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Does the time of feeding affect the diurnal rhythms of plasma hormone and glucose concentration and hepatic glycogen content of rainbow trout?

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Abstract

The diurnal rhythms of plasma glucose, cortisol, growth hormone (GH) and thyroid hormone (T_4, T_3) concentrations and hepatic glycogen content were measured in rainbow trout that had been entrained to a specific time of daily feeding (post-dawn, midday, pre-dusk); the purpose of the study was to investigate the significance of feeding time on hormones and metabolite patterns. Plasma GH, cortisol and T_4 concentrations all showed evidence of a diurnal rhythm in some treatment groups. There was a significant interaction between the time of feeding and plasma GH and cortisol concentration rhythms; for GH, this appeared to be related to the phase-shifting of the post-prandial increases in plasma GH concentrations, and for cortisol, the rhythms were only evident in fish fed in the post-dawn period [diurnal rhythms were not evident in treatment groups fed in at midday or pre-dusk]. Peak plasma T_4 concentrations were evident during the photophase in all three treatment groups; however, the time of feeding had a negligible effect on the timing of those peaks. There were no apparent diurnal rhythms of plasma T_3 and glucose concentrations, hepatic glycogen content or hepatosomatic index in any of the three treatment groups.

Introduction

Diurnal rhythms of plasma and tissue metabolites and plasma hormone levels have been described in several species of teleosts (see Boujard and Leatherland 1992a for references). Of the several known abiotic entraining factors, diel rhythms, feeding time and photoperiod (light/dark alterations) have received most attention from investigators to date. Growth performance of several species of fish has been shown to be influenced significantly by the time of feeding, and feeding time is recognised as a major synchronizer of many physiological rhythms (see Boujard and Leatherland 1992a; Meier 1993; Spieler 1993 for reviews). However, it is usually difficult to compare the results from the various studies since the different researchers employ radically different feeding regimes.

In a series of experiments with rainbow trout, *Oncorhynchus mykiss*, given access to self-demand feeders, the food demand was recorded throughout the 24 hour day in an attempt to better understand the interactions between feeding time and diurnal rhythms of several plasma metabolites and hormones (Boujard and Leatherland 1992b, c; Boujard *et al.* 1993). In those studies, the fish exhibited a clear diurnal pattern of feeding activity and demanded food preferentially during the photophase,

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with a main peak at dawn. When the fish were held under three different photoperiod regimes and given access to self-demand feeders, all the measured parameters (plasma growth hormone (GH), T_4, T_3, cortisol and glucose concentrations) showed significant diurnal rhythms, and dawn appeared to be the main synchronizer. In these studies, dawn was represented by the concomitant onset of both light and feeding and it is possible that photoperiod and feeding had both influenced the rhythms. When trout were given only restricted access to food from the self-demand feeders (food was available only during the first hours of the photophase or during the middle part of photophase) the diurnal rhythms for some hormones (GH and cortisol) were phase-shifted, whereas for some hormones (T_3) the rhythms disappeared altogether. The diurnal rhythms for plasma T_4 concentrations were retained regardless of time of feeding (Boujard *et al.* 1993). The above studies suggest that the time of feeding and method of feeding have significant effect on the changes in the diurnal hormonal rhythms.

In the present study, we determined whether the diurnal plasma hormone rhythms that are characteristic of rainbow trout entrained to a single daily feeding time are influenced by the time of feeding (post-dawn, midday, pre-dusk), an in particular, whether the time of feeding can phase-shift plasma hormone rhythms.

Materials and methods

Source and maintenance of fish

Immature rainbow trout (*Oncorhynchus mykiss*) of both sexes were used in the study; the fish were obtained from a stock reared at the Alma Research Station, Alma, Ontario, and all experiments were conducted at that facility.

The fish were maintained indoors, in an experimental holding facility under artificial light and the light cycle was adjusted weekly in order to simulate natural photoperiod of the season. Prior to starting the experiment, the fish were maintained in 1000 l grey fibreglass aquaria supplied with constantly running aerated water pumped from deep wells. The fish were fed daily (approximately 2% of the body weight) in the morning (between 09:00h and 11:00h) with a commercial trout pellet (Martins Feed Mills, Elmira, Ontario) by means of automatic vibrating feeders.

