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A comparative study of different preparations of decapsulated *Artemia* cysts as food for tench (*Tinca tinca* L.) larvae

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ABSTRACT

Two 30-day experiments were conduced to evaluate different preparations of decapsulated *Artemia* cysts as food for tench larvae from the onset of exogenous feeding or after an initial period feeding on live nauplii. In the experiment 1, four dietary treatments were tested: *Artemia* nauplii-only (control group), nauplii for the first 7 days and fresh cysts thereafter, nauplii for the first 7 days and brine cysts thereafter or nauplii for the first 7 days and dried cysts thereafter. The same feeding treatments were replicated in experiment 2, but fresh, brine or dried cysts were supplied from the first day of exogenous feeding. In overall, survival was high. Tench larvae fed decapsulated cysts had higher growth ($P<0.001$) and lower food conversion ratio ($P<0.001$) than larvae fed live nauplii only. The highest growth (19.2 mm TL, 88.7 mg W, 18.33%/day SGR) was achieved with fresh cysts from the onset of exogenous feeding. The relation between the behaviour of the different preparations of cysts in the rearing tanks and their suitability for tench larvae is discussed. Both fresh and brine cysts are a suitable food from the onset of exogenous feeding and dried cysts can be successfully used after 7 days feeding on nauplii.

1. Introduction

Tench, *Tinca tinca* (Linnaeus, 1758), a freshwater fish belonging to the family Cyprinidae, has a great potential for aquaculture (Steffens, 1995; Kamler et al., 2006; Wang et al., 2006; Wolnicki et al., 2006). Originally occurring in the waters of Europe and Siberia, today tench occurs in the inland waters of all the continents (Freyhof and Kottelat, 2008). In Europe, tench has a history of pond culture since the Middle Ages. However, the intensification of culture techniques has recently started and has been mainly focused on controlled reproduction. At present, the major aim is the development of rearing larvae systems until these animals reach size large enough for grow-out or restocking purposes. The optimal conditions from the onset of exogenous feeding are not well established, and one of the main bottlenecks is in providing food appropriate for their small mouth gape and nutritionally adequate. Acceptable results have been achieved by feeding tench larvae on *Artemia* nauplii as the sole food (Wolnicki and Myszkowski, 1998; Fleig et al., 2001; Wolnicki et al., 2003; Celada et al., 2007, 2008).

The daily production of *Artemia* nauplii is laborious, expensive and usually requires dedicated facilities. To overcome these operational drawbacks, the use of *Artemia* cysts may offer a viable alternative to live nauplii. The small particle size of cysts (200–250 μm) is suitable for small predator stages and, after the nondigestible shell has been chemically removed prior to feeding-out, decapsulated cysts can be handled as an inert diet, they are disinfected and do not leach nutrients

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(Vanhaecke et al., 1990). After the decapsulation process, the cysts can be used readily (fresh cysts) or dehydrated in brine solution for storage (brine cysts), or subjected to a drying process for longer term storage (dried cysts).

Considering that the use of fresh cysts requires a frequent preparation of new stocks, an interesting option may be process fresh decapsulated cysts for storage by dehydration in a saturated Na–Cl brine solution or by air-drying, allowing their preparation and storage before the beginning of rearing period and thereby reducing the heavy frequently labour. However, the use of cysts both fresh and subjected to preservation methods could involve negative effects on the performance of tench larvae. If this were the case, although cysts may not be adequate to start the first feeding, probably could be successfully used after a first period feeding on nauplii. The present study evaluates possibilities of the use of different forms of preparation of decapsulated *Artemia* cysts for rearing tench larvae compared with the current larval diet of live *Artemia* nauplii.

