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# GROWTH AND DIURNAL VARIATIONS IN METABOLIC PARAMETERS IN THE STARVED BASS, *DICENTRARCHUS LABRAX*, AFTER EXPERIMENTAL FEEDING

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**Abstract**—1. Young bass (*Dicentrarchus labrax*) were fed either a natural diet (filleted bogue) or a commercial diet for 107 days and studied after fasting for 7 days.

2. The food consumption index and daily caloric intake was lower in the bass on the natural than on the commercial diet but the growth related parameters were much greater in the first group.

3. Plasma acetoacetate levels showed daily rhythmicity in the bass on a bogue diet and these changes showed a trend similar to that of glucose.

4. Plasma glycerol levels were greater in the bass on a commercial than on a natural diet probably as a result of enhanced lipolysis due to the limited efficiency of energy utilization in the first group.

5. Some individual plasma amino acids showed significant daily rhythms in the bass on either diet. The compiled values of plasma amino acids were very similar in fish on either diet, indicating that in both groups lipid and carbohydrate metabolisms are more sensitive than those of protein-amino acids to the differences in the efficiency of energy utilization.

## INTRODUCTION

Significant differences have been reported in the energy and nitrogen utilization efficiency of juvenile bass, *Dicentrarchus labrax* (L.) fed on natural and artificial diets (Stirling, 1976, 1977). The high market value of this fish in Europe and recent findings about many aspects of its biology (Barnabé, 1976, 1980) led us to study the effects of natural and artificial feeding on growth and metabolic parameters in juvenile bass maintained in a captive environment since birth. As daily rhythms in metabolic parameters have been clearly shown in teleost fish kept in captivity (Delahunty *et al.*, 1978; Carrillo *et al.*, 1980), the present study was extended to determine whether different diets affected daily rhythms of specific parameters in the bass.

## MATERIALS AND METHODS

*Dicentrarchus labrax* (L.) obtained in our Institute from the same female by induced spawning were maintained in well aerated tanks at a density of 1 individual per 20 l. Aquaria were provided with a running sea water circuit (3.7‰ salinity) under natural daylight photoperiod. Water temperature was not controlled but changed naturally and gradually from $20.8 \pm 0.4$°C in late summer to $11.3 \pm 0.2$°C in winter. On September 25, when the fish were 288 days old, they were divided into two groups of 40 individuals of the same body weight ($35.1 \pm 1.7$ and $32.5 \pm 2.1$ respectively) and size ($15.0 \pm 0.2$ and $14.7 \pm 0.2$ cm). One group was fed a natural diet consisting of white filleted muscle of bogue (*Boops boops*) while the other was fed moistened commercial farina ("Food for bass", Bioter-Biona, Madrid) freshly stabilized with water (25 ml of water per 135 g of farina which gave a compact soft feed). The fish were fed once a day (between 11:00 and 12:00) until satiety. Daily food consumption was recorded. This feeding schedule was maintained for 107 days after which the fish were starved for 7 days and then killed in groups every 4 hr during the next 24 hr period. The fish were anesthetized in MS-222 (Sandoz, Barcelona) (50 mg/l) in small groups before sampling. Each fish was weighed, sized, and blood was collected from a caudal vein. The organs were rapidly dissected and immediately placed in liquid $N_2$. Blood was centrifuged at 3000 rev/min for 20 min at 4°C and plasma aliquots were stored at −75°C until assayed. One of these aliquots was deproteinized with $ZnSO_4$-$Ba(OH)_2$ (Somogyi, 1945) and the supernatant was used for glucose (Huggett & Nixon, 1957), glycerol (Garland & Randle, 1962) and both acetoacetate and beta-hydroxybutyrate (Williamson & Mellamby, 1974) evaluations. Another plasma aliquot was deproteinized with 5% sulphosalycyclic acid and amino acids were analyzed in a Chromaspeck amino acid autoanalyzer using nor-leucine as internal standard. Liver glycogen was purified with ethanol (Good *et al.*, 1933) after KOH digestion and hydrolyzed (Aranda *et al.*, 1972) for glucose assay (Huggett & Nixon, 1957). Lipids were extracted and purified (Folch *et al.*, 1957) in other portions of frozen liver or muscle. One aliquot of the lipid extract was weighed after being dried under $N_2$, another aliquot was used for lipid-phosphorus determination (Fiske & Subbarow, 1925) after digestion with 72% (v/v) $HClO_4$ (Freinkel, 1958) and a third aliquot was used for total fatty acid determination after saponification (Llobera *et al.*, 1978) according to the method of Duncombe (1963). Muscular water was determined by drying portions of tissue in an oven at 105°C until weight became constant. Proteins were measured after alkali digestion by the method of Lowry *et al.* (1951). For measurement of amino acid concentrations in the diets they were hydrolyzed in 6 N HCl for 22 hr and analyzed in the Chromaspeck autoanalyzer. One-way analysis of variance (ANOVA) (Sokal & Rohlf, 1969) was used to determine whether the parameter changed as a function of the sampling time. Two-way analysis of the variance (ANOVA) (Sokal & Rohlf, 1969) was used to compare the absolute values of each parameter between the two experimental groups. When the parameter did not show significant diurnal variation, the values obtained from fish of the same group sacrificed at different times of the day were pooled

