Effect of the establishment of dominance relationships on cortisol and other metabolic parameters in Nile tilapia (*Oreochromis niloticus*)

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Abstract

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Received January 22, 2003 Accepted August 20, 2003 The objective of the present study was to investigate the influence of the establishment of dominance relationships and social stress on plasma cortisol and metabolite levels in Nile tilapia (Oreochromis *niloticus*). During the 30-day experiment, the fish weighing 236 ± 29 g were kept in individual aquaria, except for two pairings lasting 6 h each. Blood samples were taken from the animals before and after pairing. Display, approach, attack, rebuff, chase flight, and coloration were carried out on days 16 and 30. Activities and behaviors characteristic of the establishment of dominance relationships were described. It was possible to classify all experimental fish (N = 30) as dominant or subordinate. No differences were detected between dominant (N = 15) and subordinate (N = 15) fish during isolation or after pairing in cortisol (isolated: $5.76 \pm 0.98 \text{ vs } 5.42 \pm 0.63$; paired: 10.94 $\pm 1.62 \text{ vs } 11.21 \pm 2.45 \text{ µg/dl}$), glucose (isolated: $60.02 \pm 4.9 \text{ vs } 67.85$ \pm 16.16; paired: 110.44 \pm 15.72 vs 136.26 \pm 22.46 mg/dl), triglyceride (isolated: $167.87 \pm 5.06 \text{ vs } 185.68 \pm 7.24$; paired: $210.85 \pm 13.40 \text{ vs}$ 221.82 ± 12.70 mg/dl) or total protein levels (isolated: 7.01 ± 0.42 vs 6.69 ± 0.59 ; paired: 9.21 ± 0.62 vs 9.51 ± 0.66 g/dl). However, when isolated (N = 30) and paired (N = 30) tilapia were compared, there were significant differences in cortisol and metabolite levels. The similar response presented by dominant and subordinate tilapia indicates that establishment of dominance relationships was a stressor for both groups.

Key words

- Tilapia
- Social stress
- Social relationships
- Agonistic behaviors
- Cortisol
- Dominance

Introduction

A phenomenon commonly described in territorial species such as Nile tilapia (*Oreo-chromis niloticus*) is social stress (1-3). Sometimes, social interactions stimulate an in-

crease in cortisol levels (3-5). In general, cortisol levels are of considerable value in the assessment of stress because cortisol is involved in metabolic adjustments and energy mobilization by acting on carbohydrate, protein and lipid metabolism (2,6,7).

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On a long-term basis, an increase in cortisol concentration has been directly associated with a decrease in growth rate in fish (6). Barton and Iwama (8) have also observed that exogenous cortisol administration induced a remarkable decrease in the growth rate of rainbow trout (*Oncorhynchus mikiss*). Moreover, subordinate fish showed a lower growth rate than dominant animals (2,9,10).

Some investigators have proposed that physiological responses to social stress are more marked in subordinate animals due to a larger energy expense during agonistic confrontations (5,11). Others have suggested that triglycerides are also mobilized in response to stress (12,13). An increase in plasma glucose levels has also been observed in fish submitted to acute and chronic stress (14,15). It is likely that the mass loss in animals submitted to chronic stress is the result of protein mobilization due to increased cortisol levels which enhance the availability of energy (16,17). On the other hand, Stott (18) suggested that cortisol levels can only be related to stress during and immediately after submission to stressors.

However, it is not clear whether agonistic confrontation can cause metabolic and hormonal changes. It is well known that tilapia of similar age and genetic background submitted to similar environments, nutrition and management can have different growth levels. This heterogeneity is due in part to the fact that dominant fish have priority of access to food compared to subordinate fish. However, before studying the effect of social relationships on growth it is necessary to determine how the establishment of dominance relationships can cause metabolic and hormonal adjustments. On this basis, the objective of the present study was to investigate the influence of the establishment of dominance relationships (and the social stress involved) on cortisol, glucose, triglyceride and total protein levels in dominant and subordinate Nile tilapia.