2. Materials and methods

2.1. Fish, facilities and experimental procedure

Two 30-day experiments were conducted with tench (*T. tinca*) larvae hatched under laboratory conditions. These larvae were obtained from controlled reproduction performed according to Rodríguez et al. (2004). Five days after hatching (when exogenous feeding starts), the larvae (mean of 0.35 mg weight and 5.01 mm total length) were counted and distributed at a density of 20/L in fibreglass-tanks (0.50 m × 0.25 m × 0.25 m) containing 25 L of water. To avoid the escape of both larvae and food, each tank was equipped with a 200 μm mesh filter outlet. Aerated artesian well water was supplied to each tank in an open system (inflow 300 mL/min). Quality parameters of the incoming water were pH 7.9, hardness 5.2 °dH (German degrees, calcium 32.3 mg/L), total dissolved solids 108.5 mg/L and total suspended solids 39.7 mg/L. Throughout the trial, the dissolved oxygen content was measured in the tanks with a Sension6 dissolved Oxygen Meter (Dr Lange, Berlin, Germany) and ranged between 6 and 8 mg/L. Ammonia and nitrites were measured with a Pocket-Photometer Lasa Aqua (Dr Lange, Berlin, Germany) from water samples taken inside the tanks (values were always ammonia <0.02 mg/L and nitrites <0.05 mg/L). Water temperature (measured twice a day) was 24.5 ± 0.5 °C and photoperiod was kept natural (*ca.* 15 h light:9 h dark). The water quality parameters were measured once a week. Uneaten food and faeces were siphoned from the bottom of each tank every other day.

2.2. Diets and feeding

Concerning the direct use of decapsulated *Artemia* cysts (inert food) from the onset of exogenous feeding, it should be taken into account that several authors (Dabrowski and Glogowski, 1977; Lauff and Hoffer, 1984; Munilla-Moran et al., 1990; Kolkovski et al., 1993; Walford and Lam, 1993) have suggested that the digestive enzymes from live feed play an important role in the initial period of digestion of nutrients by fish larvae. Considering this possibility, we compared cysts supplied from the onset of exogenous feeding with cysts supplied after an initial feeding period on nauplii. Feeding with *Artemia* nauplii or cysts started at the beginning of exogenous feeding (five days after hatching).

Artemia cysts (INVE Aquaculture Nutrition, EG *Artemia* Cysts, Dendermonde, Belgium, 86% hatching rate) were decapsulated according to the method described by Van Stappen (1996). The different foods were prepared as follows:

- Freshly hatched nauplii were obtained daily according to Van Stappen (1996).
- Fresh cysts (without preservation process). New stocks were prepared every 3–4 days and stored at 4 °C.
- Brine cysts. Fresh decapsulated cysts were dehydrated in saturated NaCl-brine solution according to Van Stappen (1996). Prior to feeding-out, brine cysts were thoroughly washed in fresh water to allow for cysts hydration and to rinse the brine.
- Dried cysts. Decapsulated cysts were placed on a frame with a 150 μm mesh and dried in a laminar flow cabinet at 30 °C during 24 h. In order to reduce the high buoyancy of dried cysts, before being offered to the larvae, they were hydrated in fresh water. This enabled that the different preparations of cysts had the same behaviour in water, sinking to the bottom from the moment of their supply to the culture tanks.

After verifying mean hatching rates, which corroborated the data provided by the manufacturer (250,000 nauplii g/cysts), nauplii ration assessment was performed using known amounts of nauplii hatched in determinate water volumes, and distributing them into the corresponding replicates. When decapsulated cysts were directly used as food, feeding ration assessment of the different preparations of cysts was performed from the number of cysts/g provided by the manufacturer. In all cases, feeding was made by hand in excess, in such a way that a small remainder of uneaten food could always be seen in the tanks. According to this strategy, nauplii or cysts supply per larva per day were increased during the experiment, ranging from 60 at the start to 900 at the end. Assuming that tench larvae do not feed at night (Pyka, 1997), food was supplied four times a day at regular intervals (*ca.* every 4 h) during daylight hours.

Proximate composition of nauplii and the different preparations of cysts was analyzed according to the Norms of 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), ash to ISO R-936 (ISO, 1998a) and gross energy to ISO 9831 (ISO, 1998b). Samples were stored at −30 °C