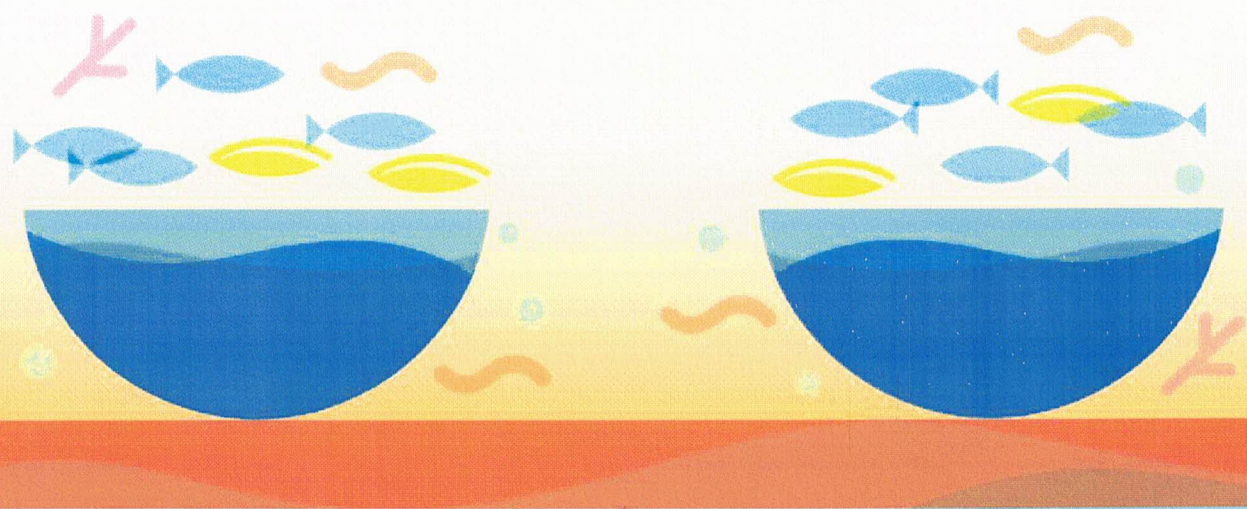


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HEMP PROTEIN POWDER FEASIBILITY AS FEED COMPONENT FOR EURASIAN PERCH *Perca fluviatilis* L.

P. Gomulka ^{1*}, H. Bober ¹, M. Woźniak, M. Kamaszewski, W. Wiechetek, P. Hliwa

¹ University of Warmia and Mazury, Faculty of Animal Bioengineering

Department of Ichthyology and Aquaculture, Oczapowskiego 5, 10-719 Olsztyn, Poland

² Warsaw University of Life Sciences, Institute of Animal Sciences, Department of Ichthyology and Biotechnology in Aquaculture, Ciszewskiego 8, 02-786, Warsaw, Poland

e-mail: pgomulka@uwm.edu.pl

Introduction

The cultivation of industrial hemp (*Cannabis sativa* L.) is a rapidly growing branch of agriculture globally with an expected annual growth rate of 16.9 % by 2030 (Polaris Market Research 2021). Hemp cultivation area has increased by 75% between 2015 and 2019 in EU and probably will rise further due to hemp environmental benefits like high carbon storage, prevention of soil erosion, increase of biodiversity and low need for pesticides. Hemp fiber is used in textile industry, construction, biofuels production and many new innovative applications. Hemp seeds are source of nutrition for both human and animals. The attempt to use of hemp seed in striped bass feed has given promising results (Sample 2022). The aim of this study was to test feasibility of hemp protein powder (HPP) as an ingredient of extruded feed for Eurasian perch *Perca fluviatilis* L.

Materials and methods

Experimental feeds. Four extruded feeds containing 0% (control group), 10% (HP10), 20%(HP20) and 30%(HP30) of HPP (50% of crude protein; commercially available diet supplement for human) were prepared. Additionally the feed containing 30% of HPP and phytase addition (2000 IU per kg) has been extruded.

