https://doi.org/10.17221/33/2024-CJAS

Influence of daily feed ration on growth and condition of juvenile pikeperch (*Sander lucioperca*) reared in a recirculating aquaculture system (RAS)

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Citation: Pěnka T., Malinovskyi O., Kolářová J., Kučera V., Policar T. (2024): Influence of daily ration on growth and condition of juvenile pikeperch (*Sander lucioperca*) reared in a recirculating aquaculture system (RAS). Czech J. Anim. Sci., 69: 230–242

Abstract: The study tested the effect of daily feed ration (DFR: 0.5%, 0.75%, 1.0%, 1.25%, and 1.5% of fish biomass) on juvenile pikeperch (*Sander lucioperca*) with an initial body weight of 21.5 ± 5.1 g and total length of 144.5 ± 8.5 mm. The pikeperch were fed floating feed at 8-hour intervals for a duration of 84 days. By the end of the experiment, the group fed DFR of 1.5% exhibited the highest body weight (51.5 ± 16.1 g) and total length (188.2 ± 17.8 mm). The weight heterogeneity, measured as the coefficient of variation (CV), ranged between 30.29 and 33.24%. The specific heterogeneity rate (SHR) ranged from 304.44 to 334.94‰/day. The group with DFR of 1.0% exhibited the highest degree of heterogeneity. Minor fin erosion was observed in the caudal fin by the end of the experiment. No significant differences were revealed in selected biochemical parameters indicating the liver, spleen, and intestinal function. All the fish tested were adequately fed, being provided sufficient nutrients for the proper growth of pikeperch. The DFR of 1.5% was evaluated as the most favourable. This amount of feed supported a higher number of values for Fulton's coefficient (FC), specific growth rate (SGR), thermal growth rate (TGR), fish weight heterogeneity, growth of total fish biomass (BG), and optimum level of biochemical parameters in blood plasma.

Keywords: feed intake; fish condition; fish production; intensive aquaculture; percid culture

Feeding is one of the most important aspects of aquaculture, as it significantly affects growth, feed efficiency, and health status of fish (FAO 2022). The amount of feed provided to fish is usually determined based on the fish feeding behaviour, growth rate, and environmental conditions (Assan et al. 2021). Overfeeding can result in nutrient wastage and reduced water quality, while underfeeding can lead to growth retardation and decreased survival (Robaina et al. 2019). Therefore, the optimal feed-

Supported within the framework of the Ministry of Agriculture of the Czech Republic (Project No. NAZV QK22020144) and by Ministry of Education, Youth and Sports of the Czech Republic – CENAKVA (Project No. LM2018099).

https://doi.org/10.17221/33/2024-CJAS

ing rate determination is essential for maximizing growth and minimizing feed wastage in aquaculture (Li et al. 2022). The proper management of feed is particularly critical in recirculating aquaculture systems (RAS). Additionally, achieving optimal water quality is integral to overall performance and health of cultured organisms. Adapting the feeding method to align with cultured fish physiological and behavioural needs is crucial (Jobling et al. 1995; Policar et al. 2013). The growth and condition of juvenile pikeperch (*Sander lucioperca*) in RAS are affected by various factors, among which daily ration plays a crucial role (Schulz et al. 2005, 2007; Steinberg et al. 2018; Rahimnejad et al. 2021, 2023). Understanding the optimal feeding regime is paramount for maximizing growth rates, ensuring proper development, and maintaining overall health in aquaculture production.

Numerous studies have investigated the feeding behaviour and rates of pikeperch in ponds and RAS (Ende et al. 2021; Kozlowski and Piotrowska 2023; Penka et al. 2023). However, there remains a significant gap in our understanding of how varying feed rations impact on the growth and physiological responses of juvenile pikeperch in RAS. Previous research has primarily examined the effects of feeding rates on the growth performance and feeding behaviour of pikeperch, with a particular focus on feeding frequency (Zakes et al. 2006; Penka et al. 2023) and the use of biculture or polyculture in RAS (Thomas et al. 2020; Penka et al. 2021; Thomas et al. 2022). It is crucial to consider the interaction between feeding frequency and feed quality, as these two factors collectively influence not only the growth performance but also the overall health and welfare of the fish (Penka et al. 2023). While numerous studies have sought to optimize feeding frequency (Zakes et al. 2006; Wang et al. 2019; Penka et al. 2023), further investigation into this aspect, focusing on the precise application of daily feed rates, is needed to ensure a more comprehensive understanding of feeding strategies in pikeperch culture.

The primary objective of the experiment was to ascertain the most effective feeding rate tailored to juvenile pikeperch within a recirculating aquaculture system (RAS), examining the impact of different feeding rates on their growth trajectory and physiological responses. This research provides valuable guidance for the development of efficient feeding protocols.

MATERIAL AND METHODS

Ethical statement

The study was in compliance with Czech animal welfare regulations and approved by the animal ethical panel at the Laboratory of Intensive Aquaculture (LIA), a part of the University of South Bohemia, Faculty of Fisheries and Protection of Waters (USB FFPW) in Vodnany, Czech Republic. These guidelines are in accordance with the prevailing legislative regulations of the Czech Republic, specifically Act No. 166/1996 and No. 246/1992, and they were approved by the Departmental Expert Committee for the Authorization of Experimental Projects of the Ministry of Education, Youth, and Sports of the Czech Republic (Permit MSMT 4394/2017-2).

Experimental environmental conditions and feed rations

The experiment was conducted in cylindrical plastic tanks (of 88.5 cm in diameter, 62 cm in height, and 380 litres in volume) for 84 days. These tanks were connected to large-scale experimental RAS at the University of South Bohemia, Faculty of Fisheries and Protection of Waters at Vodnany, Czech Republic. The light regime was set at 12 h of light and 12 h of darkness (6:00–18:00). The light intensity of 7 lux on the water surface was measured by the UNITEST 93514 luxmeter (Beha-Amprobe GmbH, Glottertal, Germany). Water temperature and dissolved oxygen content were measured three times daily (at 7:00, 14:30 and 18:00 h) using a YSI ProODO oximeter (YSI Inc., Yellow Springs, OH, USA). Additionally, pH, ammonia, and nitrite levels in the water were monitored once daily throughout the 84-day experiment. The average values were as follows: temperature: 21.5 ± 8.7 °C; dissolved oxygen saturation: 105 ± 4.56%; pH: 7.14 ± 0.173; ammonia: 0.371 ± 0.281 mg/l; nitrite: 0.402 ± 0.223 mg/l). Tanks were cleaned once daily in the morning.

