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A N I N T E R N A T I O N A L J O U R N A L

Effects of Chromium Supplementation on the Infrapopulations of *Anacanthorus penilabiatus* (Monogenoidea) and *Piscinoodinium pillulare* (Dinoflagellida) Parasites of *Piaractus mesopotamicus* (Characidae)

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ABSTRACT

This study evaluated the parasitism by Anacanthorus penilabiatus *(Monogenoidea) and* Piscinoodinium pillulare *(Dinoflagellida) in the gills of "pacu",* Piaractus mesopotamicus *supplemented with chromium in the diet. Randomised delineation by using factorial scheme (4x2x2) for Monogenoidea and 4x2x3 for Dinoflagellate with four levels of chromium (0, 6, 12, 18mg/kg diet), two stocking densities (4kg/m^3 and 20kg/m^3), two classes of length (higher and lower than 17 cm) for a period of 7, 60 and 90 days, and four replicates was used. The fishes in low density which received 12 and 18mg/kg showed decreased monogeneoidea values, seven days after the experiment begin. Six, 12 and 18 mg/kg caused reduction in the dinoflagellate number in the fishes maintained at 20kg/m^3. It was possible that chromium supplementation (12 e 18mg/kg) favoured the fishes health by reducing Monogenoidea and Dinoflagellida number in lower and higher densities, respectively.*

Key words: *Piaractus mesopotamicus*, chromium, stocking density, *Piscinoodinium pillulare*, *Anacanthorus penilabiatus*

INTRODUCTION

New technologies in intensive fish culture and diets of good quality are essential as the development of aquaculture in Brazil. This system is responsible for chronic stress due to high stocking density as supported by Tort et al. (1996), Rotlland et al. (1997) and Montero et al. (1999). Stressors may provoke alterations in the host equilibrium affecting the health of fish. (Wedemeyer, 1997). Cortisol release is the most important response stress in the fish (Mazeaud and Mazeaud, 1981; Camacho, 1999; Belo, 2002; Brum, 2003). Its elevated concentration reduces the defense response of the fish (Pickering and Pottinger, 1985; Maule et al., 1989; Belo, 2002; Brum, 2003) and results in increased susceptibility to parasitic and infeccious diseases (Barton and Iwama, 1991).

Vitamins and minerals are important in the physiological processes minimizing the effects of stressor agents. Hexavalent chromium is toxic but the trivalent form reduces the stress. The last one, improves the insulin action and reduces the

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cortisol concentration in stressed animals (Anderson, 1994, Commitee on Animal Nutrition, 1997, Hasten et al., 1997, Hossain et al., 1998). Thus, the dietary supplementation with chromium may constitute an alternative to reduce the effects of parasitism in fish (Pickering and Pottinger, 1985).

Monogenoidea presents high host specificity as related by Rhode (1993) and Kritsky et al. (1997). *Anacanthorus penilabiatus* Boeger, Husak and Martins (1995) (Dactylogyridae: Anacanthorinae) parasite of "pacu" *Piaractus mesopotamicus* Holmberg, (1887) , represent the main source of mortalities in the Northeast São Paulo (Martins et al., 2002). Elevated number of parasites indicates poor water quality, which favours the parasite reproduction (Thatcher and Brites-Neto, 1994; Noga, 1996) and induce stress in fish (Yadav and Akela, 1993; Alkahem, 1994). Moreover, the high stocking density causes food competition and lower utilization of dietary nutrients (Rotlland et al., 1997; Tort et al., 1996; Montero et al., 1999).

Low water quality and inadequate handling also favour the reproduction of dinoflagellate *Piscinoodinium pillulare* (Schäperclaus, 1954) (Lom, 1981) and high mortalities of fishes (1,500 fishes/day for 15 days) were found in São Paulo State (Martins et al., 2001).

Thus, the objective of this study was to evaluate the parasitological indexes of monogenoidea and dinoflagellate in the gills of *P. mesopotamicus* supplemented with trivalent chromium in diet during 90 days maintained in two different stocking densities.

MATERIAL AND METHODS

Fish maintenance

Piaractus mesopotamicus with averages of 100g of weight and 20cm of total length were acclimatized for 10 days in 32 aquaria of 500 l capacity, provided with dechlorinated water 1 $l.min^{-1}$. Fishes were fed *ad-libitum* with dry ration, twice a day. After this period the animals were redistributed in two stocking densities, 4 and 20 kg/m^3, as suggested by Rotlland et al. (1997) and fed for 90 days.