Experimental procedure

The experiment was carried out between March 12 and July 3, 1992 (113 days) and the photoperiod was 12:12 LD at the start of the experiment and increased to 16:8 LD by the end. Rainbow trout (initial body weight 23 g) were randomly assigned to one of 12 identical 80 l grey aquaria (150 fish per aquarium, equivalent to a stocking density of approximately 0.04 kg l^{-1}). The ambient water temperature was $9 \pm 1°C$ (for the duration of the experiment, daily variation was negligible).

The aquaria were randomly assigned to one of three treatment groups (4 replicates per treatment) and fed at dawn + 30 min, midday or dusk − 30 min with 2.5% of their live body weight of the commercial rainbow trout pellets used in the pre-experimental period; this ration level represented 120% of the recommended ration for rainbow trout by the Martin Feed Mills' guidelines. The food was delivered by means of vibrating automatic feeders that delivered the pellets in 10 sec pulses over approximately 15 min; the feeders were regulated centrally ensuring that food was delivered simultaneously to each particular treatment group (dawn, midday, dusk).

The total weight of fish in each aquarium was measured at 14 day intervals in order to adjust ration levels for each aquarium; the fish were not fed on the days of weighing.

Sampling schedule and blood collection

Fish were sampled from each treatment at 03:00h, 06:00h, 09:00h, 12:00h, 15:00h, 18:00h, 21:00h, and 24:00h. The sampling took place over a period of 5 days (between May 25 and May 29, 1992; photoperiod 14:10 LD), and samples were taken from

each treatment at each sampling time on at least two separate days. Four fish were removed from each of six aquaria at each sample time, and an interval of at least 12h was allowed to elapse between sampling from the same aquarium to minimize the possibility of measured variables being influenced by the sampling process itself.

At each sampling time, the fish were removed by dipnet with minimum disturbance to the remaining fish in the aquarium, placed immediately in a solution of MS222 (125 mg/l), and taken to a separate room for blood removal. Blood was taken by caudal severance and collected in heparinized tubes; plasma was separated by centrifugation and aliquots were stored frozen at −70°C until analysis. Total body weights and liver weights were recorded and the hepatosomatic index (HSI) for each fish was calculated as liver weight/body weight × 100. A piece of liver from each fish (approximately 0.2 g) was weighed and frozen at −20°C for subsequent determination of hepatic glycogen. A total of 16 fish per treatment per sampling time was used for plasma GH concentration, hepatic glycogen content and HSI measurement, while a total of 8 fish per treatment per sampling time were used for the measurement of plasma cortisol, T_4, T_3 and plasma glucose concentrations.

Assays

Hepatic glycogen content was determined using the method of Lo *et al.* (1970) and plasma glucose concentration was measured by a glucose oxidase colorimetric method (Sigma Chemical Co., St. Louis, MO). The glucose assay was modified to use 5 µl of plasma and the reaction was carried out in EIA/RIA microplates (Costar Corporation, Cambridge, MA) and the absorbance was measured by microplate reader (Titertek Multiskan).

Plasma GH concentrations were measured using a non-competitive enzyme-linked immunosorbent assay based on monoclonal antibodies raised against chum salmon GH (Farbridge and Leatherland 1991). Plasma T_4 and T_3 concentrations were measured using Amersham Amerlex RIA kits (Kodak Canada, Toronto, ON). Plasma cortisol levels were measured using the Incstar Gammacoat RIA kits (Incstar Co., Stillwater, MN).

Statistical analysis

Data were log-transformed wherever necessary to ensure homogeneity of variances. The data for each variable and each feeding time were separately analyzed by one-way analysis of variance. Where F values indicated significance ($p < 0.05$), individual means were compared using SNK's multiple range comparisons.

Results

Hepatosomatic index (HSI) and hepatic glycogen content

There were no diurnal rhythms in the HSI or hepatic glycogen content for any of the three treatment groups. When the 8 point mean values for the 24h period were compared, the HSIs in the midday and pre-dusk fed groups were significantly higher than in the post-dawn fed groups, but there were no differences for hepatic glycogen content (Table 1).

Plasma glucose concentration

There were no diurnal variations in plasma glucose concentration for any of the three treatment groups and the 8 point mean values were not significantly different from one another (Table 1).

Plasma cortisol concentration

There were no significant diurnal variations in plasma cortisol content in the midday fed and pre-dusk fed groups. However, in the post-dawn fed group the levels were lower during the late photophase and significantly increased during scotophase with acrophase at 24:00h (Fig. 1). The 8 point mean values were not significantly different from one another (Table 1).