According to Penka et al. (2023), the fish were fed by automatic feeders Imetronic® (Pessac, France). The feeding occurred three times daily (at 10:00, 18:00 and 02:00 h). The fish were given floating pellets Skretting Europa F 15 (Stavanger, Norway; with pellet size 2 mm). The feed composition included:

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55% crude protein, 16% crude fat, 0.7% crude fibre, 9% crude ash, digestible energy of 19.4 MJ/kg. In this way, five different feeding regimes were tested as different Daily Feed Ration (DFR) of the total fish biomass:

- T0.5: The group fed DFR of 0.5% of the total fish biomass.
- T0.75: The group fed DFR of 0.75% of the total fish biomass.
- T1.0: The group fed DFR of 1.0% of the total fish biomass.
- T1.25: The group fed DFR of 1.25% of the total fish biomass.
- T1.5: The group fed DFR of 1.5% of the total fish biomass.

Each feeding rate was tested in triplicate. It means, in total 15 experimental units were used during this study.

Experimental fish and data collection for production assessment

Pikeperch juveniles, with an initial mean body weight of 21.5 ± 5.10 g, were produced by the combination of pond and intensive aquaculture methods according to Policar et al. (2013, 2016) in LIA. Each of the 15 experimental units was initially stocked with 100 fish per tank, resulting in a density of 5.66 kg/m^3. Prior to their introduction into the RAS, the fish had already been adapted to the feed intake and the RAS conditions.

Daily monitoring of fish mortality (%) and feed consumption was performed throughout the experiment. Any uneaten pellet left on the water surface or on bottom of tanks within 24 h was collected and calculated to determine the exact feed intake (and to determine the real feed conversion ratio).

After a 28-day period, each tank was assessed for weight; the current daily feed ration for each tank was determined using a CAS PB 100/200 kg scale (CAS Corporation, East Rutherford, NJ, USA). These measurements of fish biomass were done to the nearest 0.01 kg with the aim to update tested DFR.

The biomass and number of fish in each tank were determined at the beginning and at the end of the experiment. Body weight (BW) and total length (TL) were simultaneously determined for 50 fish from each tank. BW was measured using a KERN PCB 1000-2 scale (Kern & Sohn GmbH, Balingen, Germany), while TL in millimetres was measured using a fish measuring board. These measurements were used to determine growth parameters. During the measurements, the fish were anaesthetised using a tricaine mesylate solution (MS-222; Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 80 mg/l to minimize stress, following the method described by Rozynski et al. (2018). The collected data are presented as mean ± standard deviation (SD) and the following parameters were calculated:

Coefficient of variation for body weight (%):

$$CV = 100 \times SD \times BW^{-1} \quad (1)$$

Specific heterogeneity variation rate (‰/day; Kestemont et al. 2000):

$$SHR = 100 \times (\ln CV_2 - \ln CV_1) \times D^{-1} \quad (2)$$

Fulton's condition factor:

$$FC = 100 \times BW \times TL^{-3} \quad (3)$$

Specific growth rate (%/day):

$$SGR = 100 \times (\ln BW_2 - \ln BW_1) \times D^{-1} \quad (4)$$

Thermal growth coefficient:

$$TGC \text{ (Jobling 2003)} = 100 \times (BW_2^{1/3} - BW_1^{1/3}) \times (T \times D)^{-1} \quad (5)$$

Feed conversion ratio:

$$FCR = TFC \times (FB - IB)^{-1} \quad (6)$$

Feed ingestion rate (%):

$$FIR = 100 \times FEA \times TFC^{-1} \quad (7)$$

Survival (%):

$$S = 100 \times (FN \times IN^{-1}) \quad (8)$$

Biomass gain (%):

$$BG = 100 \times (FB - IB) \times IB^{-1} \quad (9)$$

where:

SD – standard deviation
BW – body weight (g);
CV_2 – coefficient of variation of BW_2;
CV_1 – coefficient of variation of BW_1;
D – rearing period (days);
TL – total length (cm);
BW_2 – final body weight (g);
BW_1 – initial body weight (g);

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T – water temperature (°C);
TFC – total feed consumption (g);
FB – final fish biomass (g);
IB – initial fish biomass (g);
FEA – feed actually eaten (g);
FN – final number of fish (pcs);
IN – initial number of fish (pcs).

Data collection for the assessment of blood biochemistry and organosomatic indices

For the purpose of determining blood biochemical parameters, fish were randomly selected. Initially, 20 fish were chosen, and at the end of the experiment, three fish were selected from each tank in the test groups (for a total of 9 fish per group). Blood was collected from the caudal vein using a sampling material treated with heparin (Heparin inj. 5 000 IU/ml, Leciva, Prague, Czech Republic). The blood was then separated by centrifugation (at 1 073 × *g* for 10 min at 4 °C). The plasma samples were stored at –80 °C until analysis.

For evaluation of the biochemical blood analysis, the following parameters were selected: total protein (TP), albumin (ALB), globulin (GLB), amylase (AMYL), lipase (LIPA), total cholesterol (TCHOL), glucose (GLU), ammonia (NH_3), triglyceride (TAG), alanine aminotransferase (ALT), aspartate aminotransferase (AST). These parameters were measured using the FUJI DRI-CHEM NX 500i analyser (FUJIFILM Europe GmbH, Dusseldorf, Germany).