Experimental trials

The monogenoidea experiment was constituted by 16 treatments, using factorial scheme 4x2x2 wtih four levels of dietary chromium (0, 6, 12, 18 mg CQC/kg diet), two stocking densities (4 and 20 kg/m^3), two classes of length (higher and lower than 17 cm) with four replicates. The dinoflagellate experiment was designed with 24 treatments in factorial scheme 4x2x3 with the same four levels of chromium and stocking densities, and three periods of evaluation (7, 60 and 90 days) also with four replicates.

Diet, feeding and handling

The diet was composed by fish meal (13%), soy bean meal (23%), wheat meal (21%), crushed corn (25%), rice meal (16%), soy oil (1%), mineral and vitamin supplement (1%) with crude protein 26%, ether extract 6.23%, crude fibre 6.25%, crude energy 3,900 kcal/kg diet and relation calcium/phosphorus 1.45:1. Composition of mineral and vitamin supplement without chromium (nutrient/Kg) was according to A.O.A.C. (1984) as follows: iron 15,000 mg, cooper 5,000 mg, iodine 500 mg, manganese 17,000 mg, zinc 12,000 mg, selenium 70 mg, vehicle 1,000g, vitamin A 12,000 UI, vitamin D_3 1,500 UI, vitamin E 50 mg, vitamin K 4 mg, vitamin B_{12} 7 mg, vitamin B_2 7 mg, pantotenic acid 60 mg, nicotinic acid 120 mg, coline chloride 600 mg, metionin 700 mg, antioxidant 500 mg and vehicle 1,000g. Mixed diets containing chromium were pelleted and stored in black bags at $-18^{o}C$ until use. Fishes were fed once a day (9:00 h), *ad libitum*, for 90 days. Weekly, the aquatic characteristics as temperature, pH, dissolved oxygen and electric conductivity were measured. Total ammonia was measured at each 15 days.

Parasitological analysis

After 7, 60 and 90 days, eight fishes of each treatment were separated by size, anestethized with benzocaine solution (50 $mg.l^{-1}$), and their gills were removed for Monogenoidea and Dinoflagellate counts (Ghiraldelli et al. 2006). The results were treated by variance test ($P<0.01$), and Tukey test with 5% probability threshold was considered.

RESULTS AND DISCUSSION

The addition of chromium in the diet did not alter the water quality but significant difference ($P<0.05$) was observed when analysing the stocking densities (4 and 20 kg/m^3) as shown in Table 1. Except for the temperature (constant

during the experiment), high stocking density provoked worst water quality.The treatment with 20 kg of fishes/m^3 showed high concentration of ammonia and low levels of dissolved oxygen probably due to high feeding rate and fish excretion as suggested by Baskerville-Bridges and Kling (2000).

Table 1 - F values, variation coefficient (VC) and averages of aquatic characteristics of the aquaria with addition of chromium and different stocking densities (4 and 20 kg fish/m^3). Temperature (TºC), pH, dissolved oxygen (O_2D mg/l), electric conductivity (EC µs/cm) and total ammonia (TA µg/l).

Statistic	Variables				
	T	**pH**	**O_2D**	**EC**	**TA**
F to chromium levels (Cr)	1.16 ns*	0.59 ns	0.88 ns	0.99 ns	0.01 ns
F stocking densities (Sd)	0.11 ns	34.22 **	314.02 **	22.80 **	68.28 **
F interaction to CrxSd	1.11 ns	0.50 ns	1.12 ns	0.41 ns	0.11 ns
VC (%)	17.95	0.92	10.70	1.18	41.12
Averages for chromium levels					
0 mg/kg	28.3	7.49	3.78	136.51	205.86
6 mg/kg	28.9	7.54	3.65	137.55	198.20
12 mg/kg	28.9	7.50	3.61	13782	197.20
18 mg/kg	32.7	7.51	3.90	137.48	196.60
Averages for stocking densitiy					
4 kg fish/m^3	30.0a	7.58 a	4.99 a	138.71 a	61.11 a
20 kg fish/m^3	29.4a	7.44 b	2.48 b	135.97 b	337.87 b

Different letters in the same column indicate significant difference by Tukey test 5%.

After 7 days of experiment the fishes with more than 17 cm showed the highest number of *A. penilabiatus* in the gills. In spite of different length, the age of the fishes was the same, therefore, the immune system probably had the same development. Thus the higher number probably was due to higher area of gill surface for fixation of parasite (Table 2).