Fish and the experiment design. Eurasian perch from pond culture of National Inland Fisheries Research Institute in Olsztyn, Station in Żabieniec was used for test. Fish of 68.1 g ± 2.7 mean body weight (N=500) were equally distributed to ten fiberglass tanks (0.3 m³) working in RAS. Tanks were randomly assigned to experimental groups. Each group was present in duplicate. Fish were fed experimental feed appropriate for given group using belt feeders for approximately 10 hours a day for 10 weeks. Then, fish sample (n=15) from each tank was taken for body measurements. Sampled fish were euthanized and dissected. All the viscera and separately the liver were weighed. The remaining fish were weighed to determine the final total wet weight. Growth indicators (SGR, FCR, PER, VSI, HIS) were calculated based on collected data.

Statistical analysis. The analysis was done using Statistica 13 software (Statsoft, USA). Shapiro-Wilk test and Levene's test were used to assess data normality and variance homogeneity respectively. As SGR, FCR and PER data revealed lack of variance homogeneity, The Kruskal-Wallis ANOVA was used to test difference significance. For remained data, ANOVA procedure and Tuckey's post hoc test were applied.

Results

The growth of fish was very similar in all experimental groups. No significant differences were found in final body length and weight and specific growth rate. The mean body length varied between 16.7 cm and 17.0 cm and body weight between 92.8 g and 100.3 g in control and HP30F, respectively. Specific growth rate varied from 0.39 to 0.59 in HP30 and HP30F, respectively. The lowest mortality was noticed in HP30F group (1%) and the highest one in the control group (7%).

Hemp protein content did not influence significantly the feed conversion ratio (FCR)(varied between 2.00 and 3.31) and protein efficiency ratio (PER)(varied between 0.54 and 1.08) although the differences seems rather high. The only significant difference (ANOVA p<0.05) was found in the liver weight. HSI was significantly higher in the control and HP10 groups (2.44% and 2.19%, respectively) when compared to remaining groups (1.56% - 1.76%). Generally it has been noticed that results were slightly better with rising hemp powder addition up to 20%. The worst results were achieved in HP30 group (SGR 0.39, FCR 3.31, PER 0.54), however the best results were achieved in HP30F group (SGR 0.59, FCR2.0, PER 1.08).

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Conclusion

Hemp protein powder addition to feed up to 30% does not influence on the growth of Eurasian perch. However, results obtained for HP30 group suggest that higher content of HPP can limit feed conversion and fish growth. The positive effect of phytase is rather unexpected result as it is considered that hemp seed do not contain phytic acid. HPP addition did not cause increased mortality during the experiment and HSI level was significantly lower in groups fed higher levels of HPP. Presented results a quite promising, however more intensive study is needed especially to explain the role of phytase.

Acknowledgment

This work was performed in the project “ Diversification of pond-based production through semi-intensive aquaculture of Eurasian perch, *Perca fluviatilis* “ (acronym: PRO-PERCH; grant agreement no 00002-6521.1-OR1400004/17/20), financially supported by Polish Operational Programme “PO RYBY 2014–2020” within European Maritime and Fisheries Fund.

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EFFECT OF DIFFERENT PHOTOPERIOD ON GROWTH, SURVIVAL AND CANNIBALISM DURING WEANING OF EURASIAN PERCH *Perca fluviatilis* L. LARVAE REARED IN RAS SYSTEMS

P. Hliwa^{1*}, J. Król², K. Palińska-Żarska², S. Krejszef², M. Błażejowski¹, H. Bober¹, P. Gomułka¹, D. Żarski³

1 – University of Warmia and Mazury in Olsztyn, Poland

2 – National Inland Fisheries Research Institute in Olsztyn, Poland

3 – Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn, Poland

e-mail: phliwa@uwm.edu.pl

Introduction

Intensive perch culture in recirculating aquaculture system (RAS) provides optimal culture conditions for rapid fish growth, high survival rate, shorter production cycle, reduction of fish stress and cannibalism. In order to ensure a high productivity and reduced production costs, several rearing conditions have to be optimized. Larvae of percid species are strongly phototactic therefore, the varied photoperiod is a factor that can significantly affect the efficiency of rearing. Current knowledge in this matter for Eurasian perch larvae is still very limited.