Table 1. Effect of time of feeding on final mean body weight (FBW), hepatosomatic index (HSI), hepatic glycogen content (HGC) and plasma glucose, cortisol, growth hormone (GH), thyroxine (T_4) and triiodothyronine (T_3) concentrations in rainbow trout

	Feeding time		
	Dawn	Midday	Dusk
FBW (g)	74.7 ± 1.5	84.4 ± 1.6	80.8 ± 1.6
HSI (%)	1.34 ± 0.02[a]	1.48 ± 0.02[b]	1.45 ± 0.02[b]
HGC (mg g^{-1})	66.9 ± 2.3	60.4 ± 1.9	59.1 ± 1.9
Glucose (mg dl^{-1})	105 ± 2	103 ± 2	102 ± 2
Cortisol (nmol l^{-1})	56.8 ± 4.9	55.3 × 6.0	56.4 ± 5.7
GH (ng ml^{-1})	1.26 ± 0.12	1.17 ± 0.09	1.33 ± 0.11
T_4 (nmol l^{-1})	5.67 ± 0.28[a]	6.29 ± 0.28[b]	6.40 ± 0.31[b]
T_3 (nmol l^{-1})	5.05 ± 0.18[a]	6.14 ± 0.20[b]	6.22 ± 0.25[b]

Data are shown as means of all samples collected at 8 times over the 24h sampling period ± SEM [n = 4 replicates for FBW, n = 128 for HSI, HGC and GH, n = 64 cortisol, T_4, T_3 and glucose]. Means without superscripts, or with similar superscript letters are not significantly different from one another.

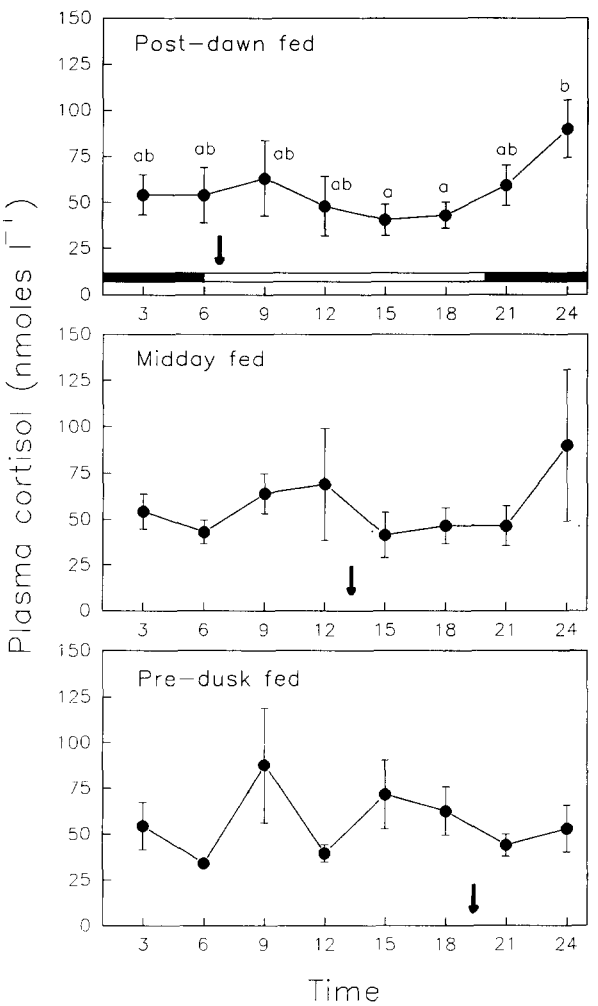

Fig. 1. Effect of feeding time on the diurnal changes in plasma cortisol concentrations in rainbow trout. The arrow indicates the time of feeding in each group, and the photoperiod is shown on the upper panel. Data are shown as mean ± SEM (n = 8). Points with similar superscript letters (post-dawn group) are not significantly different; there were no significant differences in the midday- and pre-dusk-fed groups.

Plasma GH concentration

There was a clear diurnal rhythm of plasma GH concentrations in all three treatment groups with postprandial peaks (and thus time of feeding effects) evident (Fig. 2). In the post-dawn fed group, the acrophase was seen around 6h after feeding, followed by a steady decrease reaching significantly lower value at 18:00h. There was second smaller peak in the scotophase at 03:00h. A similar trend was seen in the midday fed group, with a postprandial peak occurring around 3h post-feeding followed by a steady decrease between 15:00h and 24:00h and a second peak around 03:00h. In the pre-dusk fed group, there was only one peak with acrophase at 03:00h (approximately 6h post-feeding). There was no significant difference in the 24h mean value of GH concentrations among the three groups (Table 1).

