Effect of feeding strategy on survival, growth, intestine development, and liver status of maraena whitefish *Coregonus maraena* larvae

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**Abstract**
Optimizing larval rearing protocols is critical to successful intensive fish culture. We compared the efficacy of feeding strategies for larvae of maraena whitefish *Coregonus maraena*, a promising candidate for intensive aquaculture. Survival, growth indicators, intestine development, and liver status were compared in larvae fed live feed, commercial dried feed, and weaned from live to dried feed at 5, 10, 15, 20, or 25 days post hatching (dph). Seven experimental groups in three repetitions used 5,250 larvae (2 dph, initial body weight = 7.4 ± 0.1 mg; initial total length = 13.0 ± 0.1 mm). This 30-day trial showed initial weaning from live feed (*Artemia* sp.) to artificial diet after 15 days to be the optimal, with beneficial effects on growth, body weight, and larva yield. No differences in survival rate, size heterogeneity, and condition factor were observed among groups. Live feed and weaning to artificial diet at the appropriate time was beneficial to intestine development, while feeding on artificial feed only was associated with severe intestine impairment. Liver pathology was not seen in any group.

**KEYWORDS**
artificial diet, coregonid, larviculture, live feed, weaning
INTRODUCTION

The maräna whitefish Coregonus maräna (Bloch 1779) is a commercially and ecologically valuable species showing promise for inland freshwater aquaculture in eastern, central (Mukhachev & Gunin, 1999), and northern Europe, particularly Finland (Jobling et al., 2010), Germany (Bochert, Horn, & Luft, 2017), and Norway (Sikavuopio, Knudsen, Amundsen, Saether, & James, 2011). Several decades ago, predation by the great cormorant Phalacrocorax carbo (L.) led to dramatic decline in maräna whitefish populations in Europe (Suter, 1997). Depletion has been exacerbated by overfishing (Jackson et al., 2001), hybridization (Luczyński, Falkowski, Vuorinen, & Jankun, 1992), habitat eutrophication (Thomas & Eckmann, 2007), degradation of natural spawning sites (Winfield, Fletecher, & James, 2004), pollution, and environmental changes (Walther et al., 2002). At present, re-establishment of natural whitefish populations must be supported by culture in intensive recirculating aquaculture systems (RASs) (Matousek et al., 2017; Matousek, Stejskal, Prokesova, & Kouril, 2017). The RAS is an important model for aquaculture worldwide, given its cost-effectiveness and low environmental impact, along with allowing control of water quality and manipulation of characteristics of the final product (d'Orbcastel, Person-Le Ruyet, Le Bayon, & Blancheton, 2009). Successful whitefish production in RAS requires identification of optimal larviculture conditions and protocols, including water physicochemical parameters, stocking density, nutrition, and feeding regime (Lahnsteiner & Kletzl, 2015).

Feeding strategies can influence a range of physiological and production parameters (Geng et al., 2019; Lall, Lewis-McCrea, & Tibbetts, 2018; Orihuela et al., 2018). Farmed fish may display considerable species-specificity in feeding patterns. It is standard practice to start fish larvae on live feed (LF) before weaning to a commercial formulated diet. Brine shrimp Artemia salina (L.) nauplii comprise approximately 40% of the LF used in aquaculture and are particularly suitable for hatchery operations, as they can be stored for long periods and are readily available when needed (Lavens & Sorgeloos, 2000). Feeding on LF is essential to many fish species, including coregonids such as lake whitefish Coregonus clupeaformis (Mitchill, 1818) (Harris, 1992). Alternatively, commercial dry feed can be used for the first exogenous feeding of coregonids (Enz, Schäffer, & Müller, 2001; Leithner & Wanzenbock, 2015), usually with nutritional supplementation. For maräna whitefish, this is generally propionic acid (Lahnsteiner & Kletzl, 2015). Larval feeding on nematodes (Hundt et al., 2015), rotifers, or a combination of rotifers and Artemia (Bochert et al., 2017) has also been tested in this species.