Table 1. Composition of the diets used

| Percental composition | Bogue diet | Commercial diet |
|---|---|---|
| Water | 75.80 ± 0.60 | 11.50 |
| Lipid | 1.88 ± 0.45 | 7.03 |
| Protein | 20.60 ± 0.40 | 49.41 |
| Carbohydrate | 0.00 | 19.93 |
| Ash | 1.50 ± 0.03 | 11.46 |
| Fibre | 0.00 | 1.02 |
| Amino acids ($\mu$M/g wet weight) | | |
| Tau | 62.78 | 34.50 |
| Asp | 180.14 | 325.32 |
| Thr | 79.65 | 173.89 |
| Ser | 69.37 | 221.62 |
| Glu | 213.78 | 399.97 |
| Pro | 59.26 | 187.43 |
| Gly | 121.54 | 414.24 |
| Ala | 143.18 | 316.84 |
| Val | 101.36 | 195.09 |
| Met | 1.13 | 44.91 |
| Ile | 65.29 | 144.97 |
| Leu | 141.33 | 262.03 |
| Tyr | 29.59 | 78.61 |
| Phe | 46.75 | 140.32 |
| His | 52.85 | 98.82 |
| Trp | — | 7.34 |
| Orn | — | 15.22 |
| Lys | 112.32 | 192.72 |
| Arg | 86.09 | 147.27 |
| Total amino acids | 1566.40 | 3401.20 |

Values of bogue correspond to mean ± SEM of six determinations done at different times throughout the whole experiment while values of commercial represent the mean of triplicate determinations.

Table 2. Effect of experimental diets on *Dicentrarchus labrax*. Animals were kept on each diet for 107 days and studied after a 7 day fast. Values correspond to mean ± SEM of all the samples taken at different times during the last fasting day (number of animals in parentheses)

