



## Evaluation of a practical diet for juvenile tench (*Tinca tinca* L.) and substitution possibilities of fish meal by feather meal



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### ABSTRACT

A basal diet for juvenile tench (*Tinca tinca*) was formulated and used to test possibilities of replacement of fish meal (FM) by feather meal (FeM). A 120-day experiment was conducted with 5 month-old juveniles (27.45 mm total length, 0.308 g body weight). Three diets (500 g crude protein/kg) differing in the level of replacement of FM protein by FeM protein were prepared: 0 (basal diet), 0.25 (148 g FeM/kg) or 0.35 (210 g FeM/kg). Survival ranged from 0.96 to 0.97 ( $P=0.061$ ). The basal diet enabled higher growth (59.3 mm total length, 2.6 g body weight, 1.74%/d specific growth rate;  $P<0.001$ ) and lower feed conversion ratio (1.36;  $P=0.032$ ) than the FeM diets. There were not significant differences in body weight and specific growth rate between 0.25 or 0.35 replacement of FM protein by FeM protein. The basal diet enabled satisfactory performance and low proportion of deformed fish (0.03), showing to be feasible for future studies on juvenile tench. The FeM inclusion at the levels tested reduced growth and increased the proportion of deformed fish.

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## 1. Introduction

The tench (*Tinca tinca* L. 1758), a freshwater fish belonging to the family Cyprinidae, has a great potential for aquaculture (Wang et al., 2006; Celada et al., 2009; García et al., 2010). At present, juveniles are usually reared under extensive or semi-extensive systems using earthen ponds where fish management is difficult and yield depends on uncontrollable factors (Celada et al., 2007). The major obstacle for the increase of tench production is a deficit of young fish for stocking outdoor ponds or open waters (Wolnicki et al., 2006; Celada et al., 2009; García et al., 2010). Consequently, special attention is needed to find effective techniques for rearing juvenile tench under controlled conditions, focusing mainly on feed as an essential factor.

Tench are carnivorous (Kennedy and Fitzmaurice, 1970) and juveniles fed zooplankton and other small invertebrates (Pyka, 1997). In culture, the use of manufactured feed is limited by the lack of knowledge on nutritional requirements, forcing the use of dry diets formulated for other species. Fish meal (FM) is the main protein ingredient used for aquafeeds (Tacon and Metian, 2008). Feather meal (FeM) is an economical protein ingredient that has found increasing use in aquafeeds (Poppi et al., 2011). It has been tested with juveniles of several species as chinook salmon, *Oncorhynchus tshawytscha* (Fowler, 1990), Japanese flounder, *Paralichthys olivaceus* (Kikuchi et al., 1994), Indian major carp, *Labeo rohita* (Hasan et al., 1997) or common carp, *Cyprinus carpio* (Jahan et al., 2001), showing that adequate inclusion levels of FeM in diet are different

Abbreviations: BW, body weight; CP, crude protein; FCR, feed conversion ratio; FeM, feather meal; FI, feed intake; FM, fish meal; K, Fulton's coefficient; SGR, specific growth rate; TL, total length.

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depending on the studied species. The aim of this study was the formulation and manufacturing of a basal practical diet to test substitution possibilities of FM protein by FeM protein for juvenile tench.

## 2. Materials and methods

### 2.1. Fish, facilities and experimental procedures

A 120-day experiment was carried out with juvenile tench. Larvae were obtained by hatching under artificial reproduction techniques (Rodríguez et al., 2004) and were reared for five months until the juvenile stage in which the experiment started. From five days after hatching, when first feeding started, larvae were maintained in outdoor fiberglass tanks (2500 L) and fed decapsulated *Artemia* cysts for two weeks (Celada et al., 2013). Then, fish were fed a combination of a dry carp starter diet and decapsulated *Artemia* cysts. After 150 days, 932 juvenile tench with a mean initial body weight (BW) of  $0.308 \pm 0.011$  g and a total length (TL) of  $27.45 \pm 0.47$  mm ( $n = 120$ ) were transferred to indoor facilities and stocked in nine fiberglass tanks ( $0.5 \text{ m} \times 0.25 \text{ m} \times 0.25 \text{ m}$ ) containing 25 L of water. Juvenile tench were randomly distributed to obtain replicates. Fish were anesthetized with tricaine methanesulfonate (MS-222; Ortoquímica S.L., Barcelona, Spain), bulk weighing for each tank was carried out (to the nearest 0.001 g) and number of fish was counted. The stocking density was 1 g BW/L and the mean number of animals in each tank was  $103 \pm 1.6$  (mean  $\pm$  standard error). All experimental groups were in triplicate (three tanks per treatment).