Research into effects of diet and feeding approaches on coregonids is critical to their productive culture. The goal of the present study was to identify feeding strategies optimal for survival, growth, intestine development, and liver status of maräna whitefish larvae to support intensive culture for commercial exploitation and conservation efforts.

MATERIALS AND METHODS

2.1 Eggs and larvae

Maräna whitefish broodstock were obtained from lagoons in Szczecin in the River Odra, north-western Poland. Gametes of 35 female and 35 male naturally spawning (no hormone stimulation) fish were stripped manually by commercial fishermen in December 2016 shortly after capture and transported to local hatcheries for fertilization and incubation. Eggs (100 mg) were fertilized with 0.5 mL milt mixed with 50 mL hatchery water and incubated at the ambient water temperature of the river (2–3 °C) with initial water inflow 3 L/min, oxygen saturation to 90%, and pH near 7.0. In February 2017, the eggs were taken to the Department of Lake and River Fisheries (Olsztyn, Poland).
where they were distributed among five 8-L Zug jars (Sebesta, Kucharczyk, Nowosad, Sikora, & Stejskal, 2018; Sebesta, Stejskal, Matoušek, & Lundova, K., 2018) \((n = \sim 150,000\) eggs/jar) in a recirculating system and incubated at 3.0–3.5°C with water inflow 3 L/min, oxygen saturation to 90%, and pH near 7.0. In total, \(\sim 750,000\) eggs were incubated. After 60 days, eggs were transferred to a second set of 8-L Zug jars and incubated at 8–9°C to accelerate development and hatching. After 5 days, temperature was increased to 10°C for mass hatching. Hatching success was estimated at 90%, and \(\sim 675,000\) larvae were available for the experiment. Hatched larvae swam into a 1 m³ tank underlain with 0.2 mm mesh. Larvae at 2 days post hatching (dph) were transferred to tanks in the RAS.

### 2.2 Experimental system and rearing conditions

Seven groups of larvae in three repetitions were transferred to the experimental system consisting of 21 two-L tanks, \(96 \times 154 \times 200\) mm. Two-hundred-fifty larvae (initial body weight, \(7.4 \pm 0.1\) mg, mean ± standard error of mean (SEM); initial total length, \(13.0 \pm 0.1\) mm) were placed in each tank. A total of \(5,250\) larvae were used in the experiment lasting 30 days.

Oxygen level, water temperature, and pH were checked daily at 8.00 and 16.00. The pH range was monitored using an OXYGUARD H04PP Handy pH meter (OXYGUARD International, Denmark). The initial temperature without supplemental heat was 10°C. Temperature was elevated to 15°C by 24 hr (0.2°C/hr), 19°C at 48 hr (0.2°C/hr), and maintained at \(~19\)°C by an HC-1000A cooler (HAILEA, China). Oxygenation was maintained using two Syncra 5.0 pumps (5,000 L/hr) (SICCE, Italy). Temperature and oxygenation were monitored using probes connected to a central electronic software program, Pacific Insatech A/S (OXYGUARD, Denmark). Ammonia, nitrate, and nitrite concentrations were checked twice weekly using LCK 304, LCK 339, and LCK 341 kits (HACH, Germany) with a DR5000 spectrophotometer (HACH, Germany). Sodium chloride was added at 1 g/L weekly to maintain a 16:1 chloride: nitrogen ratio to prevent nitrite toxicity. A constant inflow of 0.4 L/min was ensured. Dead larvae were removed and counted during daily cleaning. Over the course of the 30 day trial, basic physico-chemical parameters were temperature \(= 19.1 \pm 0.0\)°C, pH \(= 8.7 \pm 0.0\), O₂ saturation \(= 85.8 \pm 0.9\)%, O₂ concentration \(= 7.9 \pm 0.1\) mg/L, NH₄⁺ \(= 0.1 \pm 0.0\) mg/L, NO₂⁻ \(= 0.8 \pm 0.1\) mg/L, and NO₃⁻ \(= 21.2 \pm 5.4\) mg/L.