| | Bogue diet | Commercial diet | $P$** |
|---|---|---|---|
| Body weight (g) | 46.6 ± 1.8 (35) | 38.2 ± 1.6 (35) | <0.001 |
| Body size (cm) | 16.5 ± 0.2 (35) | 15.6 ± 0.2 (35) | <0.01 |
| Condition factor* | 0.633 ± 0.014 (35) | 0.601 ± 0.006 (35) | <0.05 |
| Liver weight (g) | 0.915 ± 0.053 (35) | 0.590 ± 0.051 (34) | <0.001 |
| Liver somatic index (% of body weight) | 1.94 ± 0.09 (35) | 1.48 ± 0.09 (35) | <0.001 |
| Mesenteric fat bodies (g) | 1.69 ± 0.08 (30) | 0.826 ± 0.077 (35) | <0.001 |
| Mesenteric fat bodies somatic index (% of body wt.) | 3.40 ± 0.10 (30) | 2.16 ± 0.15 (29) | <0.001 |
| Liver total lipids (mg/g) | 308 ± 12 (34) | 167 ± 16 (33) | <0.001 |
| Liver phospholipid phosphorus ($\mu$g/mg) | 5.51 ± 0.25 (34) | 6.11 ± 0.37 (34) | NS |
| Liver total fatty acids ($\mu$M/100 mg) | 24.9 ± 1.2 (34) | 17.6 ± 1.4 (32) | <0.001 |
| Liver glycogen ($\mu$g/mg) | 20.5 ± 1.7 (35) | 30.8 ± 3.2 (34) | <0.01 |
| Muscle phospholipid phosphorus ($\mu$g/mg) | 4.11 ± 0.18 (32) | 4.25 ± 0.20 (30) | NS |
| Muscle total fatty acids ($\mu$M/100 mg) | 1.09 ± 0.12 (33) | 1.49 ± 0.14 (31) | <0.05 |

* Condition factor $= \dfrac{\text{body weight (g)} \times 100}{(\text{length in cm})^{3.18}}$.

** $P$ statistical comparison between both diets, by Student's $t$-test.

to give mean ± SEM values and comparison with values from the other group was done by the Student's *t*-test.

## RESULTS AND DISCUSSION

Juvenile bass were fed *ad libitum* a natural diet (filleted bogue) or a moistened commercial diet for 107 days. The food consumption index (total weight of unmoistened food eaten/increase of body weight per day) at the end of the last month of treatment was 9.22 g for those fish receiving filleted bogue and 28.58 g for those on the commercial diet. Table 1 shows the composition of each diet. Minor differences were observed in the composition of the filleted bogue captured at different times of the year, as shown by the small SEM variation of the observed values. The percental composition found for bogue was similar to that reported by Stirling (1976) for filleted saithe and in both cases, water was the major component, while protein was the main component of the organic matter. The commercial diet had much lower water content and consequently a higher proportional organic content. The total amino acid content per g was much greater in the commercial than in the bogue diet (Table 1) although the distribution of individual amino acids differed. These findings and the fact that *Dicentrarchus labrax* ate three times more commercial than natural diet means that the daily caloric intake of the commercially fed fish was much greater.

### *Structural parameters and liver composition*

The bass were sacrificed in groups every four hours during the day after a 1 week fast. None of the growth related parameters differed significantly among these groups and their values were compiled (Table 2). In spite of the greater caloric intake in fish receiving the commercial diet, both the body size and weight of fish on bogue were significantly greater. The condition factor which is an index of corpulence was also higher (Table 2). These findings demonstrate the much greater energy utilization efficiency of bogue fed *Dicentrarchus labrax* (L.) and coincide with the findings of Stirling (1977) obtained with a different natural diet and experimental protocol. To determine the contribution of both liver and mesenteric fat bodies to these body weight differences, they were also weighed. As shown in Table 2, the weight of these organs was much greater in bogue-fed than commercial diet-fed fish, both in absolute and somatic-index values, and these values were highly significant. The difference in liver corresponded to changes in its intrinsic composition, since total lipid concentration in liver was higher in the bogue-fed fish (Table 2). This augmented lipidic liver content was related not to an increase in structural lipids, because phospholipid–phosphorous concentration was similar in both groups (Table 2), but to a parallel rise in the total fatty acids concentration (Table 2). These findings indicate that there is a specific increment of neutral glycerides in the liver of bogue-fed fish. These lipids are the most active from the metabolic point of view (Wakil, 1970) and they probably originate from endogenous synthesis from amino acids coming from the diet protein, since the fat content in bogue is very low (even lower than in the commercial diet) and the