Material and methods

Larvae from wild Eurasian perch spawners were obtained followed reproductive protocol and reared up to 13 DPH according to standardized procedure described by Palińska-Żarska et al. (2020). Afterwards, 28 days experiment was set, where four variants of different photoperiod (L:D – light:dark) i.e. 24L:0D; 20L:4D; 16L:8D and 12L:12D were tested. Throughout the entire rearing period, the intensity of light, measured at the water surface was 900 lux. The sudden weaning procedure at 21 DPH was used, 7 days after photoperiod variants were established. At the end of the experiment 30 perch post-larvae from each group were euthanized, individually weighed, measured and relevant growth parameters were assessed: coefficients of body length variation [CVL(%) = 100 SDL L⁻¹, where L is the mean body length and SDL is the standard deviation of body length]; coefficients of body weight variation CVM(%) = 100 SDW W⁻¹, where W is the mean body weight and SDW is the standard deviation of body weight]; Fulton's condition factor K = 100 W × L⁻³, where W is the mean body weight and L is the mean total length]; specific growth rates SGR (% day⁻¹) = 100 × (Ln·WF - Ln·WI) t⁻¹, where WF is the mean final body weight and WI is the mean initial body weight (g); t is 28 days]. Moreover, cumulative mortality were calculated including the percentage of fish prey of cannibals. The data expressed in percentages were *arcsin* transformed before the statistical analysis. Data were compared using one-way ANOVA. A non-parametric Kruskal-Wallis' test was used to evaluate the differences in analysed parameters (p<0.05). Analyses were performed using Statistica software (StatSoft).

Results

The analysis revealed no statistically significant differences (p<0.05) between groups for final length and weight of larvae, Fulton's condition factor, SGR index, as well as in mortality (Table 1). On the other hand, such differences in the final values of coefficients of body weight and length variation were revealed with the smallest value recorded for the 20:4 photoperiodic regime.

Discussion

Lack of significant differences of perch larvae growth parameters reared under different photoperiod conditions suggest the limited influence of this abiotic factor on rearing efficiency. However, increased mortality of perch post-larvae after the change in the type of food and the violent transition from *Artemia nauplii* to artificial feed was confirmed. According to data Kestemont et al. (2015) perch larvae cultured under 24L:0D light conditions had significantly higher survival (56.2%) compared to those that had 12L:12D and 16L:8D photoperiod (45 and 49%), respectively, as well as on the final fish mean mass. It is likely that increased day length impacts the behaviour of both potential cannibals and potential prey. The day length did not significantly influence the survival of Eurasian perch but the proportion of mortality due to cannibalism was significantly reduced when day length was decreased from 24:0 LD to 8:16 (Kestemont et al. 2003; 2015) which was also reported in the present study.

(Continued on next page)

Table 1. The values (mean \pm SD) of the studied indexes of perch larvae at the end of the experimental rearing. Data in rows marked with different letter subscripts indicate statistically significant differences between groups ($p < 0.05$).

parameter	group / photoperiod			
	24:0	20:4	16:8	12:12
initial body weight (g)	0.0047 \pm 0.0012			
initial total length (cm)	0.71 \pm 0.058			
final body weight (g)	0.151 \pm 0.066	0.153 \pm 0.045	0.138 \pm 0.059	0.134 \pm 0.063
final total length (cm)	2.39 ^a \pm 0.32	2.46 ^a \pm 0.22	2.23 ^b \pm 0.32	2.30 ^b \pm 0.31
Fulton's condition factor	0.61 \pm 0.19	0.62 \pm 0.13	0.60 \pm 0.21	0.56 \pm 0.18
CVL (%)	13.9 ^a \pm 1.9	9.2 ^b \pm 0.8	15.0 ^a \pm 2.2	13.5 ^a \pm 1.7
CVM (%)	53.0 ^a \pm 23.1	32.3 ^b \pm 10.0	52.5 ^a \pm 24.8	56.0 ^a \pm 22.8
SGR (% d ⁻¹)	12.06 \pm 1.6	12.30 \pm 1.06	11.73 \pm 1.62	11.65 \pm 1.53
survival (%)	40.4 \pm 6.6	38.3 \pm 10.2	38.7 \pm 7.7	39.3 \pm 4.9
cannibalism (%)	15.3 \pm 4.6	11.8 \pm 5.8	12.5 \pm 6.0	10.7 \pm 3.6

