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Effect of feeding strategy on digestive tract morphology and physiology of lake whitefish (*Coregonus lavaretus*)

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ABSTRACT

The common whitefish (*Coregonus lavaretus*) plays an increasingly important role in European and North American aquaculture industry. At the same time a considerable decrease in whitefish abundance in natural waters is observed. Therefore, it is necessary to develop methods of whitefish rearing not only for commercial but also reintroduction purposes. Fish feeding with natural food (*Artemia* sp. nauplii) is much more expensive compared to the use of commercial feeds, and thus the attempts to develop optimal feeding strategies have been undertaken to obtain most satisfactory rearing effects at the lowest costs. The aim of the present study was to establish the best moment of commercial feed introduction (Otohime B1) and to evaluate the effects of various feeding strategies on morphological and physiological development of digestive tract in whitefish larvae. This was done using histological analysis of the digestive tract and measurements of digestive enzyme activity. The lowest mortality occurred in the group fed exclusively *Artemia* sp., while the highest was recorded in the group fed Otohime B1 diet exclusively. Histological analyses revealed no significant pathological alterations in the digestive tract, in all experimental groups. The analysis of digestive enzyme activity revealed the fastest development of gastric glands in fish fed only natural food, however, the intestinal epithelium developed at a similar rate in all groups. The results indicate that the initial feeding of larvae with natural food is not necessary in common whitefish pre-rearing when using the commercial feed Otohime B1, but the highest larval growth was observed in the group which was initially fed *Artemia* nauplii for one week before the transition to Otohime B1.

1. Introduction

Common whitefish (*Coregonus lavaretus*) belongs to salmonid fish and occurs in Europe, Asia and North America. It is a cold water species of Arctic origin. This species includes landlocked lacustrine populations inhabiting deep lakes that are cool in summer, and anadromous forms that migrate to freshwaters (rivers and lakes) for spawning.

Nowadays a considerable decrease in whitefish prevalence in natural waters is observed due to overfishing, aquatic pollution and other anthropogenic changes of the natural environment that interfere with whitefish reproduction (Thomas and Eckmann, 2007). Also, hybridization of various species of *Coregonus* spp. causes gonadal degeneration and developmental anomalies (Bernet et al., 2004) which results in the extinction of original, wild-living populations (Todd and Stedman, 1989). This poses a need for the development of efficient methods of common whitefish rearing and breeding not only for commercial, but also reintroduction purposes.

Due to its tasty meat, fast growth, wide use in fish processing industry and high market price, the importance of common whitefish in European and North American aquaculture increases constantly. The world production of this species is about 118,863 tons (Food and Agricultural Organization of the United Nation, 2015). The whitefish is

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reared in Norway, Sweden, Finland, Great Britain, Canada, Russia, Czech Republic and Poland (Hayden et al., 2013; Kahilainen et al., 2003).

Feeding is a key factor affecting the success in fish aquaculture. In Coregonid fish, exogenous feeding starts between 2 and 10 dph (days post hatching), but it is also strongly related to the water temperature. The onset of exogenous feeding is correlated with yolk sac material depletion. Enzymes present in natural food are believed to be necessary for efficient food digestion, nutrient assimilation and proper development of the digestive tract in fish larvae (Kolkovski, 2001). Whitefish larvae at the onset of exogenous feeding show not fully developed digestive tracts, with low activity of digestive enzymes or their lack (Dabrowski, 1984). For this reason, natural food: nauplii of *Artemia* (Bochert et al., 2017) or other zooplankton (Luczynski et al., 1986; Mahmoudzadeh et al., 2009) are the first food recommended for *Coregonus* sp. Feeding natural food in aquaculture is economically inefficient when compared to the use of commercial feeds. Feeding is the highest component of aquaculture costs, thus attempts to optimize whitefish feeding strategies are undertaken to obtain the best production effects at the lowest cost. The choice of an appropriate commercial feed is crucial for the fast fish growth and considerably affects the profitability of fish farming. The timing of commercial feed introduction is very important for the larval digestive tract development, as it may accelerate or delay metamorphosis. Some studies concerning common whitefish pre-rearing under controlled conditions did not provide satisfactory results (Eckmann and Kausch, 1976; Koskela and Eskelinen, 1992; Rösch and Dabrowski, 1986). However, the results of other studies proved that using natural food and commercial feed until the juvenile stage was possible to achieve successful rearing of whitefish larvae (Dabrowski and Kaushik, 1985; Dabrowski et al., 1984; Luczynski et al., 1986; Mahmoudzadeh et al., 2009).

Therefore, the aim of present study was to establish the optimal moment of commercial feed introduction, and to evaluate how various feeding strategies affect morphological and physiological development of common whitefish digestive tract.

