeast might be justified by the low concentration of that microorganism in the diet ($9x10^5$ UFC g^{-1}). According to Stefe et al. (2008), a probiotic microorganism only exerts influence in an ecosystem when its population is greater than or equal to 10^7 UFC mL^{-1} of the contents. The inclusion of the probiotic and phytogenic additives did not promote changes in piavuçu's body composition, presenting mean values of 26.18 g Kg^{-1}, 38.86 g Kg^{-1}, and 4667 Kcal Kg^{-1}, for dry matter, crude protein, and gross energy, respectively. The results obtained in that study were different than those found by Ahmad et al. (2011), who observed greater values for dry matter and crude protein, and lower values for crude mineral in Nile tilapias supplemented with cinnamon at 5 g Kg^{-1}. Abdel-Tawwab et al. (2008), after supplementing Nile tilapias' diets with 1,0-5,0 g kg^{-1} of yeast for 12 weeks, obtained better levels of crude protein and crude mineral, in addition to lower fat contents, as compared to the control treatment.

No significant differences were observed in the hematocrit values or hemoglobin levels for fish provided with feeds containing garlic, cinnamon, and yeast in the periods prior to stress, during the stress from capture, and 24 hours thereafter. The hematocrit and hemoglobin values found in this study were below those considered normal for the healthy adult piavuçu. Those values are, respectively, 32.4 ± 4.5 % e 10.8 ± 1.2 g dL^{-1}, (TAVARES-DIAS et al., 1999). Those alterations may arise from differences in the environmental conditions and cultures employed in each experiment, acute infections, anemia (SILVA et al., 2008), intoxication, environmental stress (VERAS et al., 2013), or due to hemodilution by anticoagulant agents (HOUSTON et al., 1996).

There were no statistical differences observed for leukocyte, monocyte, or lymphocyte values (Table 3) for the fish across all treatments, regardless of when they were evaluated, or of the blood parameters. Nevertheless, right after stress, the number of leukocytes was reduced by 32.8%, on average, across all treatments, with a recovery happening after 24 hours ($p<0.05$). Physiological leukocytosis happens due to acute stress, significantly diminishing the leukocyte count (TAVARES-DIAS et al., 1999). This happens due to the primary stress action, the release of corticosteroids (FALCON et al., 2008) and catecholamines. These hormones favor hemoconcentration, due to the increased interstitial pressure that promotes an increase in fluid passage to the interstitial space (ALLEN; PATTERSON, 1995). This, in turn, redistributes leukocytes from blood vessels to the tissues, causing immunosuppression (KOSER; OLIVEIRA, 2011). Falcon et al. (2008) also found alterations in leukocyte levels after stress. The researchers observed a drop in the leukocyte numbers in Nile tilapias submitted to stress. Garcia et al. (2012), after submitting Nile tilapias to an acute and chronic stress challenge, also observed a reduction in erythrocyte, erythroblast, and leukocyte levels.

No differences were observed in the number of segmented neutrophil numbers among the treatments across the different periods (Table 4). Variations in the percentages of thrombocytes were observed for fish given either the garlic and or the control treatments, as compared to the periods prior to stress, during stress, and 24 hours after stress ($p<0,05$). Martins et al. (2004a) found an increase in the number of neutrophils during the stress period in Nile tilapias. Barton and Iwama (1991) stated that neutrophilia is a typical response triggered by the presence of a stress-inducing agent.

Table 3. Mean leukocyte and monocyte values in piavuçu juveniles fed with diets containing garlic, cinnamon and yeast.

	Total leukocyte (10^3 µL^{-1})			CV (%)	(p)	Monocyte (%)			CV(%)	(p)
	Before	Stress	After 24 h			Before	Stress	After 24 h		
Control	18317^{A}	5100^{B}	18633^{A}	16.34	0.001	2	1	1	54.55	0.111
Garlic	19358^{A}	8133^{B}	17600^{A}	18.01	0.005	2	1	1	74.23	0.921
Cinnamon	17633^{A}	5967^{B}	18767^{A}	12.23	0.002	2	2	2	60.00	1.000
Yeast	20250^{A}	6000^{B}	18700^{A}	21.68	0.003	1	2	1	47.24	0.178
VC (%)	20.86	25.01	6.81			57.74	57.28	70.59		
(*p*)	0.855	0.190	0.644			0.330	0.389	0.821		

Mean values followed by different capital letters on the lines are different among one another, at a 5% probability level, as per the Tukey test (P<0.05).

Table 4. Mean segmented neutrophil and thrombocyte values in piavuçu juveniles fed with diets containing garlic, cinnamon and yeast.

	Segmented neutrophil (%)			CV (%)	(*p*)	Thrombocyte (%)			CV (%)	(*p*)
	Before	Stress	After 24 h			Before	Stress	After 24 h		
Control	67	55	78	14.69	0.070	4^{AB}	6^{A}	2^{B}	34.36	0.044
Garlic	67	63	66	16.23	0.905	7^{A}	7^{A}	4^{B}	20.41	0.022
Cinnamon	79	40	61	27.72	0.138	6	5	4	15.97	0.196
Yeast	74	68	65	14.10	0.597	5	7	3	30.59	0.074
CV (%)	16.14	35.86	2.74			24.12	24.12	26.72		
(*p*)	0.553	0.576	0.163			0.071	0.501	0.133		

Mean values followed by different letters on the lines are different among one another, at a 5% probability level, as per the Tukey test (P<0.05).

Thrombocyte reductions of 33 and 57% were observed in garlic and control treatments, respectively, 24 hours after stress was imposed. Garcia et al. (2007), observed a reduction in thrombocyte numbers in pacus challenged with *A. hydrophila* 24 hours after said challenge, suggesting that this phenomenon resulted in the migration of those cells to the inflammation site. In Nunes et al. (2014) study, the researchers analyzed the effect of yeast on macrophage migration after the inoculation of *Saccharomyces cerevisae* in the coelomic cavity of *Pseudoplatystoma* spp. catfish. They observed the two-hour interval after the inoculation to promote maximum macrophage activity and migration into the cavity. In addition, Garcia et al. (2012), after submitting Nile tilapias to chronic stress, obtained a 69% increase in the values of those cells after 10 days of stress in fish fed with diets containing the additive Ergosan. Tavares-Dias and Moraes (2007) posited that thrombocyte function in teleost fishes is not limited to blood coagulation, because those cells have a great phagocytic activity and are also responsible for body defense.

Conclusion

1. The inclusion of 10 g Kg^{-1} of garlic or cinnamon in the diets of piavuçu juveniles provides a better performance for the fish, without altering the carcass characteristics. However, those additives also showed no effects on stress reduction.

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