Acknowledgment

This work was performed in the project “ Diversification of pond-based production through semi-intensive aquaculture of Eurasian perch, *Perca fluviatilis* “ (acronym: PRO-PERCH; grant agreement no 00002-6521.1-OR1400004/17/20), financially supported by Polish Operational Programme “PO RYBY 2014–2020” within European Maritime and Fisheries Fund.

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IS THE CHOICE OF WEANING STRATEGY OF EURASIAN PERCH (*Perca fluviatilis* L.) LARVAE DEPENDS ON DOMESTICATION LEVEL OF SPAWNERS?

J. Król¹, K. Palińska-Żarska¹, S. Krejszefł¹, P. Hliwa², A. Długoński², D. Żarski³

¹ – National Inland Fisheries Research Institute in Olsztyn

² – University of Warmia and Mazury in Olsztyn

³ – Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn

*e-mail: j.krol@infish.com.pl

Introduction

Information on how domestication affects early life stages in fishes is limited. One of the recent studies showed that domestication has an impact on the digestive capacity of Eurasian perch larvae that coincided with a higher growth rate recorded for domesticated ones (Palińska-Żarska et al. 2020). Weaning is a critical step for cultured percid species as the transition from live food to commercial feed often results in high mortality. Further studies are required for the optimization of the weaning protocol at the earliest possible stage of perch larvae e.g., is full substitution of live food by commercial diet should be preceded by co-feeding period or not? (Król and Zieliński 2015). Therefore, the aim of the study was to determine the effect of a different weaning strategy (sudden switch vs co-feeding) of perch larvae originated from domesticated or wild spawners on the results of their subsequent rearing parameters.

Material and methods

Eurasian perch larvae from wild and domesticated spawners were obtained followed by separate reproductive protocols described by Żarski et al. (2019). Incubation of eggs and larvae rearing procedures, except feeding scheme, were the same for domesticated and wild stocks and conducted according to standardized protocols described by Palińska-Żarska et al. (2020). Four experimental groups (in triplicate each), differed by origin of the larvae (domesticated or wild) and two weaning strategies (sudden switch or co-feeding), were reared in RAS system during subsequent 34 days. From 4 dph until 17 dph, two groups of larvae (domesticated and wild) were fed exclusively by *Artemia* nauplii and the others two groups (also domesticated and wild) were co-fed using *Artemia* nauplii and commercial diet. Afterwards, fish were switched entirely to commercial feed. At the end of the experiment all survivors (34 dph) per tank were counted, weighed, and measured. Other rearing parameters were assessed according to the scheme described by Król and Zieliński (2015). A two-way ANOVA was used to test the effects of origin of the perch larvae (domesticated or wild), weaning strategy (sudden switch or co-feeding) and the interactions of both these factors on subsequent rearing parameters of perch larvae. Differences were considered significant at $P < 0.05$.

Results

At the end of experiment, domesticated and co-fed (*Artemia* nauplii and commercial feed) perch larvae characterized by better growth, lower mortality caused by type II cannibalism and final bigger biomass, whereas these wild perch larvae which were fed only by *Artemia* nauplii, until were switched entirely to commercial feed, had better final survival, lower mortality other than cannibalism and final bigger biomass. Based on two-way ANOVA, origin of perch larvae (domesticated or wild) affected individual body weight, mortality caused by type II cannibalism and mortality other than cannibalism. Weaning strategy significantly effected on individual body weight, mortality caused by type I cannibalism and final survival of the fish. None of the factors had a significant impact on the final fish biomass. However, significant interactions between origin of the larvae and the weaning strategy were found in all tested rearing parameters (Table 1).