After 7 days of experiment, significant interaction was observed between chromium levels and stocking densities as the monogenoidea (Table 2). The fishes maintained at 4 kg/m^3 showed lower number of parasites ($P<0.05$) than those at 20 kg/m^3. Nevertheless, fishes fed 12 and 18 mg CQC/kg diet presented reduced number of *A. penilabiatus* in the first 7 days (Table 3). Apparently, there was the effect of chromium on fish parasite in the initial days of experiment. According to Hertz et al. (1989), the chromium is an essential mineral that acts in the glucose and lipid metabolism. Dietary supplementation with the chromium was proved to reduce the effects of stress in domestic animals (Mowat, 1997). In dairy cows supplementation of chromium caused insulin and cortisol release. As a result, the increased number of lymphocytes in vitro suggested its immunostimulant action (Chang et al., 1996). In different animal species, chromium may modulate the humoral and cellular factors by interacting with insulin and cortisol. Decreased cortisol concentration in chromium supplemented animals suggests its immune-endocrine modulation. It could be inferred that corticosteroids and insulin modulated the development of acute inflammatory reaction by antagonistic hormones. Elevated levels of corticosterone inhibit inflammation while insulin favours this phenomenon (Moraes and Garcia Leme, 1982). Thus, since chromium reduce cortisol in stressed animals, it will be less susceptible to infections by improving its immune system and inflammation.

In carp, the addition of 2 mg $CrCl_3$/kg diet inhibit gluconeogenesis and improve the insulin action (Hertz et al., 1989). Increased utilization of glucose and improved growth was demonstrated in tilapia fed 2 mg Cr_2O_3/kg diet (Shiau and Chen, 1993). Moreover, the addition of 2 mg chromium oxide/kg diet of the hybrid tilapia enhanced the fish weight gain, energy stocks and hepatic glycogen probably due insulin action (Shiau and Lin. 1993).

In the second fish collection (60 days), the fishes maintained at 20 kg/m^3 showed higher number of monogenoidea parasite (P<0.05) than those at 4 kg/m^3. (P<0,05) (Table 2). The high stocking density is one of the most common stressor in aquaculture that determines the competition between the animals and decreased utilization of food nutrients (Papoutsoglou et al., 1998). Anderson (1994) reported enhanced fish excretion during the stress and rise demand for minerals. The elevated excretion and poor utilization of food provoke low water quality, reduced growth and diminish the immunological response (Baskerville-Bridges and Kling, 2000; Montero et al., 1999). This phenomenon might have occurred in this work with pacu since fishes of present work maintained at high stocking density with low water quality presented higher number of parasites.

Analysing the Table 3, the fishes fed with chromium supplementation showed decreased *P. pillulare* number in both densities, but in the 4 kg/m^3 no statistical differences was observed. In the density of 20kg/m^3 and fishes supplemented from 6mg/kg of diet, lower number of dinoflagellate was observed.

The time of experiment also influenced the average number of the parasite. Decreased number was observed after 60 days and increased after 90 days (Table 2). This occurred probably due to worsening water quality during the experiment. Fishes maintained at 20 kg/m^3 without chromium supplementation showed higher number of *P. pillulare* than the fishes maintained at low density, but no significant difference was observed (Table 3).

Table 2 - F values, variation coefficient (VC) and average number of *Anacanthorus penilabiatus* (7, 60 and 90 days) and *Piscinoodinium pillulare* in the gills of *Piaractus mesopotamicus* maintained in two stocking densities and supplemented with chromium.

Statistic	**Average number of parasites***			
	7 days	60 days	90 days	*Piscinoodinium*
F to chromium levels (Cr)	4.93**	0.43ns	0.50ns	9.58**
F to stocking (Sd)	637.97**	14.06**	1.57ns	0.14ns
F to length (Lt-monogenea) or F to Periods (Pr-Piscinoodinium)	9.15**	0.05ns	0.75ns	20.33**
F interaction to CrxSd	6.51**	0.43ns	0.67ns	2.76*
F interaction to CrxLt	2.20ns	0.54ns	0.79ns	0.79ns
F interaction to SdxLt	0.71ns	0.53ns	2.55s	2.89ns
CV (%)	19.31	18.83	18.88	15.22
Averages for chromium levels				
0 mg/kg de ração	60.48	85.73	90.00	9.19
6 mg/kg de ração	52.42	79.44	87.41	7.67
12 mg/kg de ração	49.84	82.64	84.62	7.89
18 mg/kg de ração	47.52	82.97	82.58	7.82
Averages for stocking densitiy				
4 kg de peixes/m^3	20.53	75.39b	85.54	8.10
20 kg de peixe/m^3	84.50	90.00a	89.86	8.18
Averages for length				
Below of 17 cm	48.72 a	82.24	84.89	X
Up to 17 cm	56.41 b	83.14	87.31	X
Averages for period				
7 days	X	X	X	8.79b
60 days	X	X	X	7.13a
90 days	X	X	X	8.50b

Different letters in the same column indicate significant difference by Tukey test (P<0.05). Averages were transformed in alog (x+0.5).

