Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

DOI: 10.4025/actascibiolsci.v31i4.5814

Circadian changes in thyroid hormones of piau, *Leporinus obtusidens* Valenciennes, 1847 (Osteichthyes, Anostomidae) after feeding

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ABSTRACT. To evaluate circadian changes in thyroid hormones of piau, *Leporinus obtusidens* Valenciennes, 1847, (Osteichthyes, Anostomidae) after feeding were determined the plasma levels of thyroid hormones (TH) of 128 fishes, same age, immature, both sexes, distributed into four classes of weight. They were kept in 16 aquaria (100 L), with artificial aeration and 2 L $min.^{-1}$ water flow, from March to August, 1996, in Aquaculture Station of the Universidade Federal de Lavras (UFLA). Daily, feed was supplied at 5% body weight. For blood samples, the fishes were anesthetized with benzocaine 10%, and plasmatic levels of TH were determined at 1, 4, 7, 10, 13, 16 and 22h after food supply during 4 consecutive days in June and in August, period of lowest temperature. The classes of weight 63, 75, 82 and 91 g were considered homogeneous, showing that plasmatic concentrations of T_3 and T_4 were body weight independent for this fish species. The daily cycle of plasma TH were correlated with feed intake, reaching the highest levels 7 h after feeding supply (T_3 = 1.75 ± 0.07 ng mL^{-1} and T_4 = 14.9 ± 1.59 ng mL^{-1}). It was also possible to verify that the daily intake is directly correlated with water temperature which is affected by day-light cycle.

Key words: T_3, T_4, teleost fish, temperature, photoperiod.

RESUMO. Variações circadianas dos hormônios tireoidianos de piau, *Leporinus obtusidens* Valenciennes, 1847 (Osteichthyes, Anostomidae) após alimentação. Para avaliar as variações circadianas dos hormônios tireoidianos do piau, *Leporinus obtusidens* Valenciennes, 1847, (Osteichthyes, Anostomidae) após alimentação, foram determinados os níveis plasmáticos dos hormônios tireoidianos (HT) T_3 e T_4 de 128 peixes, de mesma idade, imaturos, ambos os sexos, distribuídos em quatro classes de peso e mantidos em 16 aquários (100 L), com aeração artificial e fluxo constante de água de 2 L $min.^{-1}$, de março a agosto de 1996, na Estação de Aquicultura da Universidade Federal de Lavras (UFLA). Diariamente, a ração foi fornecida a 5% do peso corporal. Nas pesagens e amostragens de sangue, os animais foram anestesiados com benzocaína a 10% e os níveis plasmáticos dos HT foram determinados 1, 4, 7, 10, 13, 16 e 22h após o fornecimento da ração, durante quatro dias consecutivos, no mês de junho e de agosto, período do ano que apresenta menor temperatura. As classes de peso de 63, 75, 82 e 91 g foram consideradas homogêneas, mostrando que as concentrações plasmáticas dos HT são independentes do peso corporal para esta espécie. O ciclo diário dos HT está relacionado com a ingestão alimentar, atingindo o pico 7 h após o fornecimento do alimento (T_3 = 1,75 ± 0,07 ng mL^{-1} e T_4=14,9 ± 1,59 ng mL^{-1}). Também foi observado que a ingestão diária de alimento está diretamente correlacionada com a temperatura da água que é afetada pelo fotoperíodo.

Palavras-chave: T_3, T_4, peixes teleósteos, temperatura, fotoperíodo.

Introduction

The plasmatic levels of thyroid hormones in teleosts has been studied in last decades by Spieler and Noeske (1979; 1981; 1984) with goldfish; Stacey et al. (1984) with white sucker; Rydevik et al. (1984; 1989), Youngson and McLay (1989) with salmon; White and Henderson (1977), Leatherland et al. (1978), Osborn et al. (1978), Eales et al. (1981), Flood and Eales (1983), Mc Cormick and Naiman (1984), Cook and Eales (1987), Reddy and Leatherland (1994; 1995) with trout and Leiner et al. (2000) and Leiner and Mackenzie (2001; 2003) with red drum.

Many works have shown the influence of food as a factor that produces alterations in biological rhythms of fishes; rhythms that are controlled by hormones such as thyroid hormones.

Feeding has an influence in thyroid function (DONALDSON et al., 1979), in the plasma level of thyroid hormones (OSBORN; SIMPSON, 1972;

HIGGS; EALES, 1978), and in plasmatic lost and peripheral metabolism of T_3 and T_4 (EALES; SINCLAIR, 1974; HIGGS; EALES, 1977).

