Theses of Doctoral (PhD) Dissertation

DEVELOPMENT OF INTENSIVE FARMING AND FEEDING TECHNOLOGY OF EUROPEAN PERCH (*PERCA FLUVIATILIS*)

Áron Molnár
Ph.D. candidate

Dissertation supervisor:
Dr. Milán Fehér

UNIVERSITY OF DEBRECEN
Doctoral School of Animal Science

Debrecen, 2023.

1. INTRODUCTION AND AIM OF THE THESIS

The world is experiencing a global protein deficit due to increasingly dynamic population growth (KIM et al., 2019; de SOUZA-VILELA et al., 2019). Fish is one of the most important sources of protein for humanity, accounting for about 16% of the animal protein consumed by the world's population. The demand for fish meat is increasing in line with population growth, which can only be solve by intensifying the production (TIDWELL & ALLAN, 2001).

In 2021, the fish consumption per person in Hungary was only 6.52 kg, which is extremely low by global standards (MAHAL, 2022). In order to improve the overall health of Hungarian society, it is essential to promote and increase fish consumption, which cannot be achieved without the introduction of new fish species that offer consumers a better gastronomic experience and can also have a positive impact on the fisheries sector. European perch (*Perca fluviatilis*) is a native predatory fish that meets modern consumer demands thanks to its excellent meat quality.

The overall aim of the PhD research is therefore to scientifically support the introduction of perch into production and market introduction, which is still new in intensive aquaculture.

The research focused on the development of two main pillars of intensive fish farming, namely the optimal farming technology and the development of feeding protocol. The high sensitivity of fish species to stress is well known from literature (ALANÄRÄ and STRAND, 2015), and therefore the set experiments were conducted to minimise the stress effects on fish.

The other main focus of the PhD research is the development of a feeding technology for perch by introducing Yellow mealworm (*Tenebrio molitor*) larvae as an alternative protein source. Afterward, an efficient enrichment protocol was developed to enrich the insect larvae with essential trace elements for fish.

Before starting the experimental phase of the research work, the following objectives were defined:

1. <u>Develop a technology for enrichment of Yellow mealworm larvae with trace elements with beneficial physiological effects:</u>

My research aimed to develop a simple and quick, yet effective trace element enrichment protocol that would result in the addition of various bioactive compounds to cobalt and manganese, or a combination of these, in addition to cobalt and manganese in the mealwormlarvae used as fish feed.

2. Development of intensive farming techniques for perch:

The aim of my studies was to optimize intensive farming technology, which would result in minimizing stress effects on fish and improving growth, survival and uniformity of the stocks.

3: Development of a feeding protocol of perch:

During the feeding studies, I aimed to determine the optimum feeding intensity and to incorporate mealworm larvae into the perch diet. In the final phase of the research, I aimed to determine the effect of live mealworm larvae enriched with trace elements on perch growth and to investigate the uptake of trace elements accumulated in the larvae by fish.

2. MATERIALS AND METHODS

2.1. Experiments with farming of European perch

2.1.1. Effects of the tank colour on the production and antioxidant parameters of the European perch

A range of different tank types is recommended for the production of European perch at different developmental stages. Although there are several studies on the different tank sizes, their combination has not yet been investigated (STRAND et al., 2007; TAMAZOUZT et al., 2000; PALIŃSKA-ŻARSKA et al., 2013; JENTOFT et al., 2006). Therefore, the aim of this study was to evaluate the effect of combinations of different tank colours (grey; black bottom and grey sidewalls; grey bottom and black sidewalls) on the production and antioxidant parameters of European perch.

Test environment

The experiment was conducted at the Laboratory of Aquaculture, University of Debrecen. The duration of the experiment was 8 weeks. The experimental recirculation system consisted of three main parts: the rearing units and the mechanical and biological filters. Mechanical filtration was performed using sponges, while biological filtration was performed using a plastic biofilter followed by UV sterilization.

