

ALTERNATIVE TREATMENT FOR *ANACANTHORUS PENILABIATUS* (MONOGENEA: DACTYLOGYRIDAE) INFECTION IN CULTIVATED PACU, *PIARACTUS MESOPOTAMICUS* (OSTEICHTHYES: CHARACIDAE) IN BRAZIL AND ITS HAEMATOLOGICAL EFFECTS

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Summary :

The present work, studied the effect of 0, 1,000, 1,500 and 2,000 mg of garlic powder/kg dry ration for *Piaractus mesopotamicus* (Osteichthyes: Characidae), weighting 73.6 ± 39.4 g and measuring 15.0 ± 2.7 cm, fed for a period of 15, 30 and 45 days. Fifteen days after treatment with 1,000 and 2,000 mg of garlic/kg dry ration, significant reduction of *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) in the gills was related. Nevertheless, the addition of garlic to the ration caused significant increase in the erythrocyte number and in the thrombocyte percentage in the circulating blood. However, a decrease in the lymphocyte percentage was also observed. After 45 days, fish fed with garlic showed significant increase in the erythrocyte number, leucocyte, haemoglobin rate, hematocrit and thrombocyte.

KEY WORDS : Brazilian fish, *Piaractus mesopotamicus*, garlic, treatment, haematology.

Résumé : TRAITEMENT ALTERNATIF POUR L'INFECTION À *ANACANTHORUS PENILABIATUS* (MONOGENEA: DACTYLOGYRIDAE) DU PACU D'ÉLEVAGE, *PIARACTUS MESOPOTAMICUS* (OSTEICHTHYES: CHARACIDAE) DU BRÉSIL ET SES EFFETS HÉMATOLOGIQUES

Ce travail a étudié l'effet de 0, 1 000, 1 500 et 2 000 mg d'ail en poudre par kg de ration sèche sur *Piaractus mesopotamicus* (Osteichthyes: Characidae), pesant $73,6 \pm 39,4$ g et mesurant $15,0 \pm 2,7$ cm, alimenté pendant une période de 15, 30 et 45 jours. Après 15 jours d'alimentation avec 1 000 et 2 000 mg d'ail, une diminution significative du nombre d'*Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) sur les branchies a été observée. L'addition de l'extrait d'ail dans la ration alimentaire a provoqué une augmentation significative du nombre d'érythrocytes et de thrombocytes dans le sang du poisson. Toutefois, une diminution des lymphocytes a été aussi observée. Après 45 jours de traitement, le poisson alimenté avec ail a montré une augmentation significative des taux d'érythrocytes, de leucocytes, de thrombocytes et d'hémoglobine.

MOTS CLÉS : poissons brésiliens, *Piaractus mesopotamicus*, ail, traitement, hématologie.

INTRODUCTION

In the last years the Brazilian aquaculture has shown significant interest that culminated with increase of fish farming and fee fishing facilities. The dispute for fingerlings and juveniles of the most important freshwater cultivated fishes was responsible for its development. When the animals are transported from the nature to the captive environment, factors such as nutrition deficiency, alterations in the water quality, handling stress or infectious diseases may occur. In Brazil, monogeneans have occupied a special place causing significant mortalities in cultured freshwater fish either alone or associated with other parasites (Martins *et al.*, 2000). Therefore, a great number of therapies have been applied. These involve formaldehyde

or organophosphate that are dangerous to the human health and alter the ponds' ecosystem such as phytoplankton and zooplankton essential to the aquatic environment (Noga, 1996; Sipaubá-Tavares & Rocha, 2001), mebendazole, albendazole and praziquantel (Schmahl & Taraschewski, 1987; Schlotfeldt *et al.*, 1988; Buchmann & Bjerregaard, 1990; Tojo & Santamarina, 1998). This is specially true when such products are applied in the water of tropical climate that are present in a special aquatic system. Alternative products have been used in aquaculture facilities around the World, such as sodium chloride, lime, onion, garlic (Roth *et al.*, 1993), in the treatment of carp *Cyprinus carpio* parasitized with *Capillaria* sp nematode (Pena *et al.*, 1988), addition of vitamin C in the diet against bacterial or protozoan infections (Wahli *et al.*, 1995, 1998), and to reduce the monogenean number in the gills of cultivated fish (Martins, 1998). In addition, intraperitoneal injection of garlic extract in sea bass *Dicentrarchus labrax* experimentally infected with *Mycobacterium marinum* (Colorni *et al.*, 1998) has been used and bath treatment with garlic against trichodinids of the eel *Anguilla anguilla* has proved successful (Madsen *et al.*, 2000).