There are several relationships of food ingestion and hormonal release, nevertheless the sequence of involved mechanisms is not established. Until today, the action of food ingestion in teleosts hypothalamic-hypophysis-thyroid axis, in peripheral metabolism of thyroid hormones, is not well described, such as the nutritional conditions that affect the thyroid system (CUENCA; GALLEGO, 1987).

The positive effects of thyroid hormones on appetite, detected by Gross et al. (1963) and Higgs and Eales (1977), seems to be an answer of these hormones as interpose up to food conversion. The hormonal effects are also dependent of factors like previous nutritional state and environmental conditions (DONALDSON et al., 1979; CUENCA; GALLEGO, 1987).

Many fishes clearly exhibit a daily pattern of feeding activity and food demand, related to photoperiod. Significant alterations on daily levels of T_3 and T_4, observed in trout and goldfish, indicate a correlation with feeding and photoperiod (REDDY; LEATHERLAND, 1994), and also with temperature (ROZIN; MAYER, 1961; CUENCA; GALLEGO, 1987; HIGUERA, 1987; SMITH, 1989).

In this context, the objective of this work was to determinate the daily levels of plasmatic T_3 and T_4 of piau, *Leporinus obtusidens*, a native Brazilian fresh water fish, after feeding, in natural conditions of temperature and photoperiod.

Material and methods

Maintenance of experimental animals

The experiment was carried out in Aquaculture Station Laboratory of the Animal Science Department at Universidade Federal de Lavras (UFLA), Minas Gerais State, Brazil, from 7 March to 7 August 1996. The city has geographic localization defined between coordinates 21°15' South latitude and 44°52'30" West longitude, mean altitude of 918.8 m, climate characterized by mean annual temperature of 19.4°C and total rain precipitation of 1,530 mm, mainly concentrated from October to March. The study used 128 fishes obtained from UFLA Aquaculture Station, which were immature, with the same age from both sexes. The fishes were distributed in 16 indoor aquaria (100 L each), which were distributed in line, two by two and covered with a net to avoid insect entrance.

The aquarium water flow was 2 L $min.^{-1}$, oxygen and temperature were measured daily and air pump to avoid decrease of dissolved oxygen below of 5 mg O_2 L^{-1}.

The food was made and pelletized in the Food Factory of the Animal Science, UFLA University, and was balanced to 29% of total protein and 3900 kcal of total energy. It was supplied at 5% of body weight directly in aquaria, and adjusted in each weight of the fishes, but before feed supply the feces were removed by siphoning.

To minimize handling stress during blood sample collection, the fishes were anesthetized with alcoholic solution of benzocaine (10%) as recommended by Ross and Ross (1984) and Anderson (1974) in a separate aquarium.

Fishes were individualized using adaptations of mark Lea (CECARELLI et al., 1991) that consist in an operculum perforation which permits to fix a color plastic sphere with nylon.

Food was supplied once a day at 10:00 AM, as described by Dias-Júnior and Mourgués-Schurter (2001), in previous study with this species. Daily, after two hours of food supply, excess food was removed by siphoning each aquarium and the rest of food was separated after filtering, and subsequently were dried indoors before weighing to quantify food consumption.

The fishes were acclimated to aquarium, to food supply, cleaning routine and data collecting of water parameter measurements (O_2 and temperature) since March 1996, totalizing three months of adaptation to management, before blood sampling.

Experimental procedures

Data from water temperature (collected before food supply), photoperiod (supplied by climatologic station of UFLA) and food consumption, previously standardized by Dias-Júnior and Mourgués-Schurter (2001) were collected from March to August. The fishes were separated in four body weight groups: A = 63 g; B = 75 g; C = 82 g and D = 91 g to isolate a possible influence of body weight on food consumption and thyroid hormones levels. Each body weight class was composed by four aquaria with 8 fishes each, totalizing 16 aquaria and 128 fishes. The variable studied was plasmatic concentration of T_3 and T_4 after 1, 4, 7, 10, 13, 16, 19 and 22 hours after food supply. Blood was not collected in time zero due to interference of handling procedures and anesthesia that blocks food behavior of fishes.

Blood collection was done in autumn (June) and winter (July/August) because this period, temperatures reach lowest values of the year.