Experimental stock

The initial average body weight and body length of the experimental stock was 32.01 ± 0.79 g and 11,8 ± 0,81 cm. The experimental stock was artificially propagated by H&H Carpio Halászati Kft. and reared in a recirculation system prior to the experiment, taking into account the needs of the species and the age classes.

Treatments

The experimental system consisted of 9 units (180 individuals; /3 tanks/treatment; 20 fish/tank, 60 fish/treatment). We investigated the effect of a light grey (control), a black bottom and light grey sidewalls (SO) and a light grey bottom and black sidewalls (SA) tank on the production (survival rate, body weight, specific growth rate, feed conversion) and antioxidant parameters (cortisol, glucose, MDA, catalase, vitamin C, GPx, GR, GSH, GSSG and HSP70) of the European perch.

Feeding protocol

Before the experiment started, the fish were acclimatised to the environmental and technological conditions for two days. The daily feed ration was 1% of the total biomass of the fish (ALLER AQUA IVORY EX: 2 mm) and was distributed manually twice daily.

Water quality parameters

The dissolved oxygen content of the water, water temperature and pH were monitored daily (HACH HQ30d). The concentrations of the different nitrogen forms in the water were measured weekly (N-NH3+, N-NO2- and N-NO3-) using a spectrophotometer (HACH DR3900).

Production parameters

At the end of the experiment, the survival rate (S), final body weight (BWf), specific growth rate (SGR), feed conversion ratio (FCR), Fulton's condition factor (K-factor) and stock homogeneity (CV%) were determined.

- Survival (%): S = (number of individuals harvested/number of individuals stocked) × 100,
- SGR (%) = (lnWf - lnWi) / t x 100, where: Wf: final body weight (g), Wi: initial body weight (g), t: number of days,
- FCR (g/g) =F/(Wf-Wi), where: F: amount of feed in dry matter (g) fed during the experiment, Wf: final body weight (g), Wi: initial body weight (g),
- Fulton condition factor (K-factor) = W/L3 × 100, where W is the wet weight (g), L is the standard length (mm) and
- Homogeneity (CV%) = (standard deviation/average) × 100.

Determination of antioxidant parameters

The antioxidant concentration of the samples was determined using commercially available ELISA kits and colorimetric assays. Absorbance data for ELISA and colorimetric assays were collected using a SPECTRO-star Nano Microplate reader.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 for Windows software. The homogeneity of variances between experimental groups was checked by Levene's test. The effect of treatments on growth performance and antioxidant parameters of fish was analysed by one-way analysis of variance (ANOVA). Tukey's multiple comparison was used to determine significant differences between treatments. A value of $p<0.05$ was considered significant in the analyses.

2.1.2. Effect of different light conditions on production parameters

The experiment investigated the effects of light intensity and water turbidity on production parameters and cannibalism in a 3-month-old stock of pre-reared and acclimated perch in an aquarium model system.

Test environment

The experiment was carried out in a modular aquarium system at the Laboratory of Aquculture. The galvanized rack is equipped with 3 rows (8 aquariums per row, 24 in total) of square glass aquaria, each with a water volume of 12.96 litres. The experimental units were illuminated by an LED (EVERGROW LED IT5012) installed towards the aquariums. During the 4-week study, individual sponge filtration was applied per unit and the test environment consisted of 24 units in total.

Experimental stock

The experimental stock was obtained from artificial propagation by H&H Carpio Halászati Ltd. and was kept in a recirculation system prior to the experiment, taking into account the needs of the species and the age classes. The study was set up with 10 fish per tank, for a total of 240 perch. Before stocking, the wet body weight and length of the fish (1.69±0.04 g; 3,71 ± 0,8 cm) was determined individually using a digital scale (VWR LP-6501, max: 6500 g, 0.1 g accuracy) with decimal precision.

Treatments

Three treatments and two additional variables were applied in the experiment. All treatments were performed in 4-4 replicates. A part of the experimental stock was grown at 300 lux illumination (V), the second treatment at 100 lux illumination (T) and the third treatment at 20 lux illumination (S). The different intensities of illumination were adjusted using raschel nets of different densities installed between the aquarium rows. For each treatment, groups were established with water darkened with humic acid (HUMIN Aqua) (VH, TH, SH). The duration of illumination was 14 h per day (06 - 20 h). After water exchange, the turbidity of the water (NTU) was adjusted by adding 7-7 ml of humic acid for the humic acid treatments.