### 2.3 Feeding

Larvae were fed manually during the light phase (12 hr:12 light:dark) beginning at 2 dph. The artificial feed (AF) group were fed PERLA LARVA PROACTIVE 4.0 (particle size 0.1 and 0.2 mm) (SKRETTING, Nutreco, Netherlands) to excess. The LF group were fed fresh Artemia metanauplii (Ocean HE >230,000, NPG Nutrition, Belgium) (20–24 h, 0.4–0.5 mm) at 10 mL homogenous suspension/tank at approximately 3-hr intervals (08.30, 11.00, 14.00, and 16.30). AF was provided manually every 10 min during 4-hr-long feeding periods (08.30–09.30, 11.00–12.00, 14.00–15.00, and 16.30–17.30). This feeding practise was based on the character of the diet, as Artemia metanauplii, with its swimming ability, color, and enzyme secretions acting as visual and chemical stimuli, extend feeding activity. As AF has limited attraction, it is advised to present it more frequently. Feeding level was fixed at 500–700 Artemia sp. metanauplii/fish/day. The daily ration was based on a previous experiment (unpublished data) and was in slight excess, as some uneaten metanauplii were observed in tanks at the end of the day. The feeding level was adapted according to fish body weight and loss of larvae during the experiment. The nutritional composition of the commercial diet and Artemia is provided in Table 1.
FW5—first weaning from LF to artificial diet after 5 days;
FW10—first weaning from LF to artificial diet after 10 days;
FW15—first weaning from LF to artificial diet after 15 days;
FW20—first weaning from LF to artificial diet after 20 days;
FW25—first weaning from LF to artificial diet after 25 days.

2.4 | Sampling and measuring

Ten larvae from each tank (30 from each experimental group) were randomly taken for measurements of total length (TL, ± 0.01 mm) and body weight (W, ± 0.1 mg) on days 0, 5, 10, 15, 20, 25, and 30 of rearing, as described by Łaczyńska et al. (2016) and Nowosad et al. (2013). Larvae were anesthetized (Propiscin—0.4 mL/L; IRS, Poland), weighed on a digital microbalance (ABJ 220-4 M KERN, Germany) and measured manually from images taken with a Leica MZ16 A stereomicroscope and a digital camera with 5 Mp resolution for Leica DFW420 image analysis. The anesthetized larvae were lain singly on a rectangular net of known weight which was placed on paper to absorb water. The dried fish and net were placed on a balance with accuracy to 0.1 mg, weighed, and the weight of net was subtracted to obtain the weight of the fish. Fish sampled for measurements and histological analysis were humanely killed using anesthetic overdose. To avoid underestimation of final survival rate, these fish were not included in the calculation of final cumulative survival.

| Skretting          | Particle size | Mm      | 0.1–0.2 |
|--------------------|---------------|---------|---------|
|                    | Crude proteins | g/kg    | 620     |
|                    | Crude lipids  | g/kg    | 110     |
|                    | Crude ash     | g/kg    | 90      |
|                    | Crude cellulose | g/kg | 11      |
|                    | Vit A         | IU/kg   | 672     |
|                    | Vit D3        | IU/kg   | 671     |
|                    | Na            | g/kg    | 8       |
|                    | Ca            | g/kg    | 22      |
|                    | P             | g/kg    | 17      |
|                    | MnSO₄ × H₂O  | mg/kg   | 69.3    |
|                    | FeSO₄ × H₂O  | mg/kg   | 182.4   |
|                    | ZnSO₄ × H₂O  | mg/kg   | 369.8   |
|                    | CuSO₄ × 5H₂O | mg/kg   | 29.5    |
|                    | KI            | mg/kg   | 3.9     |

| Artemia           | Artemia size | NPG  | HE >230,000 |
|-------------------|--------------|------|-------------|
|                    | Crude proteins | g/kg | 540         |
|                    | Crude lipids  | g/kg | 110         |
|                    | Crude ash     | g/kg | 50          |
|                    | Moisture      | g/kg | 80          |
Experimentation was carried out in accordance the European Communities Council Directive of November 24, 1986 (86/609/EEC).