After blood collection, the fish were used for the determination of selected organs: liver, spleen, internal fat, gonads. These organs were collected and weighed using the KERN PCB 1000-2 scale (Kern & Sohn GmbH, Balingen, Germany). The following organosomatic indices were calculated:

Hepatosomatic index:

$$HSI = 100 \times (W_l \times BW^{-1}) \quad (10)$$

Fatsomatic index:

$$FSI = 100 \times (W_f \times BW^{-1}) \quad (11)$$

Spleen somatic index:

$$SSI = 100 \times (W_s \times BW^{-1}) \quad (12)$$

Gonadosomatic index:

$$GSI = 100 \times (W_g \times BW^{-1}) \quad (13)$$

where:
W_l – weight of fish liver (g);
W_f – weight of fish visceral fat (g);
W_s – weight of fish spleen (g);
W_g – weight of fish gonads (g);
BW – body weight (g).

The fin conditions of every fish in each tank were individually assessed in all used tanks by following the methodology outlined by Policar et al. (2016). The assessment of the fin condition was always performed by the same person who analysed all fish fins. This fin analysis included the left and right pectoral fins, left and right ventral fins, first and second dorsal fins, as well as the caudal and anal fins. The levels of fin erosion were:

- Degree 0: No fin erosion, ranging from 0–5% of the fin erosion.
- Degree 1: Minor damage, ranging from 6–30% of the fin erosion.
- Degree 2: Moderate damage, ranging from 31–70% of the fin erosion.
- Degree 3: Severe damage, ranging from 7–100% of the fin erosion.

Statistical analyses

All data were analysed using Statistica v14 (StatSoft Inc., Czech Republic). Prior to conducting the statistical analysis, several checks were performed. The normality of the residuals was assessed using the Shapiro-Wilk test, and the homogeneity of variance was examined by Levene's test. Additionally, data were evaluated for normality using the Kolmogorov-Smirnov test.

In each group, a two-way ANOVA was conducted to explore the potential presence of a "tank effect" among individual tanks. For statistical comparisons, analysis of variance (one-way ANOVA) was performed, followed by Tukey's honestly significant difference test to detect possible differences in biometric, production and biochemical data at the statistically significant level ($P < 0.05$). In addition, correlation analyses were performed to detect the strength of the linear relationships between the variables in the dataset.

A linear regression function was used for the correlation analyses with respect to the gradual increase in feed intake and mean body weight (in grams) between treatments (T0.5–T1.5). The correlation was

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presented between the increase in mean body weight and between treatments (T0.5–T1.5) on day 28, day 56 and day 84. The correlation between the number of steps and the mean body weight increase was calculated using Pearson's correlation coefficients.

RESULTS

After conducting the 84-day test, significant differences in fish production parameters (TL and BW) were found between all pikeperch groups (Table 1). The variability of the increase in the average body weight of the fish over the time of the experiment also gradually increased the feeding rate between the treatments (Figure 1). Figure 2 represents four graphs and their correlations between the treatments (T0.5–T1.5) and body weight over 0, 28, 56 and 84 days of the experiment, when there was a consistent increase in body weight as the treatment time increased. Group T1.5 had the highest production parameters compared to the other tested groups. The weight heterogeneity at the end of the experiment (CV_2) was higher in Groups T0.75, T1.0, T1.25, T1.5 (CV_2 = 31.3–33.2; SHR = 341–347‰/day) compared to Group T0.5 (CV_2 = 30.3 ± 0.542; SHR = 338‰/day). FC was significantly different for all test groups at the end of the experiment. The highest FC values were observed in the groups T1.25 (0.772 ± 0.079) and T1.5 (0.771 ± 0.079), and the lowest FC values were found in Group T0.5 (0.660 ± 0.082). The highest SGR was determined in Group T1.5 (1.01 ± 0.103%/day) and the lowest SGR value in Group T0.5 (0.411 ± 0.091%/day). Additionally, the highest TGC was determined in Group T1.5

Table 1. Initial and final growth parameters (n = trireplicates) of pikeperch (*Sander lucioperca*) reared in the tank for 84 days using five different feeding rates of the total fish biomass (0.5% – T0.5, 0.75% – T0.75, 1.0% – T1.0, 1.25% – T1.25, 1.5% – T1.5)

Parameters	T0.5	T0.75	T1.0	T1.25	T1.5	R^2	P-value
iTL (mm)	145 ± 8.67	144 ± 8.40	144 ± 8.30	144 ± 8.97	146 ± 7.85	0.000	0.628
fTL (mm)	166 ± 16.6^{d}	171 ± 16.2^{c,d}	175 ± 17.6^{c}	182 ± 16.4^{b}	188 ± 17.8^{a}	0.171	< 0.001
iBW (g)	21.8 ± 3.75	21.5 ± 3.78	21.1 ± 3.76	21.7 ± 3.78	22.1 ± 3.55	0.003	0.085
fBW (g)	30.5 ± 9.24^{e}	34.7 ± 11.4^{d}	39.8 ± 13.2^{c}	46.9 ± 15.0^{b}	51.5 ± 16.1^{a}	0.834	< 0.001
CV_1 (%)	16.2 ± 0.202	16.8 ± 0.201	17.1 ± 0.211	18.0 ± 0.223	16.8 ± 0.214	0.006	0.380
CV_2 (%)	30.3 ± 0.542^{b}	32.8 ± 0.664^{a}	33.2 ± 0.771^{a}	32.0 ± 0.867^{a}	31.3 ± 0.942^{a}	0.003	0.856
SHR (‰/day)	338	346	347	343	341	0.003	0.876
iFC	0.705 ± 0.051	0.722 ± 0.052	0.708 ± 0.053	0.720 ± 0.049	0.709 ± 0.042	0.001	0.620
fFC	0.660 ± 0.082^{c}	0.711 ± 0.076^{b}	0.715 ± 0.089^{b}	0.772 ± 0.079^{a}	0.771 ± 0.079^{a}	0.194	< 0.001
SGR (%/day)	0.411 ± 0.091^{d}	0.546 ± 0.059^{c,d}	0.732 ± 0.058^{b,c}	0.959 ± 0.093^{a,b}	1.01 ± 0.103^{a}	0.863	< 0.001
TGC (°C/day)	0.087 ± 0.020^{d}	0.118 ± 0.014^{c,d}	0.162 ± 0.014^{b,c}	0.220 ± 0.024^{a,b}	0.234 ± 0.029^{a}	0.858	< 0.001
aFCR	1.50 ± 0.163	1.43 ± 0.125	1.43 ± 0.094	1.33 ± 0.047	1.37 ± 0.047	0.186	0.109
rFCR	1.20 ± 0.253	1.19 ± 0.229	1.19 ± 0.103	1.04 ± 0.096	0.966 ± 0.089	0.095	0.199
FIR (%)	100 ± 0.017^{a}	98.8 ± 0.837^{a}	99.5 ± 0.293^{a}	95.8 ± 2.89^{a}	81.2 ± 4.77^{b}	0.574	0.001
S (%)	99.8 ± 0.162	99.8 ± 0.272	99.6 ± 0.387	100	99.7 ± 0.337	0.020	0.614
BG (%)	41.6 ± 11.1^{c}	58.4 ± 7.87^{c}	85.1 ± 9.18^{b,c}	125 ± 17.9^{a,b}	135 ± 20.0^{a}	0.845	< 0.001