Feeding protocol

Daily feed rations were set at 2.5% initial biomass. The feed used in the experiment was AQUA GARANT AQUA START 1 mm particle size (crude protein: 60%, crude fat: 15%). One weekly feed was pre-weighed and then applied ad libitum, by hand, 3 times daily at 9:00, 12:00 and 16:00 h.

Water quality and production parameters and statistical evaluation of the results were the same as described in the previous chapter.

2.2. European perch feeding experiments

2.2.1. Effect of feeding frequency on production parameters

In addition to the correct determinatopn of feed composition and particle size, the feeding frequency is essential during farming European perch.

Test environment

The experiment was carried out in a modular aquarium system at the Laboratory of Aquaculture, where 4-4 glass aquariums, 12 square glass aquariums in total, each with a volume of 23.5 litres, were placed in 3 rows. During the experiment, the modular aquarium unit was operated as a recirculation system.

Treatments

During the study, 3 treatments were applied in 4-4 replications. The daily feed rations were set at 5% of the initial biomass, which was fed twice daily for the first treatment (T2 treatment; 8:00am - 20:00pm), three times daily for the second treatment (T3 treatment; 8:00am - 14:00pm - 20:00pm) and four times daily for the third group (T4 treatment; 8:00am - 12:00pm - 16:00pm - 20:00pm).

Feeding protocol

The daily feed ration was 2 g per tank in all cases. Feeding was carried out with a 1.5 mm particle size feed (AQUA GARANT AQUA START 1.5 mm, crude protein: 52%, crude fat: 20%), which was fed using automatic feeders (JBL AutoFood Black).

Experimental stock

During the experiment, 10 juvenile perch were stocked per tank, with an individual mean body weight of 3.93 ± 0.06 g at the start of the study. The experimental stock was obtained from artificial propagation by H&H Carpio Halászati Ltd. and reared in a recirculation system prior to the experiment, taking into account the needs of the species and the age classes.

The water quality and production parameters and the statistical evaluation of the results were the same as described in the previous chapter.

2.2.2. Introduction of mealworm larvae into the feeding of European perch

Several studies have investigated the feasibility of replacing artificial dry feed with alternative protein sources such as plant-based protein or, in our case, insect protein. These studies have mainly experimented with the incorporation of insect protein into fish feed in dry, powdered form (insect meal). Our experiments aimed to find out whether live mealworm larvae can partially replace fishmeal-based feeds and in what proportions they can be used in fish feeds without decreasing production parameters.

Test environment

The 35-day experiment was carried out in a modular aquarium system (12 aquariums in a recirculation system) as described in *chapter 2.2.1* of the Laboratory of Aquaculture.

Experimental stock

The test was set up with 5 units per aquarium for a total of 60 individuals. The mean body weight of the fish was 24.25 ± 0.26 g. The illumination duration was 14 h per day (06 - 20 h). The experimental stock was artificially propagated by H&H Carpio Halászati Ltd. and reared in a recirculation system prior to the experiment, taking into account the needs of the species and the age classes.

Treatments and feeding protocol

During the experiment, 4 treatments were applied in 3 to 3 replicates. The treatments were named according to the mealworm larvae (L) and their percentage (%).

The LT75% group received 75% live mealworm larvae and 25% dry feed, the LT50% group received 50% live mealworm larvae and 50% dry feed. The LT25% group consumed 25% mealworm larvae and 75% dry feed during the experiment. The control group (K) was fed 100% dry feed during the experiment. The commercially available feed (AQUA GARANT UNI, 2 mm, crude protein: 47%, crude fat: 16%) and the mealworm larvae were applied by hand twice daily. A feeding intensity of 1.08% was applied during the experiment. Daily feed rations of the mealworm larvae consuming treatments were calculated on the basis of dry matter equivalence.