Table 3 - Effects of chromium interaction (Cr) and stocking densities on the average number of *Anacanthorus penilabiatus* after 7 days of feeding and *Piscinoodinium pillulare* in the gills of *Piaractus mesopotamicus*

Chromium levels	***Anacanthorus penilabiatus***		***Piscinoodinium pillulare***	
	4 kg/m^3	20 kg/m^3	4 kg/m^3	20 kg/m^3
0 mg/kg	27.8Ba	277.6Ab	9,407Aa	18,218Ba
6 mg/kg	7.5Ba	197.8Ab	3,819Aa	4,999Aa
12 mg/kg	3.5Aa	240.3Ab	3,541Aa	6,061Aa
18 mg/kg	6.6Aa	152.1Ab	3,949Aa	3,899Aa

Capital letters indicate significant difference between chromium levels and minuscule letters indicate significant difference between stocking densities.

The number of parasites in the fishes is directly related to low dissolved oxygen, high organic matter contents and overcrowding, as suposted by Martins et al. (2002) and Moraes and Martins (2004). In São Paulo State- Brazil, from cases of fishes diagnosed with parasite *P. pillulare*, 73.3% occurred in the cold season with temperatures of 17 to 24^{o}C, and the hybrid tambacu (*Colossoma macropomum* female x *P. mesopotamicus* male) was the most susceptible fish followed by *P. mesopotamicus, C. macropomum, Leporinus macrocephalus* and *Oreochromis niloticus (*Moraes and Martins, 2004). These supported the presents results when water quality were reduced due to overcrowding. The fishes not supplemented and maintained in high stocking density showed higher parasitism index than supplemented ones, as shown in Table 3. In stress condition, high cortisol levels are responsible for lost glucose sensibility (Borgs and Mallard, 1998). The reduced levels of cortisol (Anderson, 1994; Committee on Animal Nutrition, 1997), increased action of insulin (Anderson, 1981, Schwatrz and Mertz cited by Mordenti et al., 1997) and/or immustimulant effect (Anderson, 1994) provoked by chromium supplementation possibly might have reduced the number of *P. pillulare.* The results suggested that the fishes stressed by stocking density might have improved their health with the addition of up to 6 mg CQC/kg diet. According to Anderson (1994), stressed fishes have more exigency of dietary mineral since the stress was responsible for alteration of mineral and vitamin requirement (Anderson, 1994; Committee on Animal Nutrition, 1997).

However, the mechanisms of chromium action in the fishes are unknown. In conclusion, it was possible that chromium supplementation (12 mg CQC/kg) favoured the good health of the fishes as prophylaxis measure by reducing Monogenoidea and Dinoflagellida number in lower densities and the level of 18mg/kg in the higher densities.

RESUMO

O objetivo do trabalho foi avaliar a intensidade de parasitismo por monogenóide *Anacanthorus penilabiatus* e pelo dinoflagelado *Piscinoodinium pillulare* em pacus *Piaractus mesopotamicus*, cuja dieta foi suplementada com cromo. Foram utilizados dois delineamentos em esquema fatorial, consistindo de 4 níveis de cromo (0, 6, 12, 18 mgCQC/kg), duas densidades de estocagem (4 kg/m^3 e 20 kg/m^3) e duas classes de tamanho para monogenóide (maiores e menores de 17cm) e/ou 3 períodos de coleta (7, 60 e 90 dias), com quatro repetições. Os peixes que receberam 12 e 18mg CQC/kg apresentaram diminuição de monogenóides após 7 dias de alimentação na menor densidade. Nos peixes na maior densidade, os níveis de 6, 12 e 18 mgCQC/kg ocasionou redução de dinoflagelados. Os resultados demonstraram que a suplementação com cromo (12 e 18 mgCQC/kg) promoveu uma melhora na saúde dos peixes na menor e na maior densidade, respectivamente.

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