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Garlic, a member of the genus *Allium*, has been cultivated for thousands of years for medical purposes in the treatment of several kinds of human diseases. However, there is no data available regarding the haematological parameters and the treatment with extracts or minced garlic in fish culture.

In this work, different amounts of garlic powder were added to the feed of an important cultivated freshwater fish in Brazil, *Piaractus mesopotamicus*, naturally parasitized with *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) in the gills, and their effect on the level of parasitism and the haematological parameters was evaluated.

MATERIAL AND METHODS

The experiment was developed at the Laboratory of Pathology of Aquatic Organisms of the Research Center in Animal Health and Aquaculture Center, São Paulo State University, Jaboticabal, SP, Brazil. A total of 108 *P. mesopotamicus* Holmberg, 1887 (pacu) was randomly distributed in 12 flow-through aquaria of 250 L, receiving 1 L/min of water divided in four treatments (including control) and three replicates of each treatment. At the beginning of the experiment the control group had 75.3 ± 30.1 g weight and 15.0 ± 2.8 cm total length; the group fed with 1,000 mg garlic had 88.6 ± 43.3 g and 16.0 ± 2.5 cm; the group fed with 1,500 mg garlic had 80.4 ± 38.5 g and 15.4 ± 2.5 cm; and the group fed with 2,000 mg garlic had 71.0 ± 41.4 g and 14.5 ± 3.0 cm. During the experiment, the water temperature was maintained at $30.20 \pm 0.72^\circ\text{C}$ measured with a bulb thermometer, pH 7.86 ± 0.18 measured with an electronic Corning pH-meter, electric conductivity 171.00 ± 3.68 $\mu\text{S}/\text{cm}$ measured with a Corning conductivitymeter, dissolved oxygen 5.38 ± 0.70 mg/l measured with a YSI-Mod.50 oxy-meter and alkalinity 99.01 ± 32.59 mg/l according to Goltermann *et al.* (1978).

EXPERIMENTAL DIET

The fishes were fed with 1,000; 1,500 and 2,000 mg of allila disulphide (garlic) powder in 10 %/kg dry ration. Isocaloric and isoproteic experimental diets contained 3,000 Kcal energy/kg, 28 % crude protein, 6 % fat, 9 % ashes, 9 % fibre and 8 % humidity. The ration was previously crushed, mixed with the respective quantity of garlic powder and pelletized. The ration of the control group was submitted to the same procedure but without garlic. After 15, 30 and 45 days of feeding, nine animals of each treatment were anesthetized with 1 g benzocaine solution to 15 L of water. For each treatment, the samples were tasted twenty times by seven different persons for off-flavour detection.

PARASITOLOGICAL ANALYSES

The fishes utilized in this work were naturally parasitized with *A. penilabiatus* Boeger, Husak & Martins 1995 (Monogenea: Dactylogyridae). According to Boeger *et al.* (1995), the gills were removed, placed in a formalin solution 1:4,000 for one hour, shaken and fixed in 5 % formalin. To remove all the helminths, the gills were carefully scraped with the aid of a brush before the counts. Mean intensity (total number of parasite/number of infected host) was calculated according to Bush *et al.* (1997). The percentage efficacy was calculated as follows:

$$\% \text{ Efficacy} = \frac{\text{Mean number of parasites from control group} - \text{Mean number of parasites from treated group}}{\text{Mean number of parasites of control group}} \times 100$$

HAEMATOLOGICAL ANALYSES

The blood was withdrawn (0.5 ml) from the caudal vein into a syringe containing a drop of 10 % EDTA solution. This blood was utilized to measure the haemoglobin rate by using Collier (1944) method, hematocrit according to Goldenfarb *et al.* (1971) method, total leucocyte and erythrocyte number in a Neubauer chamber after dilution in 0.65 % sodium chloride solution and 1 % neutral red. For differential count of leucocytes and thrombocytes, air-dried blood smears were prepared using Rosenfeld (1947) method in which a hundred cells were counted for the establishment of each cell contents.