Blood analysis

To quantify plasmatic levels of T_3 and T_4, blood samples of anesthetized fishes were collected from the caudal vein into ice-cooled syringes that were placed immediately on ice, before centrifugation for plasma separation. Blood was collected using a 25x7 22 G1 needle during four days, every three hours, in June (19th-22nd) and August (1st-5th), and the fish taken once in June only was taken again once in August. Aquaria and fishes handling was minimal to avoid stress effect on feeding behavior. Thus, only two different fishes per day were taken by aquarium in a minimal interval of 12 hours, to avoid stress of coexisting fishes. In such case, it was possible to obtain two samples by day from each aquarium (1 and 13h or 4 and 16h or 7 and 19h or 10 and 22h) taking 4 days to have 8 points in one aquarium.

After blood samples from each fish, plasma was separated and stored in Eppendorf tubes at -45°C until analysis.

The thyroid hormones T_3 and T_4 were quantified by radioimmunoassay commercial kits (Active® T_3-free RIA (100%) Kit DSL 41100 and Active® T_4-free RIA (97%) Kit DSL 40100, from Diagnostics System Laboratories, Inc – USA). The tests were conducted by a clinical analytic laboratory (Laboratório Santa Cecília of Lavras – Minas Gerais State) because our laboratory did not have authorization to work with radioactive materials.

Statistical analysis

The experimental design was randomized blocks and the level of plasmatic thyroid hormones were statistically examined by analysis of variance (Anova) and Scott-Knott medium test, 1% for T_3 and 5% for T_4 and the data are presented as means ± SEM.

Results

Food consumption, temperature and photoperiod

In Figure 1 is presented food consumption in body weight percentage by day of piaus from March to August. It is possible to observe a decrease in food consumption from March to August, and similar mean consumption in June (2.33 ± 0.09%) and August (2.4 ± 0.14%). Parallel to this, water temperature was 16.7 ± 0.08°C in June and 15.8 ± 0.09°C in first week of August, moreover, since April to August, water temperature decrease approximately 10°C (Figure 2).

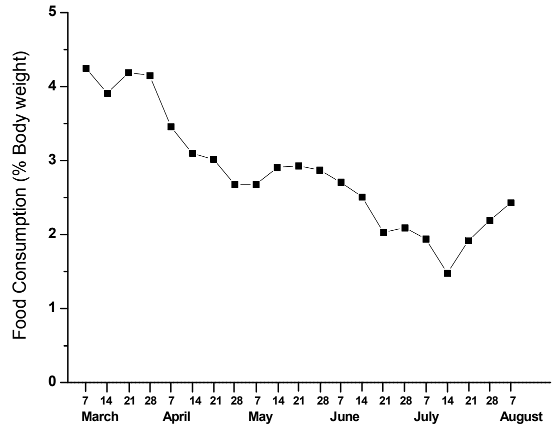

Figure 1. Food consumption (% body weight) of *Leporinus obtusidens* Valenciennes, 1847, from March to August 1996.

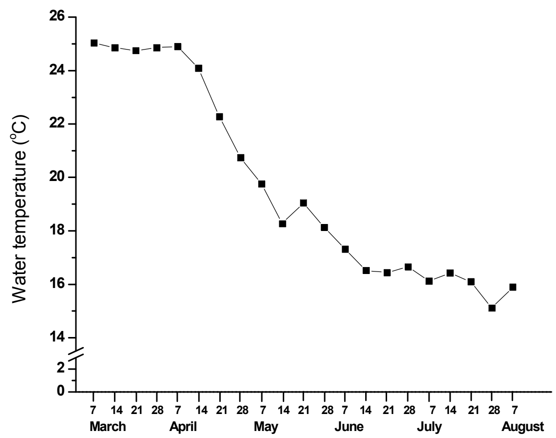

Figure 2. Aquaria water temperature (°C) of *Leporinus obtusidens* Valenciennes, 1847, from March to August 1996.

The photoperiod presented a constant reduction through this period, reaching the lowest value (10.7 hours) in 14 to 26 June (Figure 3), from that on, started a gradual increase.

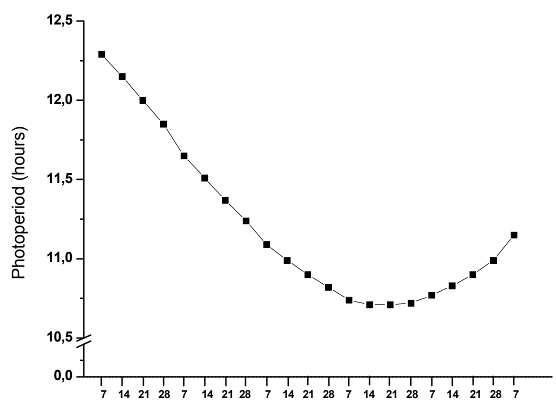

Figure 3. Photoperiod (hours) from March to August 1996.