Artesian well water was supplied in open system (flow-throughout) and each tank had a water inlet (inflow 0.30 L/min) and outlet (provided with a 250  $\mu\text{m}$  mesh filter) and light aeration. The variables of the incoming water quality (measured once a week) were: pH = 7.8, hardness 5.3°dH (German degrees, calcium 33.1 mg/L), total dissolved solids 112.2 mg/L and total suspended solids 37.3 mg/L. Dissolved oxygen content in tanks was measured with a meter HACH HQ30d (Hach Lange GMBH; Vigo, Spain) throughout the trial and values ranged between 5.7 and 7.2 mg/L. Ammonia and nitrites were measured with a spectrophotometer HACH DR2800 (Hach Lange GMBH; Vigo, Spain) from water samples taken inside the tanks (values were always ammonia <0.15 mg/L and nitrites <0.02 mg/L). Water temperature (measured twice a day) was  $24 \pm 1$  °C and a 16 h light:8 h dark photoperiod was maintained throughout the experiment. Mortality was immediately removed from the tanks. Tanks were cleaned of feces and uneaten feed every two days.

### 2.2. Diets and feeding

A basal diet was formulated according to current knowledge on carnivorous fish nutrition and juvenile tench feeding. Dietary protein was provided by FM (662 g/kg diet). Decapsulated *Artemia* cysts (100 g/kg diet) were included in this diet (García et al., 2010). Crude fat was adjusted at relatively low level, as suggested by Wolnicki et al. (2006).

Triplicate groups of juvenile tench (*ca.* 103 per replicate) were fed one of three diets in which fish meal (FM) protein was substituted by hydrolyzed feather meal (FeM) protein. FM was from anchoveta. Hydrolyzed FeM was obtained by cooking under pressure broiler poultry feathers, and then grinding and drying. Proximate composition and amino acid profiles of the FM and FeM used are presented in Table 1. The maximum level of FM substituted by FeM was chosen considering the recommendations of Yu (2008). Thus, three diets (nearly isoproteic and isoenergetic) were formulated to test different levels of replacement of FM protein by FeM protein: 0 (basal diet), 0.25 (148 g FeM/kg) or 0.35 (210 g FeM/kg). Ingredients were ground in a rotary mill BRABENDER (Brabender GmbH & Co. KG, Duisburg, Germany), mixed in a mixer STEPHAN UMC5 (Stephan Food Service Equipment, Hameln, Germany) and extruded using a stand-alone extruder BRABENDER KE19/25D (Brabender GmbH & Co. KG, Duisburg, Germany) at a temperature range between 75 °C and 90 °C. Pellets (1 mm diameter) were obtained and dried during 24 h at 30 °C. Then, pellets received a coating of cod liver oil (20 g/kg diet). Formulation, proximate composition and amino acid profiles of the diets are summarized in Table 2.

Fish were fed manually four times a day (at 10:00, 14:00, 18:00 and 22:00 h), in equal portion. A ration level of 3% live weight per day was adjusted based on the biomass calculated from the samples (see subsection 2.4). This ration was slightly in excess, thus fish were fed to satiation.

### 2.3. Chemical analysis of the diets

Proximate composition of FM and FeM (Table 1) and practical diets (Table 2) were analyzed according to the International Standards Organization: moisture to ISO R-1442 (ISO, 1979), protein to ISO R-937 (ISO, 1978), lipid to ISO R-1443 (ISO, 1973) and ash to ISO R-936 (ISO, 1998). Gross energy was determined using a calorimeter. Samples were stored at  $-30$  °C until analysis.

Amino acid profiles of FM and FeM (Table 1) and practical diets (Table 2) were analyzed by HPLC using AccQTag method from Waters (Milford, MA, USA). Amino acids were derivatized with 6-aminoquinolyl-N-hydrosysuccinimidyl carbamate reagent (AQC) by the method of Cohen and Michaud (1993) and Cohen and De Antonis (1994), and were detected by Dual  $\lambda$  Absorbance Detector Waters 2487 from Waters (Milford, MA, USA) at 254 nm. Quantification was carried out with Empower Pro 2.0 software from Waters (Milford, MA, USA). All analyses were performed in duplicate.

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