Material and Methods

Pellet ration for fish (Socil, Descalvado, SP, Brazil) was offered to the tilapia throughout the experiment at 3% of body mass. A filtering system with constant aeration was installed in the aquaria, and each aquarium was cleaned weekly. Water quality (dissolved oxygen, ammonia, nitrate, nitrite and pH) was monitored weekly. Ambient temperature was maintained with an air conditioning system and water temperature was kept at 26 ± 7°C throughout the experiment. The photoperiod used was a 12-h light-dark cycle, with lights on at 6:00 am.

Pre-experimental procedure

Experimental procedure

During the experimental period of 30 days, each tilapia remained in an individual aquarium (no visual contact, free of social stress), except during the period of pairing (social stress imposed). Each pairing took place in a neutral aquarium to avoid a "previous residence" effect. Fifteen pairs of experimental fish of similar size and body mass were transferred to this neutral aquarium for 6 h on days 16 and 30. The same pairs of individual fish were tested on the two experimental days.

On days 16 and 30, each fish was meas-

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ured and weighed, and a blood sample was removed by caudal vein puncture for individual measurements before pairing. The fish were then carefully removed from their individual aquaria and paired for 6 h (social stress imposed). At the end of pairing, all fish were submitted to a second blood sampling for measurements after exposure to social stress (paired measures). The capture, anesthetic procedure, measurement, weighing and blood sampling (approximately 1 ml of blood) lasted a total of 2 min, based on the procedure reported by Auperin et al. (19).

Dominance relationships

Behavioral observations were conducted on days 16 and 30 during the 6-h period of pairing. During this monitoring period we noted the following behaviors: display, approach, attack, rebuff, chase and flight (see Results for descriptions). Simultaneously, we noted the position (upper, intermediate or lower, and left or right) of each fish in the aquarium. These data were used to determine the social rank (dominant or subordinate) of each fish in the pairing. Thus, 15 fish were classified as dominant, and the other 15 fish were classified as subordinate. It is important to note that the dominance relationships remained the same in the second pairing.

All pairing experiments were completely recorded on videotape. Five minutes at the beginning and at the end of the video were then observed to determine the pigmentation of each fish (silver with sharp black stripes or dark gray without black stripes) and the frequency of agonistic behaviors (total number of attacks plus rebuffs) initiated by each fish.

Hormonal and metabolic analysis

Blood samples were collected into chilled tubes and immediately centrifuged at 1500 *g* for 15 min at 4°C, and plasma was stored at

-20°C until analysis. Commercial enzymatic kits were used (Weiner Laboratory, Rosario, Argentina) to determine plasma levels of glucose, triglycerides and total proteins. After validation, plasma cortisol levels were determined by enzyme immunoassay (EIA), with an interassay and intra-assay coefficient of variation of 6.1 and 10.52%, respectively, using commercial kits (Diagnostic System Laboratories, Inc., Webster, TX, USA).

Statistical analysis

Statistical comparison of the means for all parameters were carried out using GraphPad (20) and the Kruskal-Wallis test, with the level of significance set at P < 0.05. Correlations between the parameters analyzed were determined by the Pearson correlation test using the SAS System for Windows, version 6.12 (21). All data are reported as means \pm SEM.

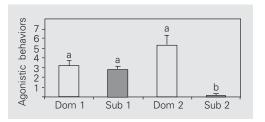
Results

At the beginning of pairing, both fish swam and displayed a silver coloration with sharp black stripes. They then swam closer to one another, approached, attacked (in general with tail beating), and fled. A few minutes later, the paired fish became more aggressive and attacked or reacted with a vigorous rebuff, such as mouth fighting and ramming. During this period of intensive interaction, both fish presented a light coloration, without black stripes. Sometimes, one of the fish chased and bit the flanks of the other fish that was fleeing. However, challenged fish mostly rebuffed attacks and another confrontation began. As shown in Figure 1, at the beginning of pairing there was no significant difference in the number of agonistic behaviors initiated by dominant or subordinate fish. Confrontations were rare after 2.5 h.

