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Productive and physiological implications of different feeding frequencies in meagre *Argyrosomus regius* (Asso, 1801)

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ABSTRACT

To gain information concerning the optimal feeding conditions for meagre (*Argyrosomus regius*) culture at the juvenile stage (170 g initial wt), four experimental feeding regimes were tested in duplicate lots. All fish were fed with a similar total weekly ration but distributed in 7, 6, or 5 days per week in the morning (lots 7M, 6M, and 5M, respectively) or 5 days per week in the afternoon (lots 5A). Over the 45 and 90-day experiment, some biometric, productive, and metabolic parameters were determined. Growth rates were very similar in all lots, the highest being in 6M fish, which exhibited the best food utilization. No major differences were detected either in body morphometry or body composition. Differences in liver and muscle activities in certain enzymes involved in the intermediary metabolism appear to reflect the impact of the intermittent food deprivation in 5M and 5A fish. In general, the feeding regime based on weekly one-day food deprivation appeared to be favourable for fish and thus could be profitable for fish farming.

1. Introduction

Meagre (*Argyrosomus regius*) is a new species with the potential for Mediterranean marine aquaculture. The total meagre production in the Mediterranean Region totalled c. 4000 tonnes in 2008 (FAO, 2010), and 10,000 tonnes in 2010, more than half of this pertaining to Spain (Monfort, 2010). Even intensively cultured individuals receiving high-fat diets, lead to high-quality commercial products, with a low fat content and healthy lipid profile (Poli et al., 2003).

Feed management in terms of feeding-rate and frequency optimization is becoming imperative for culturing marine fish and this has become one of the crucial areas of research in fish culture. Overfeeding and waste food disrupts the water quality (Ng et al., 2000) while an inadequate food supply has a direct impact on production cost (Mihelakakis et al., 2002). Optimal feeding strategies improve growth, survival, and food-conversion ratios, and minimize food wastage, reduce fish-size variation, and increase production efficiency (Güroy et al., 2006).

The effects of feeding frequency on fish growth and food-conversion efficiency have been examined for several species with different feeding behaviour. Nile tilapia (*Oreochromis niloticus*) feeding for 6 days a week had similar growth and better feed conversion with respect to 7 days fed (El Sayed et al., 2005). Similar results were reported by Eroldogan et al. (2006) in sea bream (*Sparus aurata*), although growth was lower in fish fed for 5 days compared to those fed for 7 days a week. Wu et al. (2004) reported that, when fed to satiety, channel catfish (*Ictalurus punctatus*) cultured in ponds exhibited similar growth rates and food-utilization indices irrespective of being fed 6 or 7 days a week, in the morning or in the afternoon. Blanquet and Oliva-Teles (2010) found in turbot (*Scophthalmus maximus*) juveniles a direct relationship between total feed intake and the number of days per week (from 4 to 7) the fish were fed; but overall weight gain was not significantly different for fish fed 7, 6 or 5 days a week; feeding efficiency was neither affected by feeding restriction. In juvenile Senegalese sole (*Solea senegalensis*), Rodríguez et al. (2005) recorded similar growth increases whether fish were fed during the day or at night. Takahashi et al. (2010) showed that repetitive 3-day period of food restriction and refeeding decreased growth, feed conversion rate and the protein efficiency ratio of pacu (*Piaractus mesopotamicus*).

It has been verified that fish intermediary metabolism is affected by (could be adapted to) factors such as physical activity (Lushchak et al., 2001), light/dark cycles (Polakof et al., 2007), and, primarily,

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feeding strategy in aspects such as dietary composition and energy content (Suárez et al., 2002; Pérez Jiménez et al., 2009; Enes et al., 2009), ration restriction or deprivation (Pérez Jiménez et al., 2009) or a reduction in feeding frequency (Nakagawa et al., 1995).

Feeding protocol can affect the amount of food consumed by the fish. A close link between the activity of some of the energy-metabolism enzymes and food availability has been demonstrated for several fish species (Sullivan and Somero, 1983), because the nutrients from food digestion become available to metabolic pathways for energy production. Therefore, the activity of these enzymes is likely to be correlated with growth rates. Some authors, such as Pelletier et al. (1993) studying Atlantic cod (*Gadus morhua*) and Lamarre et al. (2007) studying Atlantic wolffish (*Anarhichas lupus*), have found a strong positive correlation between growth rate and the activity of the glycolytic enzymes in the white muscle. The activities of citrate synthase (CS) in white muscle also reflected growth rates in Atlantic cod and saithe (*Pollachius virens*), two gadoid species (Foster et al., 1993; Mathers et al., 1992).

The present study assesses the possible effects and the time course of changes caused by weekly feeding frequency on meagre growth parameters and metabolic consequences on three tissues: blood, liver, and muscle, as the main sites involved in intermediary metabolism, conversion, and distribution of food energy within the body.