The survival rate (SR), size heterogeneity (SH), larval yield (LY), and condition factor (K) were assessed as follows:

\[
\text{SR} (\%) = 100 \times \left( \frac{N_f}{N_i} \right)
\]

in which \(N_i\) and \(N_f\) = initial and final number of larvae, respectively.

\[
\text{LY} (g/\text{group}) = \left( \frac{N_i}{100} \times \text{SR} \right) \times W
\]

with SR and \(W\) = % surviving and mean \(W\) (g) in larva groups, respectively.

\[
\text{SH} (\%) = 100 \times \left( \frac{\text{SD}}{W} \right)
\]

in which \(SH\) = size heterogeneity; SD = mean standard deviation of body weight of 10 randomly selected larvae/tank; \(W\) = mean body weight (mg) of 10 larvae/tank.

\[
K = 100,000 \times \frac{W}{(TL^3)}
\]

in which \(W\) = mean body weight (g) of 10 larvae/tank; \(TL\) = mean total length (mm) of 10 larvae/tank.

### 2.5 Histology

Five larvae from each group were sampled for histology on days 0, 5, 10, 15, 20, 25, and 30. Whole larvae were fixed in Bouin's fluid for 24–48 hr depending on size. The fixed samples were washed in an ethanol series (75, 80, 90, 95%), acetone, xylene, and liquid paraffin at 54°C. The obtained material was embedded in paraffin blocks, cut into 6 μm sections on a rotating microtome (Leica RM 2155), and sections were placed onto protein-coated slides. The slides were stained with Mayer's hematoxylin and eosin (Baginski, 1965). Subsequently, the stained preparations were sealed in Histokitt mounting medium (Glaswarenfabrik Karl Hecht GmbH & Co KG, Germany). After drying, the preparations were examined microscopically (Axio Scope A1, Zeiss, Germany) with AxioVs40 v. 4.8. 2.0 software (Carl Zeiss MicroImaging GmbH, Germany).
Five larvae from each group were photographed, and intestine diameter (ID), villi length (VL), villi thickness (VT), hepatocyte nucleus diameter, and hepatocyte diameter (HD) were measured. The measurements were compared among groups on days 5, 10, 15, and 20. At the completion of trial, the presence of intestine and liver pathology was assessed and compared using criteria of McFadzen, Coombs, and Halliday (1997) to categorize liver condition. Each specimen was assigned a grade (1–3), with a healthy specimen scoring 1 to degraded liver scoring 3 (Table 2). Intestinal degradation was evaluated, and each fish was assigned a grade (−, +, ++, +++), from healthy to severe degradation (Table 3).

2.6 | Statistical analysis

The data are presented as mean ± SEM. Statistical analyses were conducted using STATISTICA 12.0 (StatSoft, Praha, Czech Republic). The effects of feeding strategy on W, TL, SR, LY, SH, K, ID, VL, VT, ND, HD, and IIS were analyzed by one-way ANOVA with feeding as fixed variable. The level of significance used for all tests was \( \alpha = .05 \) (Zar, 1999). Prior to ANOVA, survival percentages were arcsin-transformed. All data were tested for homogeneity of variance using the Cochran, Hartley, and Bartlett test, and for normality with the Shapiro–Wilk normality test. Tukey’s test was used for identifying significant differences among groups. All applicable international, national, and institutional guidelines for the care and use of animals were followed by the authors.