STATISTICAL ANALYSES

The percentage from the differential count of blood cells were transformed in $\text{arc sin}(\sqrt{P + 0.5})$. The averages were compared by the Tukey test, at 5 % probability (Steel & Torrey, 1980).

RESULTS

At the end of the experiment no difference was observed on fish development, which was found to be as follows: the control group showed 66.8 ± 35.9 g and 14.5 ± 2.3 cm; the group fed with 1,000 mg garlic showed 82.1 ± 39.1 g and 15.1 ± 2.4 cm; the group fed with 1,500 mg garlic showed 59.3 ± 27.9 g and 13.8 ± 1.6 cm; and the group fed with 2,000 mg garlic showed 71.1 ± 29.4 g and 14.5 ± 1.8 cm.

PARASITOLOGICAL ANALYSES

Forty five days after treatment with the experimental diet containing garlic, a drastic reduction in the mean

Mean intensity of parasites		Days of feeding			
Treatment (mg of garlic/kg dry ration)	15	30	45	F test	
0	107.73 Bb	39.27 Ab	15.40 Aa	45.21**	
1,000	50.87 Ba	19.40 Aab	13.13 Aa	8.05**	
1,500	38.07 Ba	13.27 Bab	12.13 Aa	4.23*	
2,000	44.15 Ba	10.67 Aa	5.60 Aa	8.64**	
F test	20.21**	3.28*	0.35 ns	–	

Thrombocyte		Days of feeding			
Treatment (mg of garlic/kg dry ration)	15	30	45	F test	
0	64.88 Bb	80.38 Aa	77.88 Aa	7.84**	
1,000	65.88 Bb	89.00 Aa	81.00 Aa	15.6**	
1,500	84.33 Aa	87.89 Aa	88.11 Aa	0.51 ns	
2,000	85.77 Aa	82.33 Aa	90.11 Aa	1.72 ns	
F test	12.18**	1.65 ns	3.15*	–	

Lymphocyte		Days of feeding			
Treatment (mg of garlic/kg dry ration)	15	30	45	F test	
0	32.55 Aa	17.22 Ba	20.78 Aba	5.97*	
1,000	29.89 Aa	7.55 Ba	17.88 Ba	11.58**	
1,500	11.78 Ab	8.44 Aa	10.55 Aa	0.26 ns	
2,000	10.77 Ab	15.49 Aa	7.89 Aa	1.37 ns	
F test	9.77*	1.75 ns	2.67 ns	–	

Table I. – Mean values of the interaction between treatments and days of food upon mean intensity of *Anacanthorus penilabiatius*, thrombocyte and lymphocyte percentages in *Piaractus mesopotamicus* fed with garlic for a period of 15, 30 and 45 days. Capital letters for comparison between days of feeding and minuscule letters for comparison between treatments (ns: not significant, * $P < 0.05$, ** $P < 0.01$).

intensity of parasites *A. penilabiatius* in the gills was observed. On the other hand, no difference ($P < 0.01$) between the animals fed with garlic was seen. Fifteen days after treatment with 1,000 and 2,000 mg of garlic/kg dry ration, a significant reduction of the mean intensity was observed. Although the treatment with 1,500 mg of garlic/kg dry ration caused reduction of parasite after 45 days of feeding, the Table I shows that the parasite population was reduced 30 days after feeding with 2,000 mg of garlic/kg dry ration. Nevertheless, it is interesting to comment that the animals fed without garlic showed significant decrease in the mean intensity of parasites after 30 days. But it was not significant between 30 and 45 days after the feeding.

Moreover, no flavour alteration in the samples tasted by different persons was noticed.

HAEMATOLOGICAL ANALYSES

Significant increase ($P < 0.01$) in the total count of leucocyte and in the haemoglobin rate during all the time of collecting was observed (Table II). In all animals fed with garlic it was possible to notice significant increase ($P < 0.01$) in the erythrocyte number 30 days after feeding. However, no difference in the hematocrit and haemoglobin rate between the treatments with garlic was found. Table II shows that haemoglobin rate

has presented a progressive increase in the collected samples.