Discussion and conclusion

Our study supports the hypothesis that the type of food is the main challenging factor in intensive Eurasian perch larviculture conditions affecting its final efficiency. Recent study suggested that the production of digestive enzymes does not matter when easily digestible high-quality food, such as *Artemia*, is offered, although digestion capability was higher in wild than in domesticated perch larvae (Palińska-Żarska et al. 2020). However, in the presented study, significant changes in growth between perch larvae originated from wild and domesticated spawners were observed even before *Artemia* was completely replaced with commercial feed using co-feeding scheme. This suggests that other processes than only the production and activity of digestive enzymes are modified by progressive domestication. In conclusion, our results indicate, that choice of the weaning strategy in Eurasian perch larvae may depend on the domestication level of their parents and could significantly affect their subsequent rearing parameters.

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Table 1. F-values and P-values (* P<0.05) from the two-way ANOVA analysis used to study the effects of origin of perch larvae (domesticated or wild), weaning strategy (sudden switch or co-feeding) and the interaction of these two predictors on dependent rearing parameters.

Predictor Dependent	Origin		Weaning strategy		Origin x Weaning strategy	
	F-value	P-value	F-value	P-value	F-value	P-value
body weight	7,59	0,02*	6,43	0,03*	11,72	0,01*
fish biomass	1,68	0,23	0,31	0,59	15,22	0,01*
survival	0,10	0,76	8,50	0,02*	6,11	0,04*
cannibalism type I	3,13	0,11	11,91	0,01*	15,85	0,01*
cannibalism type II	15,53	0,01*	3,26	0,11	16,72	0,01*
other type mortality	12,59	0,01*	2,60	0,14	6,90	0,03*

Acknowledgment

This work was performed in the project “ Diversification of pond-based production through semi-intensive aquaculture of Eurasian perch, *Perca fluviatilis* “ (Grant agreement no 00002-6521.1-OR1400004/17/20, acronym: PRO-PERCH), financially supported by Polish Operational Programme “PO RYBY 2014–2020” within European Maritime and Fisheries Fund.

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DOES THE LIGHT COLOR MODULATE STRESS DURING OUT-OF SEASON REPRODUCTION OF EURASIAN PERCH FEMALES – FIRST STEP TO UNDERSTANDING

K. Palińska-Żarska^{1*}, J. Król¹, P. Hliwa², S. Krejszeff¹, M. Błażejewski², D. Żarski³

1 – National Inland Fisheries Research Institute in Olsztyn

2 – University of Warmia and Mazury in Olsztyn

3 – Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn

e-mail: k.palinska-zarska@infish.com.pl

Introduction

Since the 1990s, the Eurasian perch (*Perca fluviatilis* L.) has been considered one of the key species for diversifying aquaculture in Europe (Fontaine and Teletchea, 2019). Despite the fact that commercial production of this species began over 30 years ago and is already established in some countries, research on its biology, larviculture, and reproduction technology in controlled conditions is still being optimized and developed (Żarski et al., 2017; Palińska-Żarska et al., 2020). One aspect that still requires in-depth research is determining the optimal lighting conditions, especially during out-of-season reproduction in Eurasian perch. This is especially important when considering the modulation of light as a tool to reduce stress during this critical period. In the present study, selected genes related to stress and immune response were investigated in response to different light colors (white, blue, and red) used during the controlled out-of-season reproduction of this species.

Material and methods

Pond-reared Eurasian perch females, obtained in early November, were transferred to a recirculating aquaculture system (RAS). They were then subjected to a 40-day wintering period before spawning. Throughout this entire period, the fish were exposed to three different light colors: white (W), blue (B), and red (R). At five specific sampling points (S), the females were anesthetized, and their livers were collected for RNA extraction ($n=6$ for each S) (Fig. 1.). Subsequently, qPCR was conducted to assess the expression of selected genes: *hif1 α* (hypoxia-inducible factor 1 α), *tnfa* (tumor necrosis factor α), *hsp70* (heat shock protein 70), *hamp* (hepcidin), and *lyz* (lysozyme). The obtained data were compared using a two-way ANOVA, and differences were further analyzed through Tukey's *post-hoc* test ($p<0.05$). All statistical analyses were performed using Statistica software developed by StatSoft.