3 | RESULTS

3.1 | Growth performance, survival, size heterogeneity, condition factor, yield

At the conclusion of the trial, the highest values of W (171.4 ± 8.9 mg), TL (32.2 ± 0.3 mm), LY (23.1 ± 1.24 g/tank), SH (28.4 ± 2.0%), and K (0.51 ± 0.01) were observed in the FW15 group (Figure 2, Table 4). The W (\( p = .00083 \)), TL (\( p = .00052 \)), and LY (\( p = .00075 \)) differed significantly among some groups, while no significant differences were observed in SH (\( p = .317 \)), K (\( p < .146 \)), and SR (\( p < .658 \)) (Table 4). Significantly greater W (\( p < .05 \)) was observed in FW15 compared to LF, AF, FW5, and FW10 and in FW20 compared to the AF and FW5 groups. Similarly, significantly greater TL (\( p < .01 \)) was observed in FW15 and FW20 in comparison with the LF, AF, FW5, and FW10 groups. The LF group showed the greatest growth/body weight at the first 20 days of rearing, and AF showed poorest

| Tissue                        | Grade 1. Healthy                                                                 | Grade 2. Intermediate                                                   | Grade 3. Degraded                                                        |
|-------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------|
| Liver nuclei                  | Nuclei lightly granular, small and indistinct                                   | Nuclei with abundant dark granules; nucleoli                            | Nuclei small dark and pyknotic                                         |
| Liver hepatocyte cytoplasm    | Structured: Varied texture, scattered granules with eosin positive patches     | Homogenous, granular, slight variability in staining property           | Hyaline, lacking texture, dark small and often separated from the cell boundary |
| Intestine mucosa              | Enterocytes intact, villi with deep, longitudinal folds, cytoplasm homogenous, no vacuolation, microvilli intact | Separation of enterocytes in basal region, coarse dark cytoplasm, frequent areas of microvilli degeneration | Enterocytes small dark and separated, extensive intercellular cells may be present, microvilli often indistinct |

Note: Adapted from McFadzen et al. (1997).
### Table 3: Classification of degradation and histomorphometry of intestine of maraena whitefish Coregonus maraena larvae at the end of a 30-day trial

| Lesion                                      | Groups | LF       | AF       | FW5      | FW10     | FW15     | FW20     | FW25     |
|---------------------------------------------|--------|----------|----------|----------|----------|----------|----------|----------|
| Hyperplasia of mucosa                       | +      | +        | ++       | ++       | ++       | ++       | +        | +        |
| Villus oedema                                | –      | +++      | ++       | +        | +        | +        | +        | –        |
| Exfoliation of intestine epithelium         | +      | ++       | ++       | +        | +        | +        | +        | –        |
| Intestine diameter (μm)                     | 629.3 ± 18.30 | 690.1 ± 23.24 | 717.6 ± 32.42 | 744.9 ± 58.31 | 686.7 ± 82.21 | 646.7 ± 11.92 | 672.0 ± 22.46 |
| Length of villi (μm)                        | 148.4 ± 1.83<sup>a</sup> | 133.9 ± 4.10<sup>b</sup> | 151.4 ± 5.21<sup>b</sup> | 136.2 ± 5.53<sup>a</sup> | 163.5 ± 9.48<sup>a</sup> | 152.9 ± 9.71<sup>b</sup> | 176.6 ± 9.03<sup>b</sup> |
| Width of villi (μm)                         | 54.5 ± 2.42  | 52.4 ± 3.01  | 58.2 ± 1.79  | 56.5 ± 3.04  | 54.5 ± 1.12  | 51.1 ± 3.39  | 53.1 ± 0.74  |
| Intestine injury score                      | 0.18 ± 0.05<sup>a</sup> | 2.03 ± 0.41<sup>b</sup> | 0.49 ± 0.05<sup>a</sup> | 0.22 ± 0.05<sup>a</sup> | 0.18 ± 0.04<sup>a</sup> | 0.17 ± 0.03<sup>a</sup> | 0.06 ± 0.02<sup>a</sup> |

Note: Histomorphometry parameters indicate mean ± SEM (n = 3). Different letters indicate significant differences (p < .05). Degradation score: hyperplasia of mucosa, villus oedema, and exfoliation of intestine epithelium ranges from - (none) to +++ (severe). Intestine injury score was calculated using information presented in Table 2. First weaning (FW) from live diet to a commercial diet at 5 days (FW5), 10 days (FW10), 15 days (FW15), 20 days (FW20), and 25 days (FW25).