2. Materials and methods

2.1. Animals, experimental conditions, and sampling

Juvenile meagre from a wider batch, hatched and raised in IFAPA Centre "El Toruño" facilities, were used in this experiment. Mean initial fish weight and length were c. 170 g and c. 26 cm, respectively. Fish were distributed at random in 8 tanks of 4 m^3 (40 fish per tank) continuously supplied with 5–10 L min^{-1} of filtered and aerated seawater kept at 22.3 ± 0.1 °C. During an adaptation period (14 days long) fish were fed on a dry pelleted diet, seven days a week, once a day (0900), on a daily ration of 1% wet body weight, previously considered by Cárdenas (2010) as optimal for this fish age/size. Time-programmed belt feeders (Innovaqua España, Sevilla, Spain) were used (one per tank). After this adaptation period an experimental period (90 days long) was initiated. Four different feeding regimes (two replicates each) were assayed by using the same diet and the automatic feeders just described. The feeding regimes assayed were: 7, 6, and 5 days/week morning feeding (starting at 0900) (lots 7M, 6M, and 5M, respectively) and 5 days/week afternoon feeding (starting at 1400) (lots 5A). Lots 6M were not fed on Sundays, and lots 5M neither on Saturdays or Sundays.

All the experimental lots were provided with a similar weekly ration based on 0.7% of wet body weight per day, chosen to maintain fish slightly below the optimal daily ration previously applied to ensure full food consumption. The total weekly ration was equally distributed in seven (lots 7M), six (lots 6M) and five (lots 5M and 5A) days a week. In, both periods, adaptation and experimental, a commercial feed (SKRETTING, C.V. 6), specific for meagre, was used (see composition in Table 1). Dry matter, crude protein, crude fat, and ash were analyzed according to AOAC (2000) methods. Gross energy content was calculated by applying standard conversion factors as recommended by Bret and Groves (1979). Actual feed intake was estimated from the difference between feed offered to the fish and the uneaten feed recovered from the bottom of the tanks 30 min after the end of the feeding period and desiccated at 105 °C, 20 h. This procedure was required only very occasionally.

Based on the time course of the weight data, the ration size was periodically adjusted.

Table 1
Proximate composition of the diet (g kg^{-1} on dry weight).

Component	
Crude protein	472.0
Crude lipid	204.7
Ash	77.2
Nitrogen-free extract (NFE)[a]	246.1
Energy[b] (MJ kg^{-1})	235.0
Protein/energy ratio (g MJ^{-1})	20.1

[a] Calculated as 1000 – (crude protein + crude lipid + ash).
[b] Calculated on the basis of 23.7, 39.5 and 17.2 kJ g^{-1} of protein, lipid and NFE, respectively.

The initial day, the fish of each tank were wet individually weighed and the total length was measured (external parameters). At days 45 and 90, fish (n = 40 per tank, n = 80 per treatment) were sampled for external parameters measurement and six fish for each tank were killed (abiding by Directive 2010/63/EU for experimental animal use; 2-phenoxyethanol 0.35 mL L^{-1}), three of them (n = 6 per treatment) were used for plasma analysis, enzyme activities, glycogen content, and muscle biochemical composition while the other three (n = 6 per treatment) were used for whole body composition. All the samples were used for biometric parameters determinations (n = 12 per treatment).

Growth performance and feed efficiency were determined in each tank (n = 2) by evaluating Total Weight Gain (TWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Protein Productive Value (PPV).

2.2. Blood and tissue processing

For samplings, fish were killed after a 24-h food-deprivation period. Blood samples were rapidly taken from the caudal vein and kept in heparinized tubes under cold conditions, and then were centrifuged at 690 × g for 10 min. The supernatant was separated from the plasma fraction of blood and stored at −20 °C until analyzed.

The liver and muscle (anterior part of dorsal white muscle) portions were immediately dissected, frozen in liquid nitrogen and stored at −80 °C until being used for metabolic studies at University of Granada (UGR) facilities. Tissues were homogenized (dilution 1/10) in ice-cold 100 mM Tris–HCl buffer containing 0.1 mM EDTA and 0.1% (v:v) Triton X-100, pH 7.8. After centrifugation (30,000 × g for 30 min at 4 °C), the resulting supernatant was aliquoted and stored at −80 °C for later enzyme assays.

For glycogen assays, samples of both liver and white muscle were homogenized in 0.6 mol/l perchloric acid and then neutralized.

2.3. Biometric parameters and chemical composition

Fish sampled for this purpose were stored in ice and rapidly taken to the facilities of UGR, where all were weighed and measured. Then, the muscle (after the skin was removed) and the viscera (whole viscera and separated liver) were dissected and weighed. The Hepato-Somatic Index (HSI), Digestive-Somatic Index (DSI) and Fillet Yield (FY) were computed. Morphometric indexes such as Condition Index (CI) were also calculated.

Additionally, both whole body and the right side of the muscle of eviscerated fish were used for chemical composition determinations following AOAC (2000) standard procedures: water content by desiccation in an oven at 105 °C until constant weight; ash by incineration in a muffle furnace at 450 °C for 16 h; crude protein by the Kjeldahl method (crude protein = N × 6.25) and total lipid extraction by Soxhlet's method.