Results

Two-way analysis of variance ($p < 0.05$) indicated significant interactions between light color and the expression of the genes encoding *tnfa*, *hsp70*, and *hif1 α* , while there was a lack of significant interactions ($p > 0.05$) between light color and the expression of the genes encoding *hamp* and *lyz*. Furthermore, Tukey's *post-hoc* analysis revealed significant differences ($p < 0.05$) in the expression of *hif1 α* at S2 for all tested light colors, as well as *hsp70*, but solely in the case of R light. Additionally, a higher expression of *hif1 α* in S3 was observed, specifically in W light (Fig. 2).

Discussion

One of the factors that significantly impact the induction of stress reactions in fish is the light color during rearing conditions (e.g., Maia and Volpato 2013). Therefore, the aim of this study was to address the question: Can different applied light colors during the out-of-season reproduction of pond-origin Eurasian perch, kept in RAS, somehow influence their stress levels? The used light colors did not yield significant differences in the maturation of females. However, the analysis of selected genes related to fish reactions to stress and their immune responses indicated that the light colors in which the spawners were kept significantly affected the expression of the *hif1 α* and *hsp70* genes (see Fig. 2). It was revealed that these light colors stimulated stress reactions in fish at the beginning of the wintering phase, before reaching the final oocyte maturation. Importantly, just before spawning, the levels of all tested genes were comparable. The results obtained suggest that varying light colors indeed significantly influence stress reactions in fish during their adaptation to rearing conditions in RAS. However, these light colors do not directly impact the final reproductive outcome. Similar stress reactions to different colors were observed in goldfish (*Carassius auratus*), where B and R colors induced chronic stress and immunosuppression (Eslamlo et al. 2013). These preliminary observations need in-depth exploration of additional stress and immune-related parameters. Considering the distinct reactions of Eurasian perch females to different light colors and their welfare, it is advisable to employ W light to minimize stress associated with fish adaptation to RAS conditions, whenever possible.

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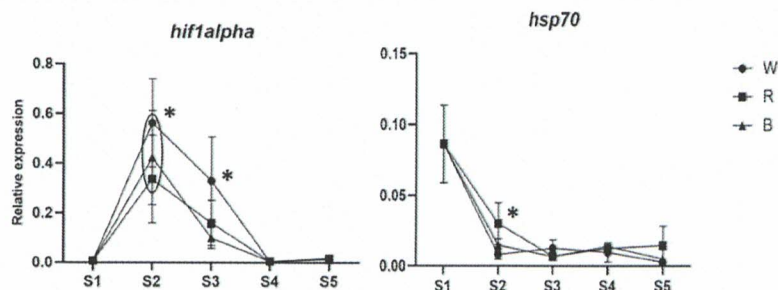


Fig. 1. Diagram of sampling points (S) during out-of season reproduction of perch. S1 – acclimation; S2 – beginning of wintering faze; S3 – beginning of “heating” faze; S4 and S5 – hormonal stimulation, first and second dose, respectively.

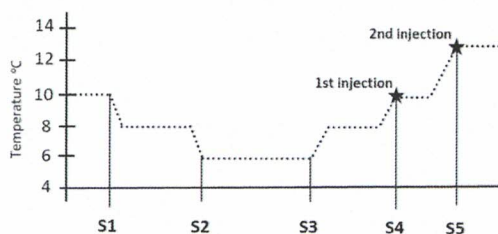


Fig. 2. Relative expression of chosen genes, during rearing Eurasian perch females in three different light colors. Data (mean \pm SD) marked with asterisk were significantly different ($p < 0.05$). W – white, R – red, B – blue, *hif1alpha* - hypoxia inducible factor 1 α , *hsp70* – heat shock protein 70, S – sampling point.