Water quality and production parameters and statistical evaluation of the results were the same as described in the previous chapter.

2.2.3. Trace element enrichment of mealworm larvae

In order to develop a trace element enrichment protocol for mealworm larvae, I have set up 2 different but related experiments. The fundamental difference between the two experiments is that in the first experiment I sampled larvae reared in trace element enriched substrate only between days 7 and 14, and in the second experiment I sampled larval element uptake continuously every day between days 7 and 14, to assess exactly how larval trace element accumulation varies over this time period.

In the experiments, the enrichment of larvae with cobalt and manganese, trace elements essential for fish, was carried out during a 14-day experiment, with sampling and determination of trace element uptake on days 7 and 14.

The 150 ml plastic enrichment units were filled with 40 g of wheat flour. I applied a solution of cobalt chloride and manganese chloride or a combination of the two trace elements at a concentration of 100 mg/kg to the substrate (3-3 replicates). After drying and homogenization, 10-10 larvae with a body weight of 0.03 ± 0.003 g at the initial developmental stage were placed in each unit (240 larvae in total). Larval growth and trace element uptake were determined for days 7 and 14 (2 x 12 treatments).

I determined the individual body mass of mealworm larvae at both the beginning and end of the experiment, as well as the survival rate. Larvae were stored in plastic sampling bags in the freezer (-18°C) until laboratory measurements. After sample preparation, the cobalt (mg/kg) and manganese (mg/kg) content of the larvae was determined. Elemental analysis of the samples was performed using inductively coupled plasma optical emission spectrometry (ICP-OES).

The statistical evaluation of the results was the same as described in the previous chapter.

2.2.4. Uptake of cobalt and manganese by European perch as a result of feeding with enriched mealworm larvae

In our experiment, 100% of the fish feed was replaced with live mealworm larvae. The insects were enriched with two essential trace elements for fish, cobalt and manganese, and their combination. The aim of this study was to investigate the indirect trace element accumulation by fish, our hypothesis being that trace elements bound by mealworm larvae in organic form can be accumulated by fish through consumption of insects. The duration of the experiment was 28 days, during which 4 treatments were set up in 3 replicates.

Test environment

The experiment was set up in the recirculation system of the Laboratory of Aquaculture. The technical and technological parameters of the system are described in detail in *chapter 2.1.1*.

Experimental stock

At the beginning of the experiment, 15 per tank and 45 per treatment, a total of 180 European perch with a mean individual body weight of 83.15 ± 12.20g were stocked. The experimental stock was artificially propagated by H&H Carpio Halászati Ltd. and reared in a recirculation system prior to the test, taking into account the needs of the species and the age classes.

Treatments

The experiment used the trace element enrichment protocol described previously, with 3 replicates per treatment: cobalt enriched treatment (Co), manganese enriched treatment (Mn), a combination of the two trace elements (CoMn) and control (K).

Feeding protocol

The fish were fed 1% of the diet in the form of enriched live mealworms. The amount of mealworm larvae was determined on the basis of dry matter equivalence (40% dry matter). Seven days before the start of the experiment, the required amount of mealworm larvae for one week's feeding was placed on the substrates enriched with trace elements and continuously fed to the fish after 1 week (for 7 days). Then, at the beginning of each week during the 4 weeks of the experiment, a new enrichment was set to provide the following week's feed.

Water quality and production parameters and statistical evaluation of the results were the same as described in the previous chapter.

Trace element uptake study

At the end of the experiment, fillet and whole fish body samples were taken from each tank of 2 fish to determine trace element accumulation in the fish. Fish meat samples were analysed after lyophilisation. The elemental composition of fish samples was determined using an inductively coupled plasma optical emission spectrometer (Agilent ICP-OES 5110 VDV).