Abbreviations: -, none; +, mild; ++, moderate; ++++, severe.
results over the course of the trial. The W and TL increments in 5-day periods are shown in Figure 2. Significantly higher LY ($p < .01$) was obtained in FW15 compared to LF, AF, FW5, and FW10, and in FW20 compared to AF.

### 3.2 Histology

Significantly lower ID was observed in AF compared to LF ($p = .0026$) and FW5 ($p = .0063$) on Day 10, as well as in AF compared to LF ($p = .022$), FW5 ($p = .0065$), and FW10 ($p = .0012$) on Day 15. Significantly longer villi were observed in LF compared to AF ($p = .00018$), FW5 ($p = .0030$), and FW10 ($p = .0035$) and in FW5 ($p = .0013$) and FW10 ($p = .00025$) compared to AF on Day 15. Significantly greater villi width was observed in LF compared to AF ($p = .019$) and FW15 ($p = .019$) on Day 20 (Figure 3). At the conclusion of the 30-day trial, the LF, FW5, FW10, and FW15 groups exhibited no serious intestine degradation. The AF group was the only treatment to receive a (+++) grade on any aspect of intestinal degradation scoring (Table 3 and Figure 4).

Significantly greater hepatocyte nucleus diameter was observed in AF compared to FW5 ($p = .040$) on Day 10 and in LF compared to FW10 ($p = .0066$) on Day 20. Significantly greater HD was observed in LF compared to AF on Day 5 ($p = .0018$), and in AF compared to LF ($p = .00022$), FW5 ($p = .00019$), FW10 ($p = .0085$), and FW15 ($p = .0091$) on Day 20 (Figure 3). Over the 30-day trial, liver of fish from all groups was normal (Grade 1).

### 4 DISCUSSION

The present study reported a feeding strategy in which LF (Artemia) was applied for 15 days with subsequent abrupt weaning to dry feed as favorable to maximize growth and survival rate in Coregonus maraena larvae. Larva survival
TABLE 4 Effects of feeding strategy on growth and survival of maraena whitefish *Coregonus maraena* larvae in a 30-day growth trial

| Group | LF  | AF  | FW5 | FW10 | FW15 | FW20 | FW25 | SS  | df | F  | MS  | p     |
|-------|-----|-----|-----|------|------|------|------|-----|----|----|-----|------|
| SH (%)| 22.5 ± 1.0 | 22.7 ± 3.1 | 23.9 ± 2.5 | 18.4 ± 1.5 | 28.4 ± 2.0 | 23.0 ± 1.2 | 25.7 ± 5.0 | 168 | 6  | 28 | 1  | .32  |
| K     | 0.49 ± 0.01 | 0.47 ± 0.02 | 0.49 ± 0.01 | 0.51 ± 0.01 | 0.48 ± 0.01 | 0.50 ± 0.01 | 0  | 6  | 0  | 2  | .13  |
| SR (%)| 90.5 ± 0.3  | 85.8 ± 1.1  | 90.0 ± 2.1  | 90.2 ± 0.4  | 90.8 ± 2.0  | 89.7 ± 1.0  | 88.2 ± 2.9 | 42  | 6  | 7  | 0  | .66  |
| LY    | 17.3 ± 0.73ab | 15.2 ± 0.62a | 16.9 ± 1.19ab | 17.2 ± 0.85ab | 23.1 ± 1.24c | 20.7 ± 0.32abc | 18.7 ± 1.27abc | 127 | 6  | 21 | 7  | 0    |

Note: Data are means ± SEM. Identical letters indicate no significant differences (p > .05) among groups. First weaning (FW) from live diet to a commercial diet at 5 days (FW5), 10 days (FW10), 15 days (FW15), 20 days (FW20), and 25 days (FW25).