$^{a–e}$Different letters in the same row indicate statistical differences (P < 0.05); Data are presented as mean ± SD. Analysed parameters are labelled with an r^2 value that indicates the strength and direction of the correlation and P-value

aFCR = apparent feed conversion ratio; BG = biomass gain; BW = body weight; CV_1 = initial coefficient of weight variation; CV_2 = final coefficient of weight variation; f = final; FC = Fulton's condition factor; FIR = feed ingestion rate; I = initial; rFCR = real feed conversion ratio; S = survival; SGR = specific growth rate; SHR = specific heterogeneity variation rate; SL = standard length; TGC = thermal growth coefficient; TL = total length

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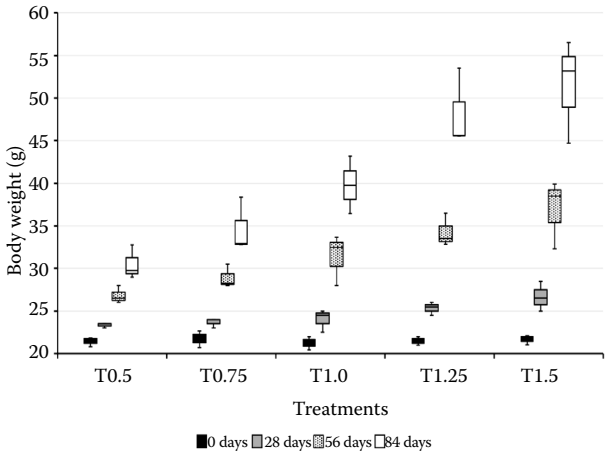

Figure 1. Boxplot graph of the body weight of juvenile pikeperch (*Sander lucioperca*) in five treatments (0.5% – T0.5, 0.75% – T0.75, 1.0% – T1.0, 1.25% – T1.25, 1.5% – T1.5) over four time periods (0, 28, 56, 84 days), illustrating variability and weight gain over time

(0.234 ± 0.029 °C/day) and the lowest in Group T0.5 (0.087 ± 0.020 °C/day). The lowest FIR was observed in Group T1.5 (71.2 ± 4.77 %), which was lower than in the other groups (95.8–100%). No significant differences were determined in apparent FCR (1.33–1.50), real FCR (0.966–1.20), and survival (99.7%–100%). The highest BG was determined in Group T1.5 (135 ± 20.0%) and the lowest BG in Groups T0.5 (41.6 ± 11.1%) and T0.75 (58.4 ± 7.87%).

No significant differences were observed in HSI (1.39–1.69) and FSI (1.79–2.73) in all tested groups (Table 2). Additionally, significant differences were observed within the tested groups in SSI. The results indicated that Groups T0.75–T1.5 had higher SSI (0.502–0.609) compared to Group T0.5 (0.323 ± 0.105). Conversely, significant differences were observed for GSI, with the highest values determined in Group T0.5 (0.745 ± 0.317) and the low-