3. RESULTS

3.1. Experiments with European perch rearing technology

3.1.1. Effect of the tank colour on the production and antioxidant parameters of the European perch

During the experiment, the survival rate was 100% for all treatments. In terms of fish weights at the end of the experiment, fish reared in the darkened bottom tank (SA) had significantly higher final body weight compared to the other two treatments, while no statistically verifiable difference was observed between the darkened sidewall tanks (SO) and the control group (K) The specific growth rate (SGR) of SA treatment was higher compared to SO group, while the control treatment did not differ from either. There were no significant differences between treatments in terms of FCR, condition factor (K) and stock homogeneity (CV%). The production parameters of the fish are summarised in **Table 1.**

Table 1: The production parameters of perch in different tank colours

	S (%)	BWf (g)	SGR (%/day)	FCR (g/g)	CV%	K
SO	100	48,70 ± 11,21^{a}	0,76 ± 0,08^{a}	1,40 ± 0,19	22,44 ± 4,66	2,66 ± 0,25
SA	100	54,24 ± 9,70^{b}	1,00 ± 0,06^{b}	1,29 ± 0,13	17,51 ± 2,46	2,70 ± 0,27
K	100	49,22 ± 11,21^{a}	0,84 ± 0,14^{ab}	1,38 ± 0,24	22,40 ± 6,56	2,62 ± 0,25

Data are given as mean ± standard deviation. **Control**: light grey tank colour; **SO**: darkened sidewall tank; **SA**: darkened bottom tank; mean values within columns marked with different letters are significantly different ($p < 0.05$).

Antioxidant parameters

Table 2 shows the effects of different combinations of the two tank colours (SO, SA) on the antioxidant parameters of fish. We measured cortisol and glucose, which are excellent stress biomarkers. The fish reared in dark bottom tanks (SA) (55.79 ± 3.20 g) and the control group (56.62 ± 2.89 g) showed significantly lower cortisol concentrations than fish reared in dark sidewall tanks (SO = 66.33 ± 2.08 g). Glucose levels correlated with cortisol levels in the different treatments. Fish in the SA group showed significantly lower glucose levels than fish in the SO group, while fish in the control group did not differ (SO = 5.33 ± 0.94 g; SA = 4.13 ± 0.83 g; control = 4.48 ± 0.95 g).

Among antioxidant enzymes, catalase levels were significantly lower in the control group (4.95 ± 0.51 g) and SA group (4.68 ± 0.91 g) than in the SO group (6.94 ± 0.51 g); however, GR values showed a completely opposite trend. The GR values of the SO group were significantly lower than those of the control and SA groups. For the third antioxidant enzyme (GPx), no significant difference was found between the groups (SO = 220.09 ± 37.62 g, SA = 169.48 ± 17.66 g, K = 182.52 ± 14.11 g). As for the different forms of glutathione (GSH, GSSG) that were measured, both GSH and GSSG levels did not differ between the control and SA groups, which were significantly higher than in the SO group.

The results obtained for low molecular weight antioxidants showed that vitamin C levels in fish were significantly higher in the SA group (92.32 ± 59.99 g) than in the SO group (30.38 ± 9.67 g), while in neither group were significantly different from the control (46.54 ± 13.60 g). The highest vitamin C levels were measured in the SA group, presumably due to the fact that the fish did not use their body vitamin stores to protect against oxidative stress. In terms of MDA levels at the end of the experiment, the control group (1029.31 ± 183.83 g) was significantly lower than the SO group (1604.59 ± 412.06 g), while the levels of neither group were significantly different from the SA (1172.85 ± 239.08 g).

The levels of heat shock proteins (HSP70) were determined to investigate the third level of antioxidant protection. No differences were found between the groups when HSP70 levels were assessed (SO = 17.11 ± 1.26 g; SA = 17.05 ± 0.94 g; K = 17.03 ± 0.85 g), presumably because the fish were not exposed to heat shock. We hypothesise that the lack of difference in the production of heat shock proteins may be due to the fact that the defence system was already able to cope with the stress at the first two levels and that activation of the third level was not necessary.