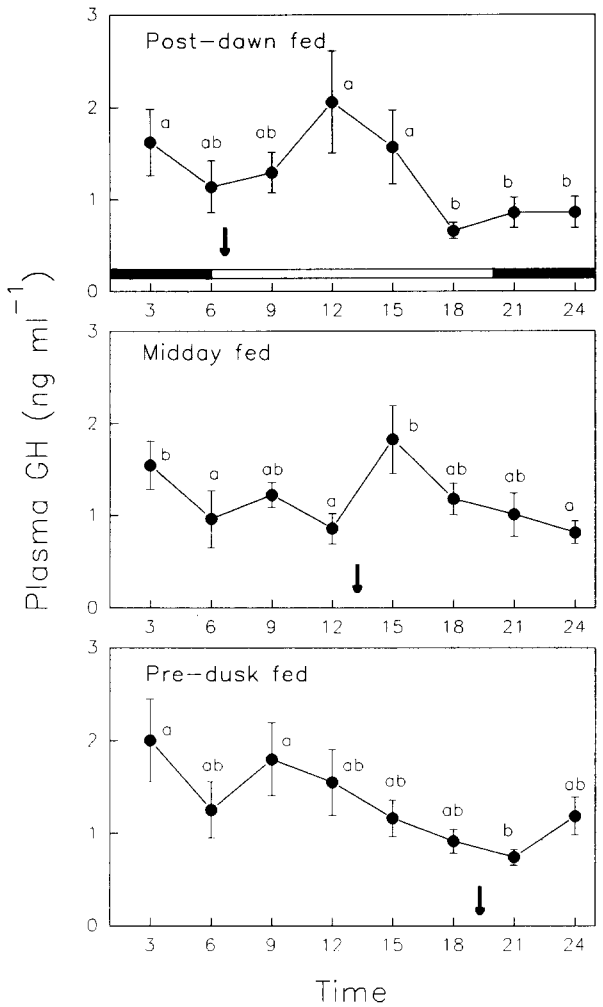

Fig. 2. Effect of feeding time on the diurnal changes in plasma growth hormone (GH) concentrations in rainbow trout. The arrow indicates the time of feeding in each group, and the photoperiod is shown on the upper panel. Data are shown as mean ± SEM (n = 16). Points with similar superscript letters in the same treatment groups are not significantly different.

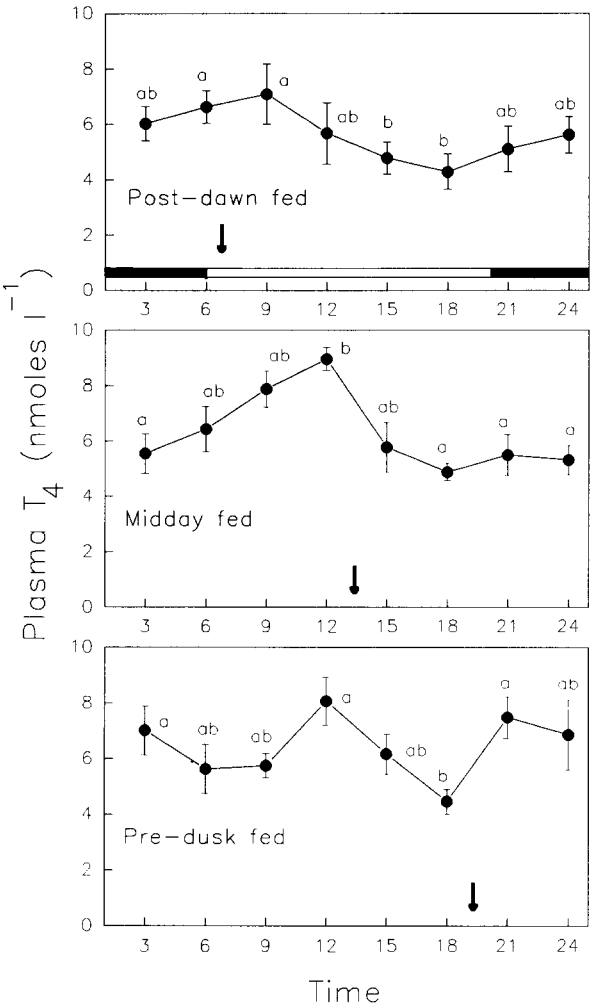

Fig. 3. Effect of feeding time on the diurnal changes in plasma T_4 concentrations in rainbow trout. The arrow indicates the time of feeding in each group, and the photoperiod is shown on the upper panel. Data are shown as mean ± SEM (n = 8). Points with similar superscript letters in the same treatment groups are not significantly different.

