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Effects of different culture systems on growth, immune status, and other physiological parameters of tench (*Tinca tinca*)

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ABSTRACT

Tench production is limited in traditional and low intensity cultures. However, its demand is increasing and makes necessary the evaluation of physiological parameters that could affect tench welfare under intensive conditions. This study analyses the growth performance and related indices, hematological and immunological parameters as well as hormone profile and electrolytes in plasma, in fish under three different culture systems: extensive (E), with initial density of 0.07 kg/m^3, no control of environmental parameters and natural feeding; semi-intensive (SI), with initial density of 0.42 kg/m^3, partial control of temperature and supplementation of artificial food; and intensive (I), with initial density of 2.5 kg/m^3, total control of photo- and thermo-period and exclusive artificial diet. Results showed that variables such as weight, length, density increase and specific growth rate (SGR) were higher in I. Higher hepatosomatic index were found in I and SI. In addition, E and SI cultures, which kept natural conditions, achieved greater gonadal increase in detriment of somatic growth. On the other hand, the analysis of hormones and electrolytes, hematological and immunological parameters did not reveal a disturbed immune function in I compared to SI and E. Thus, the general activation of the nonspecific immune system and the mobilization of energy reserves associated to gonadal maturation in fishes from S-I and E systems could be related to an adaptive, non-pathological, response to the natural environmental changes, resulting in reduction of productivity. With all in mind, it can be concluded that adequate artificial feeding and control of the environmental parameters can improve tench productivity by intensive systems without major perturbations of its welfare.

1. Introduction

The tench (*Tinca tinca*, Linnaeus, 1758) is a freshwater fish endemic from Europe, where its pond culture was developed from the Middle Ages (Steffens, 1995). This species has been cultured traditionally in Spain by extensive culture system in small ponds and swamps of agro-livestock use with poor results in production. In the rest of Europe it has been remained in polyculture with other more productive species but nowadays, it is thought to be very promising fish for diversification of aquaculture production and for regional markets. Tench represents a fish of high interest for both gastronomical use and sport fishing (Garcia et al., 2015; Gela et al., 2006; Grosch et al., 2000; Perdikaris et al., 2010) being introduced in several countries outside Europe e.g. in Africa, Asia, Australia, New Zealand and South America (Cudmore and Mandrakn, 2011; Freyhof and Kottelat, 2008). Also, in China it was introduced in 1998 as a new potential fish for farming (Wang et al., 2006). According to physiological response to culture conditions, tench is highly variable in response to dietary conditions and very sensitive to handling practices related to intensive aquaculture (Backiel, 1986; Fleig and Gottschalk, 2001). In this sense, despite tench can adapt to water recirculation systems (Celada et al., 2007b; Kamler et al., 2006; Pantazis and Apokotou, 2009; Wolnicki et al., 2006) some experimental stressing conditions could promote a poor growth (Rennert et al., 2003; Wolnicki and Myszkowski, 1998).

Research on intensive rearing of juvenile tench under controlled conditions is considered a promising approach to improve growth and production of this species (Kaminski et al., 2017).

On the other hand, crowding could affect fish welfare, as it is known that it could be influenced by a sustained physiological stress (Duncan, 2005). It is well known that stress response involves hormonal, immunological, metabolic and physiological changes to cope increased energy demand under a compromised condition. But, if this adaptive

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response persists on time, growth, reproduction and immune capacity can be affected (Barton and Iwama, 1991). Alterations of immune response are crucial determining the suitability of a culture system since animal health can be affected and in last term fish production.

Also, environmental fluctuations, whether natural or artificial, temperature, dissolved oxygen levels, type of food, food availability or photoperiod can cause considerable physiological stress on the fish and impair their health (Wedemeyer et al., 1990).

Taking into account that environmental and fish density conditions may have an effect on fish welfare, in this study we analyzed the influence of three different tench culture systems (extensive, semi-intensive and intensive) on different parameters (growth performance and related indices, hormones, glucose and electrolytes in plasma, hematological and immunological parameters) in order to identify tech culture conditions in which welfare was not disturbed to improved fish production.

2. Material and methods

2.1. Fish and experimental conditions

All procedures were conducted following the guidelines of Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, under the approval of the Bioethics Committee of the University of Granada (Spain).

The experiment was carried on the Aquaculture Center of Vegas del Guadiana (from Junta de Extremadura) sited in Extremadura (Spain). Tench of 8-months old with body weight (BW) of 7.19 ± 0.34 g, total length (TL) of 79.85 ± 1.06 mm and condition factor (K) of 1.31 ± 0.02 g/mm^3 were used (mean ± SEM; n = 100). The study was developed during 15 weeks (from middle of April to middle of July of 2013).

Three experimental groups of tench (assayed per triplicate) were cultured under different rearing conditions:

Extensive culture (E): tench were kept in three ponds of 30 m^3 (10 × 3 × 1 m) with natural vegetation in the background. Initial stocking density was 0.07 kg/m^3 and feeding regimen was natural. The photoperiod and temperature (20.5 ± 3.4 °C) were also natural. Water was constantly open circuit (5 l/min). Ponds were covered by a mesh to avoid predation.