Table 2. Antioxidant and stress parameters of perch in different tank colours

Antioxidant	Control	SO	SA
Cortisol (mg/mL)	56.62 ± 2.89^{a}	66.33 ± 2.08^{b}	55.79 ± 3.20^{a}
Glucose (mmol/L)	4.48 ± 0.95^{ab}	5.33 ± 0.94^{b}	4.13 ± 0.83^{a}
Catalase (mU/mL)	4.95 ± 0.51^{a}	6.94 ± 0.51^{b}	4.68 ± 0.91^{a}
Glutathione peroxidase(GPx) (mU/mL)	182.52 ± 14.11	220.09 ± 37.62	169.48 ± 17.66
Glutathione reductase (GR) (mU/mL)	21.42 ± 3.94^{b}	12.06 ± 1.13^{a}	22.51 ± 5.02^{b}
Reduced glutathione (GSH) (µM)	19.68 ± 2.09^{b}	14.74 ± 3.93^{a}	23.15 ± 0.57^{b}
Glutathione disulphide (GSSG) (µM)	9.76 ± 1.07^{b}	7.37 ± 1.96^{a}	11.66 ± 0.26^{b}
Vitamin C (nmol/mL)	46.54 ± 13.60^{ab}	30.38 ± 9.67^{a}	92.32 ± 59.99^{b}
Malondialdehyde (MDA) (nmol/mL)	1029.31 ± 183.83^{a}	1604.59 ± 412.06^{b}	1172.85 ± 239.08^{ab}
HSP70 (ng/L)	17.03 ± 0.85	17.11 ± 1.26	17.05 ± 0.94

Data are presented as mean ± standard deviations. **Control**: light grey tank colour; **SO**: darkened sidewall tank; **SA**: darkened bottom tank, mean values within a row with different letters are significantly different ($p < 0.05$).

3.1.2. Effect of different light conditions on production parameters

Statistical analysis showed that humic acid and illumination had an effect on the results, but their interaction (illumination*humic acid) did not affect the production parameters of fish. At the end of the experiment, the survival was above 90% for all treatments and no cannibalism was observed in any of the tanks. The best survival was achieved in the treatments reared under full illumination (V and VH).

In terms of individual body weight, the most favourable results were obtained in treatments where the tanks were covered (T: 4.66 ± 0.33 g) and where the tank water was darkened by the addition of humic acid (TH: 4.93 ± 0.15 g). The results of the T and TH groups were significantly better than those of the other treatments, but not statistically different. The final body weights of fish reared under full illumination were positively affected by water darkening (V: 3.73 ± 0.28 g; VH: 4.33 ± 0.28 g), whereas the results of S and SH treatments were not affected by humic acid supplementation (S: 3.78 ± 0.15 g; SH: 3.80 ± 0.26 g). The most favourable results for the SGR (T: 3.64 %/day and TH: 3.74 %/day) and FCR (T: 0.84 g/g and TH: 0.78 g/g) indicators were also obtained with the same two treatments. At the end of the experiment, the fish reared under the strongest light (V) showed the highest growth, while the group reared under moderate light (T) did not differ statistically from either treatment.

The results of the experiment showed that moderate lighting during the early stages of perch rearing has a positive effect on growth and feed conversion, while complete darkening has a negative effect on production parameters. Humic acid had the greatest effect on the results for the groups reared under full light, while it had no effect on the production parameters for the treatments in total darkness. The results of the study suggest that moderate light during the early stages of perch rearing has a positive effect on growth and feed conversion, while total darkness has a negative effect on production parameters.

3.2. European perch feeding experiments

3.2.1. Effect of feeding frequency on production parameters

In the study, survival was above 90% for all treatments. At the end of the experiment, the treatment that received the daily feed ration divided in two groups produced the most favourable body weight (13.96 ± 0.14 g) and growth rate (SGR: 3.08 ± 0.01%/day), but no significant differences between groups were observed (T2). Regarding the feed conversion ratio, the best result was obtained by the treatment receiving the feed in three daily rations (FCR: 1.06 ± 0.18 g/g), but no significant difference was found for this indicator neither. The results indicate that the distribution of daily feed ration does not influence the production parameters of perch juveniles.