At the end of pairing, we could identify the

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Figure 1. Number of agonistic behaviors (attacks plus rebuffs) initiated during the first (1) and last (2) 5 min of pairing of dominant (Dom) and subordinate (Sub) Nile tilapia. Data are reported as means \pm SEM for 15 fish. Data for days 16 and 30 were combined. Significantly different (P < 0.05) means are indicated by different letters (Kruskal-Wallis test).



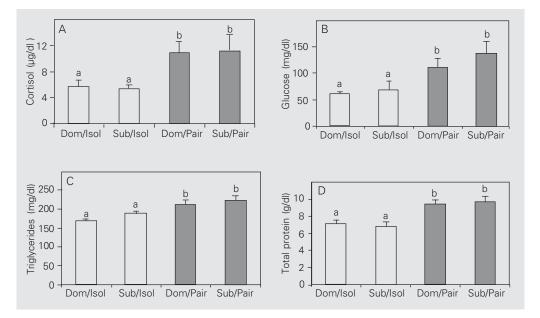


Figure 2. Plasma cortisol (A), glucose (B), triglycerides (C) and total protein (D) observed in dominant (Dom) and subordinate (Sub) Nile tilapia while isolated (Isol) and after pairing (Pair). Data are reported as means \pm SEM for 15 fish. Significantly different means (P < 0.05) are indicated by different letters (Tukey test).

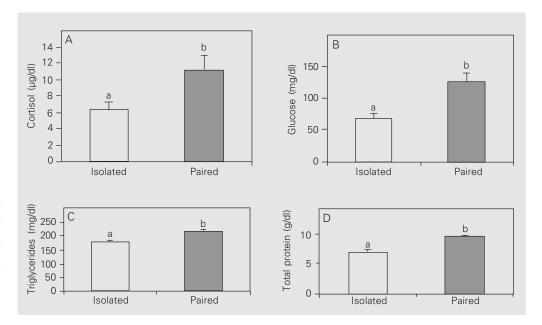


Figure 3. Plasma cortisol (A), glucose (B), triglyceride (C) and total protein (D) levels observed in Nile tilapia while isolated and after pairing. Data are reported as means ± SEM for 30 fish. Significantly different means (P < 0.05) are indicated by different letters (Tukey test).

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dominant fish as the one having a silver coloration with sharp black stripes, swimming normally, and that chased and occasionally attacked the other fish when approached. At the same time, subordinate fish had a dark gray coloration without stripes and did not rebuff the aggressive behaviors of the other fish. Furthermore, subordinate fish remained in the section of the aquarium opposite to the dominant fish and avoided the dominant fish. During this phase, subordinate fish rarely attacked dominant fish and there was a significant increase in the number of agonistic behaviors initiated by the dominant fish compared to the subordinate fish (Figure 1). In general, subordinate fish swam slowly and showed a higher number of injures (wounds with scales missing) than dominant fish.

Glucose, triglyceride, total protein and plasma cortisol levels were not significantly different between fish classified as dominant and subordinate, either during isolation or after pairing (Figure 2). However, glucose, triglyceride, total protein and plasma cortisol levels measured during isolation were lower than those measured after pairing for the fish as a whole (see Figure 3).

There were no differences in body mass, size or feed intake between dominant and subordinate fish during the experiment. Compared to the beginning of the experiment, however, there were numerical increases in body mass (from 236.10 ± 5.60 to 243.57 ± 5.80 g), length (from 19.10 ± 0.22 to 19.30 ± 0.20 cm) and feed intake (from 7.50 ± 0.25 to 9.45 ± 0.50 g/day) at the end of the experimental period.