When the percentage of the defense blood cells was analysed, significant increase ($P < 0.05$) in the thrombocyte number in fish fed with 1,500 and 2,000 mg of garlic/kg dry ration was observed. After 15 days of feeding with control diet and 1,000 mg of garlic/kg dry ration, increases in the thrombocyte number were also reported. On the other hand, 1,000 mg of garlic/kg dry ration caused reduction in the lymphocyte number in the circulating blood of pacu. The same occurred after 30 and 45 days in the animals fed with control diet and 1,000 mg garlic/kg dry ration ($P < 0.01$). Other cells contained in the blood of tropical freshwater fish did not show alteration that was related to treatment or duration of feeding.

DISCUSSION

During the experiment, it was not observed refusal of the food in fish fed with garlic in the ration. Pena *et al.* (1988) compared the effect of fresh minced garlic, hexane extract of garlic and aqueous extract of garlic (200 mg/l) in the treatment of carp infected with *Capillaria* sp. In this situation, the fresh minced garlic treatment showed 100 %

Analyzed parameters											
Treatment	Erythrocyte (x10 ³ /µl)	Leucocyte (number/µl)	Haemoglobin (g/dl)	Hematocrit (%)	Thrombocyte (%)	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)	Eosinophil (%)	S.G.C. (%)	MI
0	2,069.22 B	6,298.11 A	10.88 A	40.00 A	74.38 B	23.52 A	0.74 A	0.85 A	0.11 A	0.11 A	54.13 A
1,000	2,790.78 A	7,131.33 A	12.54 A	43.33 A	78.63 AB	18.44 AB	1.15 A	0.81 A	0.11 A	0.18 A	27.80 B
1,500	2,989.22 A	4,375.22 A	11.89 A	38.89 A	86.78 A	10.26 B	1.37 A	1.15 A	0.07 A	0.18 A	21.15 B
2,000	2,789.55 A	4,524.78 A	12.02 A	39.55 A	86.07 A	11.38 B	1.22 A	0.90 A	0.07 A	0.15 A	20.14 B
F test	11.11**	2.94 ns	2.24 ns	0.65 ns	7.57*	5.97*	1.12 ns	0.39 ns	0.08 ns	0.41 ns	14.78**
Analyzed parameters											
Days of food	Erythrocyte (x10 ³ /µl)	Leucocyte (number/µl)	Haemoglobin (g/dl)	Hematocrit (%)	Thrombocyte (%)	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)	Eosinophil (%)	S.G.C. (%)	MI
15	2,507.17 b	1,103.33 c	7.63 c	32.33 b	74.22 b	21.25 a	2.02 a	0.61 b	0.00 a	0.14 a	60.21 a
30	2,076.00 b	5,201.08 b	12.00 b	33.58 b	84.90 a	12.18 b	0.94 ab	1.32 a	0.17 a	0.25 a	20.65 b
45	3,395.91 a	10,442.60 a	15.86 a	55.42 a	84.26 a	14.28 b	0.39 b	0.86 ab	0.11 a	0.08 a	11.57 b
F test	11.61**	35.52**	47.67**	47.80**	13.30**	8.36**	4.40*	4.80*	1.40 ns	1.33 ns	52.65**
F test	0.37 ns	1.15 ns	0.29 ns	0.40 ns	4.12*	3.61*	0.62 ns	1.86 ns	0.87 ns	0.51 ns	4.49**

Table II. – Mean values of haematological analyses and mean intensity (MI) of *Anacanthoborus penilabiatius* in the gills of *Piaractus mesopotamicus* fed with garlic (T) for a period of 15, 30 and 45 days (D). Capital letters for comparison between treatments and minuscule letters for the days of feeding (S.G.C: special granulocytic cell; * P < 0.05; ** P < 0.01; ns: not significant).

reduction in egg count, significantly superior to the reduction obtained in the other groups. On the other hand, Boxaspen & Holm (1991) were not able to eliminate sea lice from the infected salmonids fed with garlic-coated salmon pellets. Recently, in eels, Madsen *et al.* (2000) observed reduction of *Trichodina jadranica* from the infected fish 24 hours after bath treatment with 200 ppm of fresh raw garlic.