Abbreviations: df, degrees of freedom; F, distribution fitting; factor parameter: FS, feeding strategy; K, condition factor; LY, larva yield, MS, mean square; SH, final size heterogeneity; SR, survival rate; SS, sum of square.
and growth are affected by starter feed, which must satisfy nutritional needs immediately after depletion of the yolk sac (Puvanendran & Brown, 1999), and feed composition and feeding strategy are of critical importance (Lee, 2003). The timing of weaning is considered to be the most important factor in successful larva feeding in peled Coregonus peled (Gmelin) (Stejskal et al., 2017), pikeperch Sander lucioperca (L.) (Hamza, Mhetli, & Kestemont, 2007), totoaba Totoaba macdonaldi (Gilbert) (Mata-Sotres, Lazo, & Baron-Sevilla, 2015), burbot Lota lota (L.) (Palićnska-Zarska et al., 2014), golden pompano Trachinotus ovatus (L.) (Ma et al., 2015), fine flounder, Paralichthys adspersus (Orihuela et al., 2018), Japanese flounder, Paralichthys olivaceus (Geng et al., 2019), and butter catfish Ompok bimaculatus (Bloch) (Pradhan, Jena, Mitra, Sood, & Gisbert, 2014). The majority of these reports also described a positive effect of LF for initial feeding, and exclusive use of starter diets in early stages of rearing is often suggested to have negative effects on later development (Bochert et al., 2017). This is supported by Leithner and Wanzenbock (2015) who observed dramatically reduced survival rate at 30–40 dph when feeding dried feed only in different strains of Coregonus lavaretus. Results were compromised by unidentified disease in some groups near the end of their experiment. A similar dramatic increase in mortality from 30 to 40 days of rearing was observed by Esmaeilzadeh-Leithner and Wanzenböck (2018). Importance of using LF during early phases of larval rearing is also highlighted by Bochert et al. (2017).

We found no significant differences in SR, SH, and K among the feeding regimes. The SR of larvae fed the commercial diet was lower than in the other groups, but did not reach significance. This was also observed by Mahmoudzadeh, Ahmadi, and Shamsaei (2009), who reported that larvae fed dry feed showed comparable SR to those fed a live and artificial diet at the first 4 weeks. Bochert and Luft (2019) found superior survival in C. maraena larvae fed exclusively with dry feed compared to those receiving Artemia and a mixture of live and dry feed from the initiation of exogenous feeding, irrespective culture temperature (6–20°C). A similar feeding technique with Artemia only for the first phase of rearing, followed by a cofeeding 50% Artemia and 50% dry feed and a final transition to dry feed only, is used in Atlantic whitefish Coregonus huntsmanui as described in a rearing handbook (Whitelaw et al., 2015).

The survival rate of the most successful group in the present study (FW15) was higher than other reported results (Beltran & Champigneulle, 1992; Bochert & Luft, 2019; Leithner & Wanzenbock, 2015) for a comparable length trial. Ostaśewska et al. (2018) reported considerably lower survival at the end of a 35-day experiment (82.9%) for a feeding strategy similar to FW15 in the present study. Our higher survival rate (91.8%) could imply an effect of the commercial feed used. Survival rate in the present study was also considerably higher than reported for C. peled (Stejskal et al., 2017, Matousek et al., 2020), as a related species.

At the end of our trial, significantly higher larva TL, W, and LY (p < .05) were observed in FW15 and FW20 compared to other treatments. The LF group showed highest length and body weight values during the first 20 days of the trial, and AF produced inferior results throughout the trial. Our results are similar to those of Bochert
et al. (2017) at 30 days, who reported enhanced growth in maraena whitefish larvae fed live *Artemia* nauplii at first feeding. Fish fed *Artemia* 6–16 dph and AF 17–42 dph displayed the highest TL and W from Day 7 to Day 42. With respect to growth, our results are superior to those obtained by Bochert et al. (2017). Growth of fish reared at low water temperatures (Leithner & Wanzenbock, 2015) has been reported lower compared to findings of the present study. Hundt et al. (2015) confirmed the highest growth at 17 dph in European whitefish larvae fed *Artemia* compared to a dry diet or live nematodes. Stejskal et al. (2017) observed the lowest W with AF in all weekly increments from 7–35 dph, with LF producing the highest W values, except at 28 and 35 dph. Fish weaned at 20 and 25 dph tended to have lower final body weight compared to those weaned at 15 days. A similar phenomenon was observed by Ostaszewska et al. (2018).