2. Material and Methods

2.1. Experimental setup

Experimental rearing of whitefish larvae was performed in the commercial fish farm Akwakultura Adrian Karczewski, in Naterki near Olsztyn (Poland). Whitefish larvae on the"0″ dph were acclimated in a recirculatory system of brackish water (~3.5‰, ~14 °C) until the onset of exogenous feeding (6 dph). Then, the larvae (body mass, BM = 6.7 ± 1.0 mg; total length, TL = 12.05 ± 0.47 mm; n = 30) were divided into four experimental groups, each in three replicates (20 L tanks), and reared for 35 days. The larvae were fed (ad libitum) either *Artemia* nauplii (crude protein 54%, crude fat 11%, crude ash 5%, moisture 8%; Ocean Nutrition, USA) or the commercial feed Otohime B1 (crude protein 51%, crude fat 11%, crude ash 15%, moisture 6,5%; Reed Mariculture, USA). Group A (Control) was fed *Artemia* nauplii during the entire rearing period; groups B and C were initially fed *Artemia* nauplii, and then Otohime B1 was gradually introduced, replacing *Artemia*; group D was fed Otohime B1 from the beginning to the end of experiment (Fig. 1). To ensure that food was constantly available to the fish during the day, both *Artemia* nad Otohime B1 were added to the tanks accordingly. The nauplii were added to the tanks in sufficient amounts, to ensure constant satiation, while Otohime B1 was portioned every 15–30 min with the use of revolving automatic feeders, which were similar to those described by Charlon and Bergot (1984). Stocking density of whitefish larvae was 200 fish per tank (10 fish/l), average water temperature was 15 ± 0.5 °C, and water pH was 8.6 ± 0.5. Ammonium concentration was below 0.1 mg/l and nitrate concentration below 0.01 mg/l. For body weight (BM ± 1 mg) and total length (TL ± 0.1 mm) measurements, five larvae were taken from each rearing tank at the end of each week of the experiment, except for the final (35th) day, when 10 fish per tank were measured. Weight measurements were used to calculate the daily Specific Growth Rate (SGR; %/day). Tanks were cleaned twice a day. Dead fish were removed and counted in order to calculate cumulative mortality. The applied photoperiod was 12/12 h (day/night).

2.2. Histological and histochemical analyses

For histological analyses, 15 fish from each group were sampled on the last day of the experiment. The fish were anesthetized using MS-222 (tricaine methanesulphonate, Sigma) and preserved in Bouin's solution. The preparations were embedded in paraffin and cut into 5 μm thick longitudinal sections using a RM2265 microtome (Leica Microsystems, Nussloch, Germany). Sections stained with hematoxylin and eosin (H-E) were used to evaluate tissue morphology and to perform morphometric measurements of hepatocytes, enterocytes and exocrine pancreatic (acinar) cells. Hepatocyte cytoplasmic and nuclear volumewere used as a measure of hepatic metabolic activity. Mucins (glycoconjugates) were identified using histochemical staining with alcian blue and Schiff's reagent (AB/PAS) (Pearse, 1985).

2.3. Immunohistochemical analyses

Proliferating cells in posterior intestine and in liver were visualized using monoclonal mouse anti-PCNA (proliferating cell nuclear antigen) antibodies (clone PC10, DAKO, Poland) in dilution 1:300, according to Ostaszewska et al. (2008). Gastrin-positive cells were detected in the digestive tract using polyclonal anti-gastrin antibodies (NCL-GASp, Novocastra, Leica, German) in dilution 1:200 (incubation 1 h). Before incubation with primary antibodies, the sections were deparaffined in xylene, dehydrated in ethanol series and a high temperature epitope retrieval method was performed using a citrate buffer (pH 6) for 30 min. Endogenous peroxidase activity was inhibited by incubation in 3% H_2O_2. For PCNA and gastrin detection, the sections were incubated with Labeled Polymer for 30 min, and then with DAB (EnVision + SystemHRP, DAKO Cytomation, Carpinteria, CA, USA), until the colour changed. The nuclei were counterstained with Harris' hematoxylin. Sections incubated without the primary antibodies were used as a negative control.

2.4. Microscopic and morphometric analyses

Microscopic analyses were done using Nikon Eclipse 90i microscope connected to the Nikon DS5-U1 digital camera (Nikon Corporation, Tokyo, Japan). Morphometric measurements were done using NIS–Elements AR 2.10 software (Nikon Corporation, Tokyo, Japan). Surface area of absorptive vacuoles in supranuclear regions of posterior intestine enterocytes, the number of goblet cells in posterior intestine epithelium, the number of gastrin-positive cells in posterior intestine, pyloric caeca and in the stomach, and the number of PCNA-positive nuclei in basal region of posterior intestine folds and in liver hepatocytes were evaluated in 5 fish from each group (25 folds × 5 fish × 3 replicates), at 400× magnification.

2.5. Enzymatic activity of digestive tract

For enzymatic analyses, 10 fish from each group were frozen in liquid nitrogen and stored at −80 °C. Digestive tracts were dissected and homogenized in dH_2O at 14000 rpm for 15 min. The activity of the following enzymes was examined: pepsin by Anson (1938), chymotrypsin and trypsin by Erlanger et al. (1961), lipase by Winkler and Stuckmann (1979), α-amylase by Foo and Bais (1998), γ-glutamyl transferase (γ-GT) by Gendler (1984), leucine aminopeptidase (LAP) by Nagel et al. (1964) and alkaline phosphatase (ALP) by Wenger et al. (1984) methods. Spectrometric measurements were conducted at 25 °C