T_3 plasmatic level

Daily concentration of plasmatic T_3 (ng mL^{-1}) of *L. obtusidens*, during June and August 1996 did not show difference between weight classes, and than, they was considered homogenous for the other analysis. Thus, data are presented in Table 1.

Table 1. Plasmatic T_3 (ng mL^{-1}) of *Leporinus obtusidens,* after food supply (h) in June and August 1996.

Time after food supply (h)	Year period	
	June/1996	August/1996
1	1.64 ± 0.06 Aa	1.22 ± 0.09 Bb
4	1.67 ± 0.05 Aa	1.44 ± 0.09 Ba
7	1.75 ± 0.07 Aa	1.58 ± 0.12 Ba
10	1.53 ± 0.09 Ab	1.35 ± 0.07 Bb
13	1.55 ± 0.13 Ab	1.48 ± 0.03 Aa
16	1.48 ± 0.11 Ab	1.38 ± 0.08 Ab
19	1.50 ± 0.07 Ab	1.37 ± 0.09 Ab
22	1.45 ± 0.18 Ab	1.37 ± 0.05 Ab

Means followed by same capital letter in same line are statistically equal ($p < 0.01$); Means followed by same lowercase letter in same column are statistically equal ($p < 0.01$), mean ± SEM (n = 16). Time after food supply: 10, 13, 16 and 19 correspond to dark phase of the day.

In June and August there was an increase in T_3 plasmatic level seven hours after food supply (1.75 ± 0.07 ng mL^{-1} and 1.58 ± 0.12 ng mL^{-1}, respectively), decreasing at 10 hours, and kept invariable in subsequent times. These results clearly show that there is a cycle in T_3 plasmatic level related to feeding.

Comparing June and August daily levels of plasmatic T_3, between 1 to 10 hours after food supply, it is possible to see a difference in this period, followed by an establishment at similar levels (Table 1), beside food intake in these months was the same (2.33 ± 0.09% and 2.40 ± 0.14% of body weight, respectively). This difference may be related to temperature variant of these two periods, 16.7 ± 0.08ºC in June and 15.8 ± 0.09ºC in August, suggesting a similar situation reported by Eales and Shostak (1986) for trout, in which temperature increase promoted a rising mobility of thyroid hormones in tissues and facilitated the release and absorption of these hormones.

T_4 plasmatic level

The *L. obtusidens* T_4 plasmatic concentration (ng mL^{-1}) after food supply, also did not show difference between weight classes, thus, June and August 1996 data are presented in Table 2.

In June there was an increase in T_4 plasmatic level (14.90 ± 1.58 ng mL^{-1}) 7 hours after food supply, decreasing and than stabilizing, and this tendency was also observed to T_3 in the same period.

Comparing June and August daily levels of plasmatic T_4, it is possible to see a relevant elevation of August hormone levels in all times after food.

Table 2. Plasmatic T_4 (ng mL^{-1}) of *Leporinus obtusidens,* after food supply (h) in June and August 1996.

Time after food supply (h)	Year period	
	June/1996	August/1996
1	12.25 ± 1.43 Bb	23.80 ± 1.32 Aa
4	11.77 ± 0.59 Bb	24.70 ± 1.57 Aa
7	14.90 ± 1.58 Ba	22.30 ± 1.50 Aa
10	12.32 ± 0.33 Bb	23.22 ± 1.92 Aa
13	11.82 ± 0.78 Bb	23.15 ± 2.18 Aa
16	12.12 ± 0.92 Bb	25.27 ± 1.63 Aa
19	11.15 ± 0.52 Bb	23.17 ± 2.71 Aa
22	11.70 ± 1.10 Bb	24.75 ± 2.21 Aa

Mean followed by same capital letter in same line are statistically equal ($p < 0.05$); Mean followed by same lowercase letter in same column are statistically equal ($p < 0.05$), mean ± SEM (n = 16). Time after food supply: 10, 13, 16 and 19 correspond to dark phase of the day.

Discution

Food consumption

Food consumption was the same in the colder period of the year, even with the temperature fall observed in the end of July. This shows that food consumption of *L. obtusidens* was not compromised with one degree fall in water temperature. Comparing the period from March to August, when temperature decreased, there was a reduction in food consumption (Figures 1 and 2), which coincides with results already mentioned by Herpher (1988), Angelini et al. (1992) and Mazur et al. (1993) for teleosts.