3.2.2. Involvement of mealworm larvae in the feeding of European perch

No mortality was observed during the experiment, the survival was 100% for all treatments. The production parameters tested were statistically verifiable differences in any of the parameters of the treatments fed with the highest dose of mealworm larvae (LT75) did not achieve a less favourable result than the treatments fed only with dry feed (K).

Regarding the results of the average body weight of the fish at the end of the experiment, the LT50 treatment (32.7 ± 7.06 g) achieved the best result, followed by the result of the LT50 group (32.5 ± 5.91 g). The treatment receiving the highest dose of live larvae did not achieve a more unfavorable result compared to the control treatment (31.9 ± 6.48 g) and the LT25 (32 ± 6.49 g) group. The biomass increase (WG%) of the treatments reached the highest value where the rate of addition of mealworm larvae was the highest (LT75=34.54 ± 1.96 g), while it was the lowest in the control groups (K=30.33 ± 7.09 g).

Regarding the specific growth rate of fish, the most favorable result was achieved by LT75 (0.85 ± 0.04 %), the most unfavorable by K (0.75 ± 15 %). In terms of FCR, the most favorable indicator was achieved by the LT75 (1.09 ± 0.06 g/g) treatment, while the control (1.27 ± 0.27

g/g) treatment was the least favorable. The LT50 (1.11 ± 0.20 g/g) and LT25 (1.15 ± 0.18 g/g) were slightly lower than the LT75 treatment, but achieved a more favorable result than the control group. Regarding the homogeneity (CV%) of the experimental stock, LT75 (19.01 ± 5.08 %) was the most uniform, while the LT50 (22.33 ± 8.05 %) treatment showed the greatest degree of diversity compared to the other treatments (LT25=20.93 ± 7.92 %), (K=20.95 ± 8.54 %).

Based on the results, it can be stated that the use of mealworm larvae as live feed proved to be suitable for replacing fishmeal-based feed, presumably due to its favorable protein content and amino acid profile similar to fishmeal.

3.2.3. Trace element enrichment of mealworm larvae

The trace elements application had no effect on the survival and growth of mealworm larvae, as the results showed that cobalt and manganese, as well as the combination of the two microelements, did not significantly affect the production parameters for either the 7-day or 14-day treatments (**Table 3).** In terms of trace element uptake by larvae, it can be stated that both cobalt and manganese accumulated efficiently after 7 days.

In all cases, cobalt supplementation significantly affected the cobalt content of the larvae, and stocks treated for 14 days accumulated significantly more trace elements than stocks treated for 7 days. The results of CoMn treatments were correlated with these results in terms of cobalt uptake. Thus, the combined application of cobalt and manganese did not negatively affect cobalt uptake.

In the case of manganese, there was a significant difference in larval trace element accumulation between the 7 and 14 day treatments compared to the control, while the 7 and 14 day manganese supplementation did not differ to a statistically.

Table 3: Growth and trace element uptake of mealworm larvae (ML)

Treatment	BWi (g)	BWf (g)	Co (mg/kg)	Mn (mg/kg)
K7	0,030±0,002	0,038±0,004	0,00±0,00^{a}	22,64±3,54^{abc}
Co7	0,033±0,001	0,463±0,002	30,19±9,71^{b}	18,36±3,56^{ab}
Mn7	0,030±0,004	0,400±0,004	0,00±0,00^{a}	35,38±9,60^{cde}
CoMn7	0,032±0,003	0,427±0,005	35,98±0,98^{b}	30,11±5,09^{bcd}
K14	0,037±0,003	0,059±0,004	0,00±0,00^{a}	15,75±0,85^{a}
Co14	0,032±0,002	0,052±0,005	53,10±8,91^{c}	15,67±0,77^{a}
Mn14	0,036±0,001	0,064±0,003	0,00±0,00^{a}	36,87±1,90^{de}
CoMn14	0,034±0,003	0,061±0,007	50,97±5,45^{c}	44,04±6,54^{e}

Data are presented as mean ± standard deviations. Mean values within columns marked with different letters are significantly different ($p < 0.05$). **Control**: wheat semolina substrate; **Co7**: cobalt-enriched ML for 7 days; **Co14**: cobalt-enriched ML for 14 days; **Mn7**: manganese-enriched ML for 7 days; **Mn14**: manganese-enriched ML for 14 days; **CoMn7**: cobalt and manganese-enriched ML for 7 days; **CoMn14**: cobalt and manganese-enriched ML for 14 days

The enrichment of mealworm larvae with various trace elements is effective and can be achieved in a short time based on the results of our experiment. It can be shown that the mealworm larva can take up large amounts of cobalt and manganese from the substrate.