Plasma thyroid hormone concentrations

Plasma T_4 concentrations were higher during the photophase in the midday and pre-dusk fed groups. In the post-dawn fed and midday fed groups, the plasma T_4 concentrations began to rise well before feeding time, reaching peak values around feeding time; in both the groups there was a postprandial decrease. In the pre-dusk fed group the plasma T_4 concentration peaked at noon and then decreased to a significantly low level at 18:00h followed by a postprandial peak with acrophase around 21:00h (Fig. 3). When the 24h mean values of three groups were compared, that of the post-dawn fed group was significantly lower than in midday fed and pre-dusk fed groups (Table 1).

Plasma T_4 concentration did not show any marked diurnal variation in any of the three groups; the 24h mean value of plasma T_3 concentration in post-dawn fed group was significantly lower than that of the two other groups (Table 1).

Discussion

In the present study of rainbow trout that had been entrained to a single daily meal given at post-dawn, midday or pre-dusk, the diurnal rhythms of plasma GH, T_4, and cortisol concentrations were markedly influenced by the time of feeding. Furthermore, fish fed early in the photoperiod had significantly lower growth rates, HSIs and plasma thyroid hormone levels compared with the other two groups.

Plasma GH concentration exhibited a distinct diurnal rhythm with a postprandial peak and a secondary peak during the later part of the scotophase. These rhythms were significantly affected by the time of feeding with the postprandial peaks being phase-shifted; however, the time of feeding did not appear to affect those peaks that were evident during the scotophase (around 3h before dawn in all the 3 groups). In the case of the pre-dusk fed group, the postprandial peak was phase-shifted and therefore superimposed on the peak which occurred during the late scotophase. These results suggest that both photoperiod and time of feeding have significant influence on the diurnal rhythms of plasma GH levels in rainbow trout. Similar high levels of plasma GH during the scotophase and a secondary peak probably associated with feeding were observed previously in kokanee salmon, *Oncorhynchus nerka* (Leatherland *et al.* 1974) and rainbow trout (Boujard and Leatherland 1992c; Boujard *et al.* 1993). The physiological significance of these two peaks (postprandial and nocturnal) is not clearly understood, although they may be related to the regulation of energy partitioning, and thus influence the growth of fish. The 24h mean plasma GH concentrations were not significantly different in any of the three treatment groups, but there was a significant reduction in growth rates of the treatment group fed early in the photoperiod (Table 1).

In general, plasma T_4 concentrations appear higher during the photophase in all the three treatment groups. This observation is in agreement with the reports from other workers (Eales *et al.* 1981; Cook and Eales 1987; Boujard and Leatherland 1992c; Boujard *et al.* 1993). It is noteworthy that the plasma T_4 concentrations were higher at the time of feeding in the post-dawn and midday fed groups, but there was a significant decrease in the levels following feeding. A similar postprandial decline in plasma T_4 concentrations was also observed in other studies (Eales *et al.* 1981; Cook and Eales 1987; Boujard and Leatherland 1992c; Boujard *et al.* 1993). Such a decrease was not seen in the pre-dusk fed group; on the contrary, the levels significantly increased after feeding. We have no explanation for such variation in different groups. It is possible due to the fact that in the post-dawn and midday fed groups the postprandial changes occurred during the photophase, whereas in the pre-dusk fed group the postprandial peak was present in the scotophase and the two different peaks in this group represent one due to a photoperiod effect, and the other due to feeding itself; in the other two treatment groups, if two peaks exist, they may well overlap.

The plasma T_3 concentrations did not exhibit any diurnal variation, and the time of feeding did not seem to have any effect on the variations.

Although the HSI did not vary diurnally in any of the treatment groups, the 24h average HSI in the post-dawn fed group was significantly lower than in the other two groups. It is possible that this low HSI is correlated with the lower growth rate of the post-dawn fed group. In previous studies with rainbow trout, we reported weak diurnal rhythms in the HSI (Boujard and Leatherland 1992c), but only when the fish were given unlimited access to a demand-feeder. When access was limited to a 2h period either at early morning or midday, these rhythms were no longer evident (Boujard *et al.* 1993); thus, the method of food delivery appears to have a major influence on the appearance of diurnal rhythms in the HSI.