Acknowledgment

This work was performed in the project “ Diversification of pond-based production through semi-intensive aquaculture of Eurasian perch, *Perca fluviatilis* “ (Grant agreement no 00002-6521.1-OR1400004/17/20, acronym: PRO-PERCH), financially supported by Polish Operational Programme “PO RYBY 2014–2020” within European Maritime and Fisheries Fund.

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EFFECT OF DIETARY PROTEIN ON LIVER ENZYME ACTIVITY AND MORPHOLOGY OF EUROPEAN PERCH (*Perca fluviatilis*)

W. Wiechetek^{*1}, M. Kamaszewski¹, J. Król², P. Gomułka³, R. Kasprzak¹, D. Adamek-Urbańska¹, H. Szudrowicz¹, A. Szczepański¹, K. Kawalski¹, J. Martynow¹, P. Bujarski¹

1. Department of Ichthyology and Biotechnology in Aquaculture, Institute of Animal Science, Warsaw University Of Life Sciences

2. Department of Salmonid Research, The Stanisław Sakowicz Inland Fisheries Institute, Oczapowskiego 10, 10-719 Olsztyn, Poland

3. Department of Ichthyology and Aquaculture, Faculty of Animal Bioengineering, University of Warmia and Mazury, Oczapowskiego 2, 10-719 Olsztyn, Poland

E-mail: Wiktoria_Wiechtek@sggw.edu.pl

Introduction

Due to the development of intensive fish production and the increasing global consumption of aquaculture products (FAO, 2022), European perch (*Perca fluviatilis*) is currently considered an interesting species for the diversification of European aquaculture (Fontaine and Teletchea, 2019; Gebauer et al. 2021). Furthermore, it can serve as a complementary species to wild stocks in niche alpine markets, thus reducing pressures on wild individuals of the same species (Cooney et al. 2021). In the wild, European perch are opportunistic, schooling predators feeding on a diverse diet (Thorpe, 1977). In contrast, the nutritional requirements under perch aquaculture conditions are currently not fully defined (Bochert, 2022). It therefore becomes necessary to develop formulations for the production of efficient feeds to produce nutritionally complete commercial fish.

The aim of this experiment was to determine the effect of dietary protein content on morphology and enzymatic activity in liver of the European perch (*Perca fluviatilis*).

Materials and methods

The study was carried out as part of the project “Diversification of the productive function of earthen ponds based on semi-intensive rearing of *Perca fluviatilis* - PROPERCH (no. 00002-6521.1-OR1400004/17/20) co-financed by the European Maritime and Fisheries Fund.

A 12-week feeding experiment was carried out, during which the fish were fed a feed with fish meal (FM) levels of: 44%, 52%, 60% and 68%. On the last day of the experiment, the fish were sacrificed, measured and weighed, and biological material in the form of liver sections was collected from 12 individuals from each of the 4 groups for analysis. Tissue sections were fixed in Bouin’s fluid and then subjected to a standard histological procedure. The sections for biochemical analysis were placed in liquid nitrogen and later homogenates were prepared from them. Paraffin-embedded tissues were sectioned and stained with haematoxylin and eosin (HE) and AB/PAS (Alcian blue - Schiff’s reagent with periodic acid). The stained slides were subjected to microscopic analysis. In addition, biochemical analysis of the activity of the following oxidative stress enzymes was performed: alkaline phosphatase, acid phosphatase, superoxide dismutase (SOD) and glutathione peroxidase. The obtained results were analysed using statistical methods.

Results

The highest body weight was observed in the fish meal-fed group at 68% and the lowest in the group with 44% FM (Kruskal-Wallis ANOVA; $p < 0.05$). In contrast, the most favourable FCR values were achieved in the FM52 and FM60 groups, which translated into the Protein Per Growth Rate (PCR), which was also the most favourable in these two groups. Increase in phosphatase activity in individuals fed diets with a fish meal content above 52%. The group with the highest proportion of fish meal in the diet showed increased superoxide dismutase activity. In addition, all study groups had high glutathione peroxidase activity.

Conclusions

The results obtained indicate that the most favourable protein content in the feed for common perch kept in RAS are feeds with a fish meal content above 52%.

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