The monogenean *A. penilabiatius* is normally found on the skin surface or in the gills of pacu, *P. mesopotamicus*. When the cultivated fish is exposed to stressors, increases in the parasite population may occur (Martins, 1998). Factors responsible for stress in tropical fish can be listed such as the presence of high organic matter, decreased dissolved oxygen, alterations in the water temperature, ammonia levels, high stocking density, stress of transport or nutritional deficiency.

Based on this work, the addition of garlic to the ration of *P. mesopotamicus* was associated with significant reduction of *A. penilabiatius* when the fishes were fed with 1,000 and 2,000 mg/kg dry ration for 30 days. The treatment with 1,500 mg/kg dry ration was the only one that caused decrease in the parasite number after 45 days of feeding. On the other hand, the reduction of parasites observed in the control group may be related to the maintenance of fish in aquarium, that does not offer sufficient nutrient intake to favour parasite reproduction, as was also observed in the same fish by Martins (1998). In fact, the water quality in aquarium is different from that observed in fish ponds. Another hypothesis is the increase of the natural response of fish. Several different substances have been described which may participate in nonspecific immunity: components in the fish surface mucus and serum as complement-like activity, lysozyme, C-reactive pro-

tein, transferrin and interferon-like molecules in antiviral responses (Evans & Gratzek, 1989). Evidence that certain fish species may produce immunity to monogenean infections has been shown in guppies against *Gyrodactylus bullatarudis* (Scott & Robinson, 1984) and sticklebacks (Lester & Adams, 1974). However, the exact mechanism is unknown and their resistance may be involved with the mucus production. Then, Moore *et al.* (1994) suggested that serum and mucus of juvenile sole, *Pleuronectes vetulus* contain factors involved in the resistance to *G. stellatus*. Further studies to identify and characterise anti-monogenean factors in fish, specially in Brazil must be carried out.

As to the haematological parameters in normal conditions, the untreated fish of this work showed higher values of erythrocyte number, haemoglobin rate, hematocrit and lower values of neutrophil, monocyte and special granulocytic cells when compared to the same fish cultivated in an intensive polyculture system (Tavares-Dias *et al.*, 1999b). The fishes that were fed with garlic showed increase in erythrocyte and thrombocyte number and, decrease in lymphocyte percentage that were the most important results obtained. Contrarily to these observations, increase in neutrophil percentage and erythrocyte number in fingerling of channel catfish, *Ictalurus punctatus* exposed to malachite green (Grizzle, 1977), in *Oncorhynchus masou* infested with *Argulus coregoni* (Shimura *et al.*, 1983); in rainbow trout exposed to copper (Dick & Dixon, 1985); in carp infested with *Argulus* sp seven days after treatment with Neguvon (Ranzani-Paiva *et al.*, 1987); increase in haemoglobin rate of *Catla catla* treated with bovine serum albumin (Vardhani, 1992); reduced erythrocyte number and haemoglobin rate in *Cyprinion watsoni* exposed to zinc and copper treatment (Shah

et al., 1995; Ranzani-Paiva *et al.*, 1997) and, in *P. mesopotamicus* infested with *Argulus* sp after organophosphate treatment (Tavares-Dias *et al.*, 1999a) were related. On the other hand, some anthelmintics such as levamisole was effective in augmenting the non-specific defence system of Atlantic salmon (Findlay & Munday, 2000) Moreover, when the same fish species *P. mesopotamicus* was supplemented with vitamin C in the diet (Martins *et al.*, 1995), no changes in haematological parameters were observed. Recent studies with cultivated freshwater fish in Brazil, showed increase in hematocrit, erythrocyte number, leucocyte and haemoglobin rate of *P. mesopotamicus* exposed to mebendazole treatment (Martins *et al.*, 2001). In this work, significant increase in the erythrocyte number and in the thrombocyte percentage was observed. This experiment encourages researchers to search alternative methods for control of fish helminth parasites. Still, further studies must be carried out with the addition of garlic to the ration of Brazilian fish, specially those cultivated in ponds. Thus, the study of aquatic parameters as temperature, pH, dissolved oxygen, ammonia and its effects on phytoplankton and zooplankton population must be encouraged.

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