Photoperiod reduction in June (14 to 28th) was preceded by a progressive fall in food consumption (up to 14th July), and this makes difficult the interpretation of photoperiod effect on food consumption, because Leiner and MacKenzie (2001), suggested that the quantity of food ingested is influenced by photoperiod. They found that higher light period stimulates an increase of food consumption of red drum feed *ad libitum*, but not observed difference on food consumption in fishes fed at 5% of body weight, despite of higher feed efficiency on higher light periods. If food consumption is influenced only by photoperiod, like reports Leiner and MacKenzie (2001), food consumption of piaus should have increased, not decreased.

Gutierrez et al. (1984), Laidley and Leatherland (1988), Boujard et al. (1993) and Reddy and Leatherland (1994) say that hyperglycemia in fishes after feed is associated to photoperiod. Boujard et al. (1993) verified lower glucose plasmatic level in evening of trout feed with automatic feeders, following a progressive increase in light phase. Nevertheless, Reddy and Leatherland (1994) did not verify any difference in glucose plasmatic level in rainbow trout, but they ascribe this to lower food availability (15 minutes).

If food consumption variations were associated only to temperature, both curves were similar. But photoperiod also has influence in food consumption of *L. obtusidens*. Thus, the isolated influence of these variables on food consumption must be subject to future works, because we studied food consumption in natural temperature and photoperiod (Figures 2 and 3).

T_3 plasmatic level

No interference of weight (63; 75; 82 and 91 g) was observed in plasma T_3 concentration. Also, Eales et al. (1981) registered in trout no correlation between body weight and plasmatic concentration of T_3 and T_4. Brown and Eales (1977) and Higuera (1987) emphasized the necessity to consider fish body weight and suggested that, in teleost thyroid study, we must be careful in maintaining weight and age homogeneity of fishes used in experiments, since they verified a significant correlation between plasmatic T_3 and body weight of trout (*Salmo gairdneri*). Thus, if there is body weight influence on plasmatic level of T_3 in piaus, future studies must consider weight classes higher of 28 g, that was the difference of minor to major weight class used in this work.

Nevertheless, food consumption during the lowest temperature period (June to August) does not have a significant variation. The difference observed on T_3 concentration until 10 hours after food supply could be related to one degree of temperature decrease. It is known that temperature promotes releasing, mobility and absorption increases of thyroid trout hormones (EALES; SHOSTAK, 1986). These authors also mentioned that temperature may influence hormone binding in tissues, action of T_4 to T_3 conversion enzymes or recognized places in hypothalamic-hypophysis axis and feedback answer.

This difference could be related to photo phase variation, observed on the same period, such as that registered by Cook and Eales (1987) and by the results of White and Henderson (1977), which showed in brook trout a level increase of thyroid hormones during photo phase, followed by a fall in dark phase, probably due food behavior and daily activity of the fishes. Eales et al. (1981) also verified a daily variation of thyroid hormones concentration during studies of the effect of food ingestion in rainbow trout. Brown et al. (1978) did not find evidence of daily variation of these hormones in rainbow trout; nevertheless, the fishes went 72 hours without food before data collection.

The existence of T_3 daily cycle was also verified by Leatherland et al. (1978), Osborns et al. (1978) and by Spieler and Noeske (1979) while studying rainbow trout and goldfish.

Daily variations of thyroid hormones occur only in feed fish, it is also observed in fasting fishes but at lower levels that are not different (COOK; EALES, 1987). These authors verified an inconsistent fluctuation of these hormones, probably as a result of extrathyroid conversion of T_4 to T_3, usual to salmonids, instead of alterations in the release of thyroid hormones. They also consider that T_3 level is a result of periphery deiodination of T_4. So, if photoperiod influence exists on *L. obtusidens*, it is less intense than temperature, whereas August photoperiod was approximately one hour higher than June (Figure 3).

In the experimental condition of this work, T_3 circadian cycle of piaus could become stronger by photoperiod, due to time of feed.

Plasmatic level of T_4

The unexpected elevation of T_4 plasmatic level observed in August was not perceived in any other studied work, and the data of piaus experiment do not allows us to explain this sudden increase in T_4 concentration, unless the elevation of approximately 1h on photoperiod from June to August is enough to provide strong stimuli in hypothalamic-hypophysis-thyroid and promote the delivery up to 50% of T_4 compared to June.