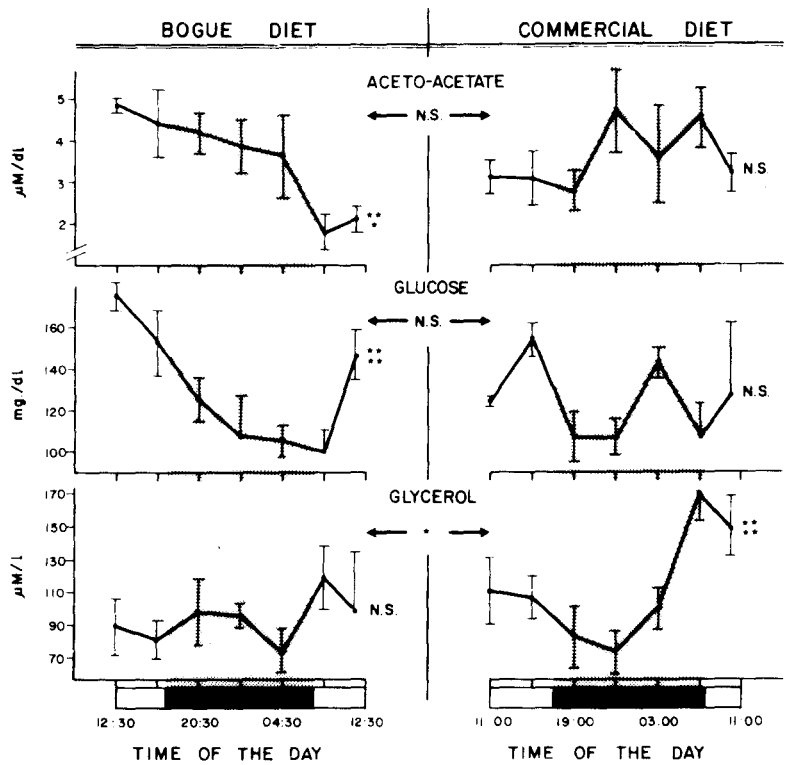


Fig. 1. Plasma level of acetoacetate, glucose and glycerol in two groups of *Dicentrarchus labrax*, fed either natural (filleted bogue) or a commercial diet, sacrificed every four hours during the day after a 1 week fast. Values correspond to means ± SEM of 5 animals. The significance of each parameter change as function of the sampling time is denoted at the side of its correspondent line and that between the two experimental groups in the middle of the figure: * = $P < 0.05$, ** = $P < 0.02$, *** = $P < 0.01$,