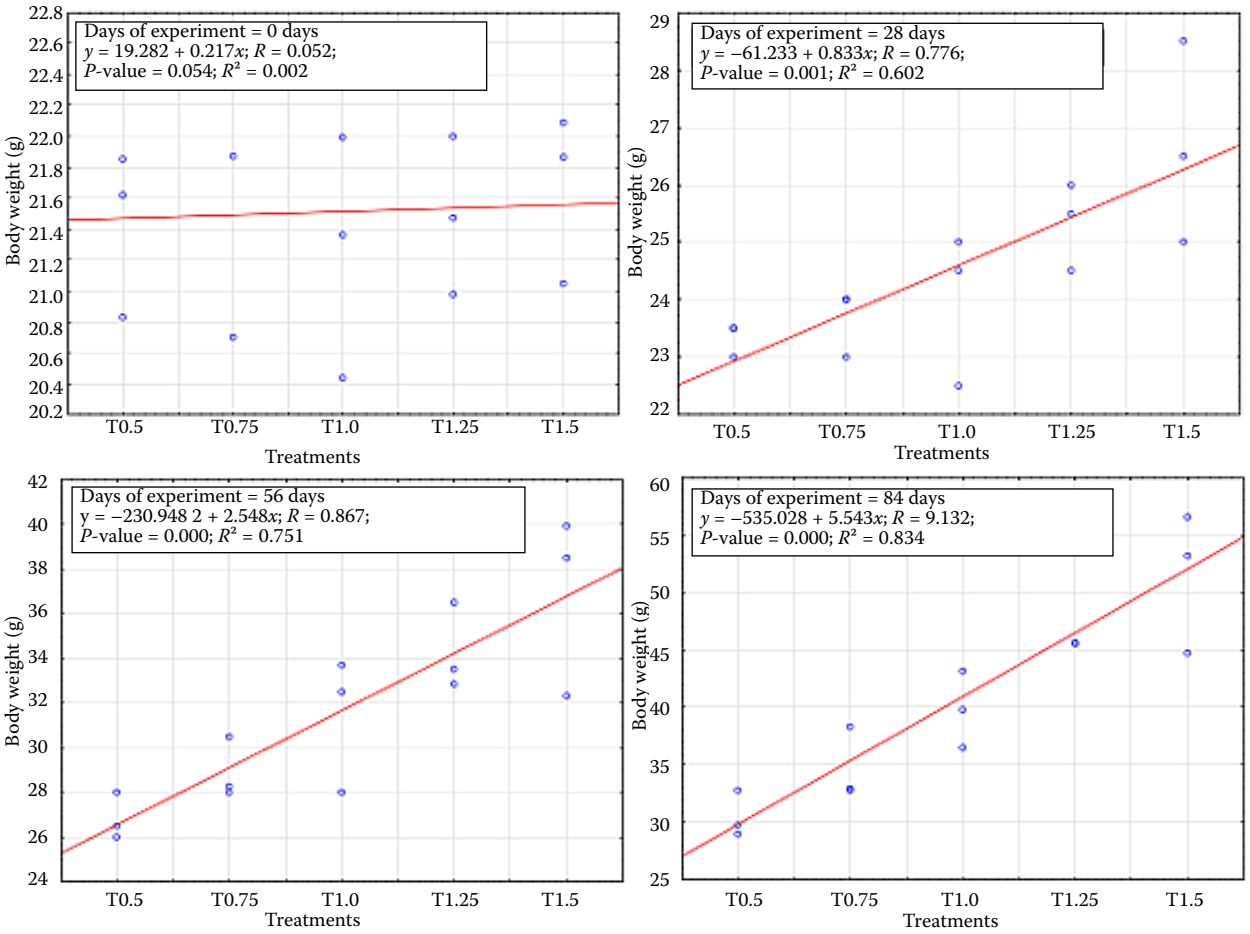

Figure 2. Correlation between body weight and treatments (0.5% – T0.5, 0.75% – T0.75, 1.0% – T1.0, 1.25% – T1.25, 1.5% – T1.5) in time periods (0, 28, 56, 84 days)

Each graph is labelled with a linear regression equation and an R^2 value that indicates the strength and direction of the correlation

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Table 2. Initial and final organosomatic parameters of pikeperch (*Sander lucioperca*) reared in the tank for 84 days using five different feeding rates of the total fish biomass (0.5% – T0.5, 0.75% – T0.75, 1.0% – T1.0, 1.25% – T1.25, 1.5% – T1.5)

Parameters	Initial	T0.5	T0.75	T1.0	T1.25	T1.5	R^2	*P*-value
HSI	1.72 ± 0.242	1.62 ± 0.709	1.39 ± 0.609	1.61 ± 0.676	1.69 ± 0.828	1.43 ± 0.805	0.000	0.922
FSI	5.55 ± 2.13	1.79 ± 1.19	1.85 ± 0.75	2.49 ± 0.85	2.73 ± 0.82	2.00 ± 0.80	0.000	0.909
SSI	0.031 ± 0.003	0.323 ± 0.105^{b}	0.502 ± 0.184^{a}	0.544 ± 0.118^{a}	0.577 ± 0.144^{a}	0.609 ± 0.259^{a}	0.192	0.003
GSI	0	0.745 ± 0.317^{a}	0.504 ± 0.094^{a,b}	0.444 ± 0.252^{a,b}	0.381 ± 0.315^{b}	0.337 ± 0.224^{b}	0.139	0.042

Initial n = 20, final n = 45; ^{a,b}Different letters in the same row indicate statistical differences ($P < 0.05$); Data are presented as mean ± SD. Analysed parameters are labelled with an R^2 value that indicates the strength and direction of the correlation and *P*-value

FSI = fatsomatic index; GSI = gonadosomatic index; HSI = hepatosomatic index; SSI = spleen somatic index

Table 3. Initial and final biochemical parameters in the blood plasma of juvenile pikeperch (*Sander lucioperca*) reared in the tank for 84 days using five different feeding rates of the total fish biomass (0.5% – T0.5, 0.75% – T0.75, 1.0% – T1.0, 1.25% – T1.25, 1.5% – T1.5)

Parameters	Initial	T0.5	T0.75	T1.0	T1.25	T1.5	R^2	*P*-value
TAG (mmol/l)	5.45 ± 1.89	6.35 ± 2.35	5.42 ± 2.66	5.07 ± 2.94	5.86 ± 2.49	6.20 ± 1.39	0.000	0.943
LIPA (μkat/l)	0.371 ± 0.051	0.477 ± 0.173	0.372 ± 0.051	0.449 ± 0.185	0.410 ± 0.043	0.381 ± 0.133	0.040	0.221
AMYL (μkat/l)	16.84 ± 2.30	12.4 ± 2.45	12.7 ± 1.51	12.9 ± 2.26	12.1 ± 1.71	12.0 ± 2.29	0.010	0.517
TP (g/l)	39.2 ± 6.00	33.0 ± 2.21^{b}	35.0 ± 2.21^{a,b}	35.6 ± 2.82^{a,b}	36.0 ± 3.40^{a}	37.1 ± 3.48^{a}	0.159	0.009
ALB (g/l)	5.43 ± 1.18	3.91 ± 0.796	3.99 ± 0.918	4.25 ± 1.05	4.10 ± 0.903	4.16 ± 0.651	0.009	0.539
GLB (g/l)	33.8 ± 3.59	29.1 ± 1.79^{b}	31.0 ± 1.59^{a,b}	31.7 ± 1.55^{a,b}	32.6 ± 2.57^{a}	33.0 ± 3.07^{a}	0.223	0.001
GLU (mmol/l)	9.79 ± 5.82	4.76 ± 2.61	3.07 ± 1.07	3.87 ± 0.753	4.30 ± 0.831	3.88 ± 0.543	0.002	0.751
NH_3 (μmol/l)	827 ± 108	962 ± 219	1054 ± 260	1085 ± 191	1152 ± 999	1165 ± 279	0.098	0.066
TCHOL (mmol/l)	4.60 ± 0.806	3.64 ± 0.946	4.20 ± 0.911	4.27 ± 1.40	5.25 ± 1.69	5.25 ± 1.48	0.069	0.076
ALT (μkat/l)	0.817 ± 0.181	0.239 ± 0.055	0.264 ± 0.052	0.354 ± 0.175	0.327 ± 0.110	0.404 ± 0.226	0.119	0.027
AST (μkat/l)	3.47 ± 0.956	0.983 ± 0.307	1.39 ± 0.423	1.45 ± 0.604	1.41 ± 0.637	1.68 ± 0.922	0.077	0.011