There was no evidence of a diurnal rhythm of hepatic glycogen content in any of the three treatment groups. In previous studies, there was no general agreement as to whether circadian rhythms of hepatic glycogen content are present; the considerable variation among these studies with regard to season, photoperiod and feeding regimes employed render comparisons difficult (Delahunty *et al.* 1978; Singh 1981; Laidley and Leatherland 1988). In previous studies in our laboratory, we reported significant increases in hepatic glycogen content in

rainbow trout, but as with the T_3 response, this effect was only found in fish given unrestricted access to a self-demand feeder (Boujard and Leatherland 1992c).

There are several reports of hyperglycaemia in fish after feeding and often these peaks are not only associated with feeding but are also synchronized with photoperiod (Delahunty *et al.* 1978; Gutierrez *et al.* 1984; Laidley and Leatherland 1988; Boujard and Leatherland 1992c; Boujard *et al.* 1993). Boujard and Leatherland (1992b) and Boujard *et al.* (1993) using rainbow trout fed by means of self-demand feeders observed the lowest plasma glucose levels just before dawn and progressive increase throughout the photoperiod. These extended higher values may be the result of the fish feeding over a prolonged period of time when they are given access to a demand-feeder. In the study represented here, in which the food was delivered at a specific time within a short period of 15 min, postprandial peaks of plasma glucose were suggested by the changes in mean values, but no significant changes were found.

Plasma cortisol concentrations are the most studied physiological variable in terms of circadian rhythms in fishes and other vertebrates. Although several investigators have confirmed the existence of diel rhythms of cortisol in fish (see Boujard and Leatherland 1992a for review), there are wide variations in the frequency, amplitude and phasing of such rhythms. Several workers have reported in rainbow trout and other fishes, that cortisol peaks occurred during scotophase with another secondary peak shortly after feeding (Garcia and Meier 1973; Peter *et al.* 1978; Baker and Rance 1980; Rance *et al.* 1982; Bry 1982; Pickering and Pottinger 1983; Spieler and Noeske 1984; Laidley and Leatherland 1988; Boujard and Leatherland 1992c; Boujard *et al.* 1993).

In the present study, peak plasma cortisol concentrations were found during the scotophase (as reported in earlier studies) in the post-dawn fed group, but not in the other two treatment groups. The shift in the time of feeding appeared to have a significant effect on the diel plasma cortisol pattern, particularly during the scotophase. A similar disappearance of the peak of cortisol in the scotophase was reported by Delahunty *et al.* (1978) in goldfish when the feeding pattern was changed from a single feed presented early in the day to a single feed administered at random. In rainbow trout given free access to self-demand feeders, a main peak of plasma cortisol concentration was apparent at the time of the major feeding episode (*i.e.*, dawn) (Boujard and Leatherland 1992c); postprandial increases were not evident in the present study possibly because of the method of feeding that was used. Postprandial peaks of plasma cortisol concentration may be caused by the presence of the experimenter during manual feeding and the associated stress due to the increased activity and competition for food. Even with the use of self-demand feeders, only a limited amount of food is delivered at a time (when one fish activates the feeder) and the competition for food and the following stress may lead to elevated cortisol levels. When automatic feeders are used (such as the vibrating type used in the present study), the food is delivered in excess over a short time period and it is spread out over the water surface; intuitively, this method would involve less competition for food and therefore be less stressful. These results suggest that not only is the time of feeding significant, but the method of feeding may also have profound effect on the diel changes in plasma cortisol diel concentrations.

Time of feeding is now widely accepted as one of the Zeitgebers influencing the physiological rhythms in fish. The feeding time has a significant influence on the energy partitioning and growth of fish since these are probably under the control of various hormones. A better understanding of circadian hormonal rhythms and the influence of time of feeding on the changes in these cycles is important for better feed utilization and in order to maximize feed conversion efficiency and growth of fish in intensive culture.

In conclusion, time of feeding seems to have significant effect on the plasma GH levels because the postprandial hormone peaks were phase-shifted with the change in the time of feeding. Photoperiod and time of feeding both appear to have effect on the diurnal rhythms of plasma T_4 concentrations, and the diurnal rhythms of plasma cortisol concen-

trations were lost when fish were fed in the middle of the day or late in the photoperiod.

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