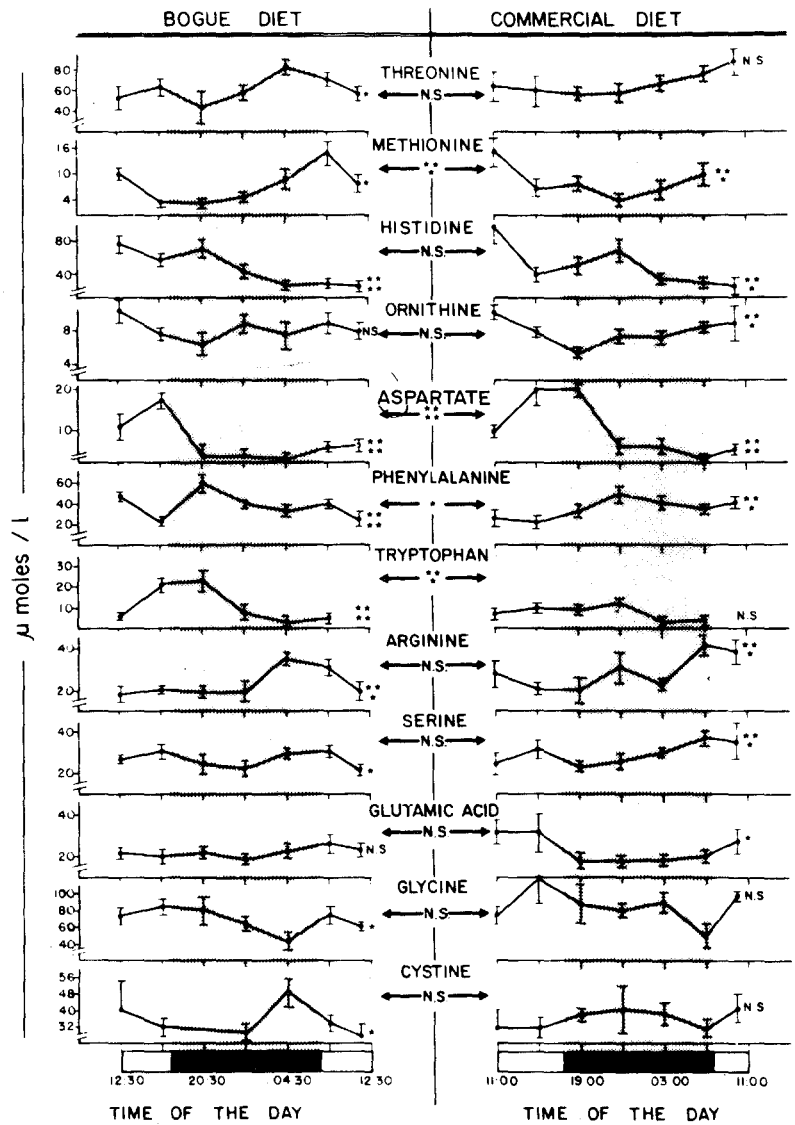


Fig. 2. Plasma level of those amino acids showing circadian rhythms in at least one experimental group in *Dicentrarchus labrax*, fed either natural (filleted bogue) or commercial diet, sacrificed every 4 hr during the day after a 1 week fast. Values correspond to means ± SEM of five animals. The significance of each amino acid change as function of the sampling time is denoted at the side of its correspondent line and that between the two experimental groups in the middle of the figure: * = $P < 0.05$, ** = $P < 0.02$, *** = $P < 0.01$, **** = $P < 0.001$, NS = not significant ($P > 0.05$).

amount of carbohydrates is negligible. Unlike the lipids, liver glycogen content was significantly lower in the bogue-fed than in the commercial diet-fed fish (Table 2). These differences in glycogen were not accompanied by intergroup changes in mean plasma glucose concentration (see below). Although further studies are required to obtain a precise interpretation of these results, they may represent differences in the rate of glycogenesis rather than in glycogenolysis because of the limited ability of fish to mobilize liver glycogen even in conditions of starvation (Nagai & Ikeda, 1971; Larsson & Lewander, 1973).

*Circulating metabolites*

Unlike structural parameters and liver components, some of the circulating metabolites studied in the two groups of *Dicentrarchus labrax*, fed either commercial or natural diet, showed circadian rhythmicity, although in several parameters the cycles were affected in a different manner as a function of diet.

Beta-hydroxybutyrate was not detected in plasma of bass and in agreement with Zammit & Newsholme (1979), ketone bodies may not be an important fuel in teleosts during starvation. Acetoacetate levels in plasma were, however, detected in the fasted bass of both groups and values are summarized in Fig. 1. In the bogue-fed group, acetoacetate levels showed significant daily rhythmicity. A trend of change similar to that of acetoacetate was observed in plasma glucose levels. (Fig. 1) and was most evident in the bogue-fed bass where the glycemia levels were lowest at the end of the dark phase. This parallel change