Initial n = 20, final n = 45; ^{a,b}Different letters in the same row indicate statistical differences ($P < 0.05$); Data are presented as mean ± SD

ALB = albumin; ALT = alanine aminotransferase; AMYL = amylase; AST = aspartate aminotransferase; GLB = globulin; GLU = glucose; LIPA = lipase; NH_3 = ammonia; TAG = triglyceride; TCHOL = total cholesterol; TP = total protein

est GSI values in groups T1.25 (0.381 ± 0.315) and T1.5 (0.337 ± 0.224). Furthermore, no significant differences were revealed in all experimental units for selected blood biochemical parameters (TAG, LIPA, AMYL, ALB, GLU, NH_3, TCHOL, ALT, AST) (Table 3). TP and GLB had a gradual upward tendency, where the highest values were determined in groups T1.25 (TP = 36.0 ± 3.40 g/l; GLB = 32.6 ± 2.57 g/l) and T1.5 (TP = 37.1 ± 3.48 g/l; GLB = 33.0 ± 3.07 g/l) and the lowest in Group T0.5 (TP = 33.0 ± 2.21 g/l; GLB = 29.1 ± 1.79 g/l).

Our study observed a minor increase in damage to the caudal fin (at the end of the experiment), escalating from degree 0 to 1, as depicted in Figure 3. Almost all fish (99–100%) in each group exhibited no damage (degree 0) to their pectoral fins (both left and right), dorsal fins (both first and second), anal fins, and caudal fin.

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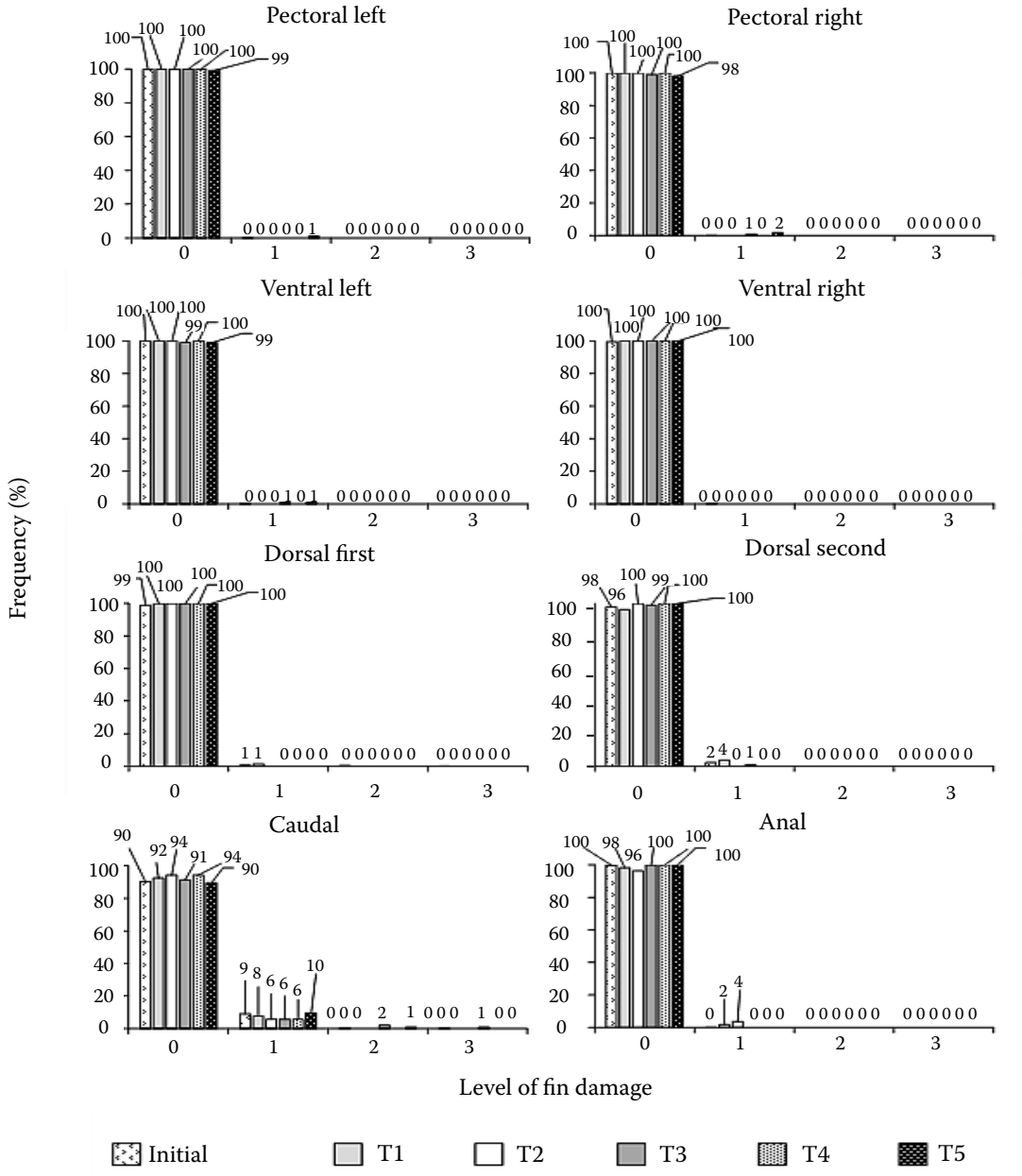

Figure 3. Frequency of fin erosion in juvenile pikeperch (*Sander lucioperca*) (degree 1 to 3, with degree 3 indicating the most severe damage according by Policar et al. 2016)

The assessment was conducted at the beginning (Initial) and at the end of an 84-day feeding test with five groups with different daily feed rations (0.5% – T0.5, 0.75% – T0.75, 1.0% – T1.0, 1.25% – T1.25, 1.5% – T1.5)

DISCUSSION

Biometric parameters

In this experiment, significant differences were found within the treatments tested, with the highest TL and BW values determined in Group T1.5 (TL = 188 ± 17.8 mm; BW = 51.5 ± 16.1 g). There was no significant "tank effect" between tanks within all tested groups. Based on these results, it can be concluded that the tested fish were able to adapt themselves to all tested daily feed rations. The lowest values of production parameters (FC, SGR) were calculated in Group T0.5. However, the highest values were calculated in Group T1.5 with growth heterogeneity (SHR = 341‰/day).