There were positive correlations between levels measured during isolation and after pairing for glucose (r = 0.29, P < 0.05) and triglyceride levels (r = 0.45, P < 0.05), but not for cortisol or total protein levels. At the same time, there was a positive correlation between glucose and cortisol levels (r = 0.65, P < 0.05). However, there was a negative correlation between glucose and triglyceride levels (r = -0.47, P < 0.05). Cortisol and

triglyceride levels were not correlated and total protein levels were not correlated with any of the other three parameters.

Discussion

Activities and behaviors characteristic of the establishment of dominance relationships were observed during all pairings and it was possible to classify all experimental fish as dominant or subordinate because dominance relationships had been clearly established by the end of pairing. This classical response to acute stress has been previously described in tilapia by others (1,3,5). Our results also agree with those reported by Peters et al. (14) and Domingues (22) who showed that, although subordinate fish suffer a greater number of attacks than dominant fish, the dominance status is not a good indicator of the stress level.

Nevertheless, in the present study, there were no differences in cortisol, glucose, triglyceride or total protein levels between dominant and subordinate fish. These results suggest that social interactions during the establishment of the dominance relationship are an important stress factor for Nile tilapia and cause similar increases in cortisol and metabolic parameters in both dominant and subordinate fish. After the establishment of dominance relationships, the dominant animal normally replaces attack behaviors with threats, and the subordinate animal replaces rebuffs with flight (2). In the present study, however, no threats were observed.

Other investigators (5) have proposed that physiological and metabolic responses are more marked in subordinate than in dominant Nile tilapia. It is important to note that Fernandes (5) worked with fish maintained in experimental groups for several days. It is possible that the marked responses of subordinate fish were influenced by both social interactions (acute stress) and the length of grouping time (chronic stress). Furthermore, during lengthy grouping of many fish, ap-

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proaches and threats lead to attacks, rebuffs and fights (2).

In the present study, experimental fish were kept isolated, except during pairing when two fish were transferred to a neutral aquarium. In this case, imposed acute stress can be hypothesized to be more intense than in a group, because the agonistic behaviors, mainly from dominants, were focused on a single animal. Consequently, we observed a clearly significant difference in cortisol levels between isolated and paired tilapia. This has been previously described in tilapia submitted to different stressful situations (1,3). In contrast, Ejike and Schreck (4) observed lower glycogen levels and similar cortisol levels when comparing dominant and solitary salmon. Thus, these investigators suggested that complete isolation is not a healthy situation. However, our results did not confirm theirs because the same tilapia had lower levels of cortisol, glucose, triglyceride and total protein when isolated than when paired, suggesting that acute stress caused a mobilization of extra energy.

As observed in the present study, work carried out with other species of fish has demonstrated that different kinds of stress cause an increase in glucose levels (2,15,23). These studies also reported that high cortisol levels were positively correlated with high glucose levels. For these reasons, it is possible to confirm that stress caused an additional mobilization of glucose. At the same time, establishment of dominance relation-

ships caused an increase in triglyceride levels in our study. This supports other studies showing that high cortisol and catecholamine levels cause an increase in lipase activity which promotes the breakdown of triglycerides in fat cells and consequently increases triglyceride levels in plasma (12,13).

In the present study, the establishment of dominance relationships also caused a mobilization of protein as a source of energy. During the increase in energy demand provoked by stress, increased cortisol levels promote the mobilization of muscular protein in trout (2,16,17). Some investigators have not observed a significant increase in total protein levels in fish submitted to social stress (5,14). It is important to note that the mobilization of protein as an energy source has been observed in animals submitted to chronic stress (7.24). In the chronic case, the increase in plasma cortisol concentrations is directly related to a decrease in growth rate (6). However, our data on food intake, body mass and length were not affected by the imposed acute stress, probably because the duration of pairing was not sufficiently long to affect fish development.

During pairing, the intense agonistic interactions caused a significant increase in the plasma levels of cortisol and the metabolites analyzed. The similarity of response shown by dominant and subordinate animals indicates that the establishment of dominance relationships was a stressor for both groups.

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