The work of Leiner and MacKenzie (2001) clearly shows the strong influence of feed and photoperiod on T_4 plasmatic levels. Fasting fishes did not show a daily cycle of T_4, but fishes fed at random times showed diminished amplitude over time, different from fishes feeding in same time that showed well characterized T_4 peaks related to feed in same photoperiod. Leiner and MacKenzie (2001) are convinced that T_4 daily cycle is regulated by an endogen biological clock. They tested different photoperiods and feeding time and these treatments only changes the amplitude of T_4 rhythm. The authors also attribute plasmatic T_4 circadian cycle in red drum as an result of an independent endogen clock that determine when hypothalamic-hypophysis-thyroid must be activated, where photoperiod and feeding time contribute with this clock. The piaus presented a T_4 answer like that described by Leiner and MacKenzie (2001), because plasmatic level of T_4 for this species was approximately 50% lower in June compared to August. This coincides with the lowest photoperiod values that were followed by a progressive fall on food consumption. Leiner and MacKenzie (2001) do not believe that only food consumption is responsible by T_4 daily cycle observed in red drum.

They also report that both, photoperiod and food consumption contribute to T_4 daily cycle, and explain that Boujard and Leatherland (1992) and Spieler (1992) already proposed an existence of multiple oscillatory system to temporal integration in fishes, in which, an oscillator coincide with light period, and another with the feed, and thereby, T_4 daily cycle is determined by both influence.

Gorbman (1969) say that in certain natural condition, species like *Umbra limi* have considerable quantities of T_3 and lower T_4, which is different from *L. obtusidens* concentrations that always shows higher levels of T_4.

T_4 probably is the main released substance of thyroid colloid of fish and the T_3 majority is derived from peripheral deiodation of T_4 (EALES, 1985).

The existence of T_4 daily cycle in fishes was verified by many authors in goldfish and rainbow trout (LEATHERLAND et al., 1978; OSBORN et al., 1978; SPIELER; NOESKE, 1979; COOK; EALES, 1987). They explain that feed stimulate a variable T_4 releasing by thyroid, raising in trout, a T_4 peak between 4 to 6 hours after food supply, without consider the photoperiod. This probably is thyroid fast answer to feed stimuli.

Results on T_4 plasmatic concentration observed in June 1996 agree with Eales et al. (1981) witch mentioned, in studies with trout kept in water temperature of 11-13ºC, that T_4 and T_3 hormones variations were parallel.

Higgs and Eales (1977) also consider that feed trout quickly use plasmatic T_3, and that consumption or the ingested energy/protein ratio stimulates the metabolism and the secretion of T_4, which increase the peripheral deiodation to T_3. Thyroid tissue sensitivity to TSH stimuli, and the activity of hepatic deiodase are strongly influenced by nutritional state and low food consumption reduce the quantity of T_4 5'deiodase (extrathyroidal enzyme that convert T_4 to T_3) (HIMICK; EALES, 1990; LE BAIL; BOEUF, 1997).

The relationship between food ingestion and thyroid hormone daily level, observed in this study for *L. obtusidens*, agree with data of Higgs and Eales (1977; 1978), Flood and Eales (1983), Rydevick et al. (1984), Himick and Eales (1990) observed for salmonid. They verified a raise in plasmatic T_4 related to food consumption.

The consumption act in absorption of plasma hormones and in peripheral conversion of T_4 to T_3 (increasing deiodation of T_4), directly altering its daily cycle (BROWN; EALES, 1977; HIGGS; EALES, 1977; BROWN et al., 1978; EALES et al., 1981; FLOOD; EALES, 1983; COOK; EALES, 1987; HIMICK; EALES, 1990).

Thus, it is possible to conclude that exist a daily cycle of plasmatic T_4 and T_3, related to feed in *Leporinus obtusidens*, where maximum level of thyroid hormone appear 7 hours after food supply during photophase. Moreover, to be sure that temperature and photoperiod also have an important participation, and the level of their influence in this species, other experiments must be conduced to quantify the isolated influence of each one on thyroid hormone cycle and on food consumption.

Acknowledgements

We are greatful to Capes for the Master grant between the years of 1994 and 1996; to the Graduate Animal Science course at the Universidade Federal de Lavras – UFLA; to Prof. Álvaro João Lacerda de Almeida – UFLA; to Dr. José Alair Couto (Santa Cecília Laboratory) for thyroid hormones analysis.

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Received on November 21, 2008.
Accepted on March 20, 2009.