The applied DFR limitation was found to be appropriate based on previous studies. Zakes et al.

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(2003) used the lowest DFR (1.2–2.0%) for juvenile pikeperch with an initial body weight of 25 g. Similarly, Zakes et al. (2006) used a DFR (0.8–1.0%) for pikeperch (BW = 21 g). Previous studies on smaller pikeperch have suggested similar trends in growth and required feed intake. These results highlight that those higher fish densities in intensive aquaculture can help reduce growth heterogeneity and cannibalism among fish. In addition, limiting DFR was an appropriate practice, consistent with previous studies where lower DFRs were used for fish of similar body weight (Zakes et al. 2003). In our study, the FC for all tested groups ranged from 0.66 to 0.77 after 84 days of rearing. This value is lower than those observed in previous studies, where comparable FC values were reported for fish of similar body weight (Zakes et al. 2003). SGR and FCR were consistent with the results of previous studies (Zakes et al. 2003; Zakes et al. 2006). The differences in these parameters were probably due to the age and body weight of fish in each group (Wang et al. 2019). The tested feeding regimes significantly affected the biometric parameters in pikeperch. Higher fish densities in intensive aquaculture seem suitable to minimize growth heterogeneity and cannibalism (Zakes et al. 2006; Policar et al. 2013). Optimal DFR is an effective strategy for optimizing the growth and performance of fish (Schulz et al. 2005; Zakes et al. 2006; Schulz et al. 2007). In our study, the fish were able to fully consume food at a DFR of 1.5%. Using higher DFR (2% and 2.5%) would result in greater feed consumption. This could have a negative impact on the local ecosystem as more unused feed would enter the water. Too high DFR could cause digestion problems and disturb the nutrient balance of the fish. This could lead to various health problems such as digestive problems or overweight. Additionally, excess feed may also cause increased levels of ammonia and other waste products in the rearing system (pond or RAS), which could lead to further environmental problems (Abd El-Hack 2022). This could negatively affect water quality and the overall health of the ecosystem (Lin et al.2022).

In this study, juvenile fish with higher SGR (0.96–1.01%/day) were observed in Groups T1.25 and T1.5. This aligns with Penka et al. (2023), who observed similar trends with the fixed DFR of 1%, but with lower BW (SGR = 0.82–0.95%/day). Schulz et al. (2005) achieved a higher SGR (1.37–1.45%/day) using Sera fish oil-enriched feed at a higher temperature of 22.6 °C. Ronyai and Csengeri (20098) conducted an 18-week experiment with the fish of average weight 84 ± 19 g, achieving an SGR of 1.07 ± 0.01%/day with DFR at 1.2%. Discrepancies between our production data and those previously published may be attributed to variations in tank specifications, water quality, light regime, stocking density, or the digestibility and quality of the applied feed. These factors play a significant role in influencing the performance of intensive aquaculture in percid fish, as emphasized by Melard et al. (1996) and Policar et al. (2019).

This experiment demonstrates that the contrast between real and apparent FCRs underscores their significance. The apparent FCR is derived from the designed feed consumption rate, while the real FCR reflects the actual observed consumption. Notably, the mean difference between these two metrics varies across groups receiving different feeding rates (T0.5–T1.5). For the first four groups (T0.5–T1.25), this difference remains at 0.3 or lower, indicating good agreement. However, in the group with the highest feeding rate (T1.5), the mean difference reaches 0.4, the highest among all groups. This finding supports our approach, especially considering that this group also had the highest leftover feed. Leftover feed, a common occurrence in pikeperch culture, provides valuable insights into assessing the feasibility and optimizing feeding strategies (Zakes et al. 2006; Wang et al. 2019; Penka et al. 2023). If DFR increases, feed consumption and fish growth efficiency increase proportionally. However, due to the experimental design, which included only limited feeding treatments (DFR = 0.5–1.5%) with a linear increase in intake, a clear dose-response calculation was performed to determine the optimal feeding level. After performing the correlation analysis, valuable insights were provided into the effect of increased DFR on the average body weight of the fish. And DFR = 1.5% was determined as suitable.

Physiology and fish condition

Several physiological and biochemical parameters were tested in different groups of fish, shedding light on potential health implications. FSI was higher in groups T1.25 and T1.5, compared to SSI, which was lowest in treatment group T0.5. This suggests that the initial assertion of not exceeding

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the maximal feeding rate may need to be reconsidered. Essentially, the data indicate a negative health trend in the group with the highest feeding rate, underscoring the need for a correlation analysis. It is important to note that the high FSI is not typically desired by farmers rearing pikeperch (Molnar et al. 2006). Therefore, apart from health implications, FSI could also impact on the economic feasibility of rearing. A significant observation was the lack of substantial differences in HSI across all tested groups. Other organosomatic parameters in the groups (T0.75–T1.5) displayed higher SSI due to spleen enlargement, with group T0.5 exhibiting the significantly lowest SSI. FSI values were higher in groups T1.25 and T1.5, with the lowest FSI observed in group T0.5. Generally, the enlarged spleen in fish can be indicative of potential health issues or problems related to inadequate nutrition (Seppanen et al. 2009). GSI presented an inverse trend, peaking in group T0.5, while the lowest values were found in groups T1.25 and T1.5. This observation could indicate a negative effect of higher DFR on the GSI. The amount of feed may affect the reproductive performance of the fish, which is an important factor in aquaculture (Izquierdo et al. 2001; Torsabo et al. 2022).