A study of the characid black tetra *Gymnocorymbus ternetzi* reported successful larva weaning prior to gastric differentiation, which results in reduced dependence on *Artemia* during early stages of rearing (Lipscomb, Yanong, Ramee, & DiMaggio, 2020). Interaction of intestine and liver function is assumed to be a key factor for growth and welfare of farmed fish. Histological examination revealed the most severe intestine degradation (Grade 3; IIS = 2.03) in the AF group, corresponding to the lowest ID, VL, and VT (Table 3). The AF group also produced the lowest growth, survival, and larval yield (Table 4).

The ID, as well as VL and VT, displayed a trend to higher values with LF and later weaning time, in conjunction with higher growth and survival in these groups. This may be attributed to the digestive enzymes obtained from LF. However, it remains to be clarified whether the digestibility of dry diets is comparable to that of live diets.

It has been reported that production of specific pancreatic digestive enzymes occurs at different times after the onset of first feeding and that their activity increases rapidly. Appearance of gastric enzymes is linked to development of the stomach toward the end of the larval period and, hence, occurs later as reported for pigfish *Orthopristis chrysoptera* (Thompson, Faulk, & Fuiman, 2019).

In our investigation, no group presented evidence of liver pathology (Table 2), and all showed a level of fat deposit within the normal range. Ostaszewska et al. (2018) also did not find significant pathologies using similar feeding techniques, but a different diet (OtohimB1, Red Mariculture, USA), Escaffre and Bergot (1986), in a study of rainbow trout *Oncorhynchus mykiss* (Walbaum), reported that the diameter of hepatocyte nuclei reflects the nutritional status of the fish. Segner, Rösch, Schmidt, and Jürgen von Pocppinghausen (1988) stated that European whitefish *Coregonus lararetus* (L.) larvae fed on zooplankton exhibited the largest nuclei, with those of larvae reared on dry diets being significantly smaller. This was confirmed by Ostaszewska et al. (2018). In our study, the hepatocyte nucleus diameter was similar among tested groups, and no significant differences ($p > .05$) were found in larvae fed LF compared to AF. LF may stimulate liver metabolic activity, in particular protein metabolism, which enhances growth of maraena whitefish larvae.

**FIGURE 4** Intestine of maraena whitefish *Coregonus maraena* (Bloch 1779) larvae after 30-day feeding trial. (a) LF (live feed), (b) AF (artificial feed), (c) FW5 (first weaning from live feed to a commercial dry diet at Day 5). (a) healthy (intestine injury score = 0.18), (b) moderate damage (intestine injury score = 2.03), and (c) slight damaged (intestine injury score = 0.49)
We used a feeding regime in which we manually provided dry food at 28 feedings per day during the first 30 days of rearing. This is labor intensive and may be excessive. Further research should be aimed at establishing optimal feeding frequency during European whitefish larval stage, as such frequent offering of dry feed may bring unsustainable labor costs.

Present study investigated a single artificial diet in combination with Artemia. However, it was demonstrated that different formulations of artificial diets can provide significantly different results during larval rearing as demonstrated for Gulf killifish Fundulus grandis (Patterson et al., 2016). Therefore, next research should be focused on comparison of performance available commercial diets in early rearing protocols for C. maraena.

5 | CONCLUSIONS

The reduction or elimination of Artemia from the early feeding protocol for maraena whitefish would be economically advantageous. However, this 30-day investigation shows initial weaning from LF to an artificial diet after 15 days to be the optimal strategy for beneficial effects on growth, body weight, and yield. Efficacy of other tested feeding strategies can be ranked FW20 > FW25 > FW10 > LF > FW5 > AF. LF and appropriate time of weaning to artificial diet is beneficial for intestine development, while a diet consisting of only AF is associated with severe intestine impairment. Live/dry feeding strategies are not associated with liver pathology.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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