Table 3. Effect of experimental diets on plasma amino acids in *Dicentrarchus labrax*. Compiled values of the plasma amino acids without rhythms of all the animals in each group fed on bogue diet and commercial food starved for 7 days and sacrificed at different times of the last day (number of animals in parentheses)

| Amino acid ($\mu$M/l) | Bogue diet | Commercial diet | $P$** |
|---|---|---|---|
| Tau | 1123 ± 114 (31) | 1316 ± 165 (32) | NS |
| Ala | 640 ± 28 (31) | 586 ± 31 (32) | NS |
| Val | 362 ± 13 (31) | 384 ± 19 (32) | NS |
| Ile | 185 ± 9 (31) | 192 ± 11 (32) | NS |
| Leu | 336 ± 13 (31) | 354 ± 19 (32) | NS |
| Tyr | 84 ± 5 (30) | 84 ± 6 (32) | NS |
| Lys | 295 ± 18 (31) | 306 ± 23 (32) | NS |

** $P$ = Statistical comparison between both diets by Student's $t$-test.

between circulating glucose and acetoacetate levels in the bass was also reported by Zammit & Newsholme (1979) following different periods of starvation, indicating either that in this fish both parameters are interrelated or that they are controlled by a similar internal clock. Plasma glycerol levels, on the contrary, showed daily rhythms in bass on commercial diet (Fig. 1) and the compiled values were significantly greater in this group than in the bogue-fed fish ($P < 0.05$). Since increasing levels of glycerol in the bass indicate mobilization of stored triglycerides in adipose tissue (Zammit & Newsholme, 1979), present results suggest that the more limited energy utilization efficiency of the bass on commercial rather than natural diet caused the augmented lipolysis of stored triglycerides which may be the main factor responsible for reducing their mesenteric fat bodies. During the day, this augmented lipolysis in starved bass that had received commercial diet occurred at the onset of the light period when plasma glycerol levels were highest (Fig. 1).

*Plasma amino acids*

The daily rhythmicity of circulating metabolites in the starved bass was clear for certain amino acids. In Fig. 2, the values in plasma are summarized for those amino acids showing significant daily rhythms. The lowest level of most of them occurred during the dark phase and increased at the end of this period, although some amino acids including phenylalanine, arginine, threonine, cystine and serine showed the opposite trend. As the present study was performed in animals starved for 7 days, these rhythms were not directly dependent on feeding schedules but on behavior or endogenous events. The activity schedule in the bass is apparently related more with feeding than with light (Barnabé, 1976, 1980) and although we have not evaluated the bass activity, it was observed to be considerable during the dark periods. Amino acid rhythms in bass differed from those we described previously in goldfish in that most of their highest values were observed during the dark periods (Carrillo *et al.*, 1980) but it is well known that unlike the bass, goldfish are definitely more active during the day than at night (Spencer, 1939).

The observed changes in some of the amino acids in the bass presented rhythms which were dependent on the diet received before the fasting period. This was the case for aspartate ($P < 0.001$), phenylalanine ($P < 0.001$), tryptophan ($P < 0.001$) and arginine ($P < 0.05$). Rhythms of other amino acids (threonine, methionine, histidine and ornithine) were, however, independent of the diet and further studies are required to explain these differences. The rest of the amino acids did not present significant daily rhythmicity and their compiled values in groups sacrificed at different times of day are shown in Table 3. In spite of the considerable variations in body size and structural components in the two groups of fish, the plasma level of most amino acids not showing rhythms did not differ between the natural and commercial diet-fed bass. As it has been shown that tissue protein synthesis is inhibited in fasting fish (Jackim & Laroche, 1973), circulating amino acids in the fasted bass probably represent changes in muscular protein breakdown. Present results suggest that protein breakdown is the same in the two groups of bass studied. White dorsal muscle protein concentration in our bass on commercial diet did not differ from that in bogue-fed fish (mean values were 207 ± 8 mg/g and 197 ± 8 mg/g, respectively). These findings, together with those of the compiled plasma amino acid levels, indicate that the important differences in energy efficiency between the diets had a much greater effect on lipid and carbohydrate metabolism than on protein-amino acid metabolism.

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