Different feeding rates (0.5–1.5%) influenced the concentration of plasma total protein (TP), with values continuously increasing from the lowest to the highest feed ration. Statistically significant differences were found for the outlying groups. When comparing TP in fish at the beginning of the test with TP of fish from all treatments (T0.5–T1.5) at the end of the test, higher values were observed in fish at the beginning of the test. This could be attributed to the natural growth of the fish that achieved a higher level of nutrient digestion during the 84 days of the experiment. Lower levels of TP could indicate malnutrition or poor health, as proteins are essential for growth and repair in organisms (El-Wahab et al. 2020; Yuan et al. 2023). In the context of aquaculture, this could potentially lead to slower growth rates and lower overall yield, affecting the economic viability of the operation (Henriksson et al. 2021). In contrast, higher TP levels, particularly those observed in groups T1.25 and T1.5, could suggest that the fish receive an adequate or excessive amount of feed. While this may initially seem beneficial for growth, it could also lead to health issues such as obesity or organ damage if the protein intake is too high (Roh et al. 2020). Additionally, overfeeding can result in waste accumulation and water quality degradation, which can further affect fish health and survival (Abd El-Hack et al. 2022). Therefore, maintaining an optimal TP level is crucial for ensuring the health and growth of the fish, as well as the sustainability and profitability of intensive aquaculture (Henriksson et al. 2021). GLB and ALB parameters are closely related to the TP parameter, and in our follow-up, GLB had similar tendencies like TP, but ALB did not indicate any statistical differences between the groups at the end of the test. TP, ALB and GLB corresponded to the values published for pikeperch in good condition (Kolarova and Velisek 2012).

TAG was comparable with lipid metabolism, which did not present any significant differences in all groups. However, we found higher values (5.07–6.35 mmol/l) in fish of all groups at the beginning and end of the experiment compared to the TAG values of the pond-based pikeperch (1.75–4.20 mmol/l) published by Kolarova and Velisek (2012). These differences in TAG values are likely due to different farming methods (RAS and pond culture) used in both groups of the pikeperch in both studies. RAS-based pikeperch has a higher lipid metabolism due to the higher fat content in the artificial granules used (16%) compared to the natural diet (zoobenthos or prey fish with fat content 1–5%) of the pond-based pikeperch (Policar et al. 2016). Lipase concentration (LIPA) did not differ significantly between the groups of fish with different feeding rates. LIPA was significantly lower only when comparing the fish at the beginning and end of the test with the daily feeding rate of T0.5–T1.5. The same trend was observed for amylase concentration (AMYL). Both enzymes are involved in lipid metabolism and are closely related to pancreatic activity, with an increase in AMYL accompanied by an increase in LIPA. GLU, NH3, and TCHOL concentrations indicate the level of stress in fish. No significant differences were observed between the groups with different daily feeding rates. The ammonia concentration in the blood plasma of the RAS-based fish was generally found lower (828–1 165 µmol/l) compared to the ammonia level in the blood plasma of pond-based fish (330–960 µmol/l) published by Kolarova and Velisek (2012). This difference could be due to the higher stress levels in pikeperch or the lower water quality and higher ammonia concentration in the water of each RAS compared

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to the pond. Therefore, while certain biochemical parameters are related to the stress level in fish, the feeding rate did not appear to influence these stress markers significantly. Thanks to these observations, we can conclude that the RAS-based pikeperch have a physiologically higher concentration of ammonia in the blood plasma than the pond-based pikeperch. This difference may be due to the higher stress of pikeperch reared in the RAS. The enclosed environment of RAS, while allowing the better control of conditions, can also lead to increased stress due to factors such as higher fish density and less natural behaviour. This stress can result in increased ammonia production as the fish metabolism changes in response to the environment. In contrast, pond-based pikeperch live in a more natural and spacious environment, which can be related with lower stress levels and thus lower ammonia production. The water in ponds is also naturally filtered through the ecosystem, which can help maintain lower ammonia levels. Therefore, while RAS offers advantages in terms of control and efficiency, it is crucial to carefully manage the system to minimize stress and maintain water quality, thereby ensuring the health and well-being of the fish. Further research is needed to optimize these systems and mitigate potential issues related to ammonia accumulation. This could include exploring different feeding strategies, improving filtration systems, or investigating ways to reduce stress in the fish. These efforts can help make RAS a more sustainable and effective method for pikeperch aquaculture.

By the end of the test, juvenile pikeperch reared in RAS exhibited more fin erosion, particularly in the caudal fins. Other fins such as pectoral, ventral, dorsal, and anal fins suffered less damage compared to the caudal fin. Compared to the previous study by Policar et al. (2016), these fins were either minimally damaged or not damaged to the same extent. In the study by Penka et al. (2023), pikeperch fin erosion was lowest when fed at an optimal 8-hour interval. In conclusion, the caudal fin appeared to be the most susceptible to fin erosion in RAS-based pikeperch, and the feeding frequency did not significantly affect the rate of fin erosion. Clayton et al. (1998) reported the fin erosion in walleye (*Stizostedion vitreum*), where mechanical injuries were caused by low light intensity and they speculated on possible causes of fin erosion. The main cause of fin erosion could be a bacterial disease (*Bacillus columnaris*), neither poor water quality nor overstocking in tanks (Clayton et al. 1998). There is evidence that diet quality influences fin erosion in fish (Lellis and Barrows 2000; Latremouille 2003). Feed manufacturers should take responsibility for developing diets that reduce fin erosion (Latremouille 2003). Future diet formulation trials should include an assessment of fin erosion and should not focus solely on growth rate and cost (Ellis et al. 2008).

CONCLUSION

This study suggests that Group T1.5 (with DFR = 1.5%) optimizes growth and performance in juvenile pikeperch, potentially enhancing yield and profitability in intensive aquaculture. It was observed that DFRs exceeding this level could lead to health issues such as organ damage or malnutrition. These health issues could, in turn, negatively impact on the economic viability of the aquaculture operation due to their effects on water quality and waste management. Therefore, it is crucial to balance the benefits of commercial growth with the fish welfare. This conclusion is well-suited for a research journal, as it summarizes the findings of the study and provides a clear direction for future research and practice in the field of aquaculture.

Conflict of interest

The authors declare no conflict of interest.

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Received: March 1, 2024
Accepted: June 6, 2024
Published online: June 26, 2024