Semi-intensive culture (S-I): tench were maintained in three ponds of 36 m^3 (6 × 6 × 1 m) in a greenhouse. The initial density was 0.42 kg/m^3 and daily feeding was constant (2.5% of total biomass), which was based on commercial cyprinids feed (42% protein and 8% lipid), although they also had some natural food available. Photoperiod was natural and temperature was partly controlled (19.3 ± 2.4 °C) due to the greenhouse structure with ventilation systems (zenith and lateral), heating and mobile shading (aluminized mesh of 35% reduction of radiation transmission) managed by a climate controller. Water was under recirculation with a renewal daily rate of 10%.

Intensive culture (I): tench were kept in three circular polyester tanks of 5 m^3 (r = 1.3 and h = 1 m) sited in an industrial warehouse. The initial density of 2.5 kg/m^3 was similar to that expressed by other authors for this initial size of fish (Pantazis, 2012). Feeding was according to 2.5% total biomass and based on a commercial feed of cyprinids (42% protein and 8% lipid). The temperature (22.4 ± 1.4 °C) was kept constant by a boiler and an automatic system of heat exchangers. The photoperiod (14:10, 41 ± 6.5 lx) was kept constant by to an on-off controller. The water was under recirculation with a daily renewal rate of 10%.

Ponds and tanks were checked every day and physicochemical properties of water were controlled twice a week (Table 1).

Once a month 100 fishes from each experimental lot were captured to determine biometric parameters such as body weight (BW), total length (TL) and condition factor (K), to adjust feeding doses and to follow the proper development of the experiment. This process was performed under sedation with fish clove oil at a dose of 33 ppm (Hamackova et al., 2004). The fish were returned to their respective lot once they were recovered from sedation.

2.2. Growth performance and nutritional indices

At the end of the experimental time, 100 fishes were sampled for each tank, according to the protocol above described. The following parameters were estimated as follow:

Condition factor (K, g/cm^3) = 10^5 × [BW (g) / TL^3 (mm)].
Specific growth rate (SGR, %/day) = 100 × [Ln final BW (g) − Ln initial BW (g)] / time of rearing (days).
Density increase (%) = 100 × (final density of lot − initial density of lot) / initial density of lot.
Feed conversion ratio (FCR) = Total food supply (g) / [final fish biomass (g) − initial fish biomass (g)].
Incidence of fish with visible body deformities (IDef, %) = 100 × (n° of fish with visible deformities) / total fish stocked.
Survival rate (%) = 100 × (n° of survival fish after experimental period) / (total fish stocked).

From the animals previously sampled, 12 fishes from each tank (36 per experimental condition) were anesthetized and blood samples were taken (described below) then fish were sacrificed by anesthetic overdose and total viscera, liver, gonads and perivisceral fat were dissected and weighted to estimate the following biometric indices:

Hepatosomatic index (HSI; %) = 100 × liver weight (g) / BW (g).
Processing yield (%) = 100 × eviscerated weight (g) / BW (g).
Perivisceral fat (%) = 100 × perivisceral fat (g) / BW (g).
Gonadosomatic index (GSI; %) = 100 × gonad weight (g) / BW (g).

2.3. Blood sampling

Blood of 12 fishes per each tank was pooled in 4 samples (n = 12 for experimental condition) after experimental time, blood was obtained from the caudal vein with heparinized syringes. After blood extraction, plasma was obtained by centrifugation of blood at 3.000*g* for 10 min and stored at − 80 °C until following use.

Hemoglobin, respiratory burst activity of phagocites, immunoglobulins, serum globulins, and all enzymatic assays were performed using a Power Wavex microplate spectrophotometer (Bio-Tek Instruments, USA) in duplicate in 96-well microplates (UVStar® Greiner Bio-One, Germany).

2.4. Plasma biochemical assays

Hormones such cortisol, T3 (triiodothyronine) and T4 (thyroxine) were assayed in plasma as well as glucose and electrolytes (Cl^-, K^+, Na^+). Plasma cortisol concentration was assayed following an in vitro immunological test (Ref.1.875.116, Roche) and measured in a Roche Elecsys System 2010. Remaining assays were performed using Roche's kits being measures followed on an analyser Roche/Hitachi Cobas C Sistem.

2.5. Hematological assay

Hemoglobin concentration was estimated by cyanmethemoglobin method using a commercial kit (Sigma Chemical Co.,) being absorbance read at 540 nm. The hemoglobin concentration was estimated using the equation relating the absorbance and concentrations of standard hemoglobin solutions. Hematocrit was determined by centrifugation of whole blood by standard microhematocrit method. Duplicate blood samples were loaded into standard heparinized capillary tubes and centrifuged in a microhematocrit centrifuge at 650*g* for 10 min.