3.2.4. Uptake of cobalt and manganese by European perch as a result of feeding with enriched mealworm larvae

Low mortality was observed for all treatments in the experiment, with no significant differences between treatments. No mortality (S=100%) was observed in fish consuming mealworm larvae enriched with a combination of the two trace elements (CoMn), while the control group had the highest mortality rate (S=95.56 ± 7.70%) for the other treatments, and the Co and Mn treatments both had a 97.78 ± 3.85% survival rate.

At the end of the experiment, the average biomass of the treatments showed an increase in all cases compared to the initial data. The CoMn treatment (1430.67 ± 8.09 g) achieved significantly better results compared to the control group (K=1254.23 ± 126.16 g), while the Co (1388.1 ± 46.94 g) and Mn (1363.3 ± 108.59 g) treatments did not differ statistically from either of them.

The mean final body weights of the treatments were correlated with these parameters (K=87.50 ± 19.26 g; Co=94.64 ± 17.29 g; Mn= 92.95 ± 18.23 g; CoMn=95.38 ± 20.81 g) at the end of the experiment. In terms of FCR, the CoMn (0.84 ± 0.08 g/g) and Mn (0.80 ± 0.08 g/g) treatments showed significantly better performance at the end of the experiment compared to the control (1.99 ± 0.34 g/g) group, while the Co (1.21 ± 0.23 g/g) treatment did not show statistically verifiable differences compared to either treatment.

In terms of specific growth rate of fish, the control group (0.19 ± 0.10%) had a significantly lower rate of growth compared to the other treatments at the end of the experiment (Co= 0.46 ± 0.09%; Mn= 0.42 ± 0.18%; CoMn= 0.46 ± 0.04%). The results of the production parameters indicate that individuals fed with live mealworm larvae enriched with the 2 trace elements essential for fish, cobalt and manganese, achieved significantly higher individual weights and specific growth rates and lower feed conversion at the end of the experiment compared to the other treatments.

Fillet samples were taken at the end of the experiment and whole body trace element analysis was also performed (**Table 4**). Regarding the cobalt content of fish fillet, the cobalt-enriched treatments were significantly more enriched in trace elements (Co = 806, 1 ± 230.4 µg/kg; CoMn = 359 ± 257.8 µg/kg) compared to the other two treatments. Enrichment with manganese resulted in reduced cobalt accumulation, as the Co treatment consuming only cobalt-enriched insects had significantly higher cobalt concentrations compared to the CoMn group.

Table 4: Cobalt and manganese concentration measured in the meat and whole body of the fish

	Treatment			
Trace element conc.	**Control**	**Co**	**Mn**	**CoMn**
$Co_{(body)}$(µg /kg)	15, 1 ± 3,12[a]	1527,8 ± 717,7[c]	7,7 ± 1,66[a]	722,3 ± 266,6[b]
$Co_{(fillet)}$(µg/kg)	0,00 ± 0,00[a]	806, 1 ± 230,4[c]	5 ± 1,16[a]	359 ± 257,8[b]
$Mn_{(body)}$(µg /kg)	5145,6 ± 917,4[a]	5263,5 ± 1716,1[a]	8911,2 ± 2388,6[b]	9153,3 ± 3351,1[b]
$Mn_{(fillet)}$(µg /kg)	807,8 ± 153,8[a]	852,7 ± 179,2[a]	1691 ± 103,56[b]	1058,5 ± 370,5[b]

Data are presented as mean ± standard deviations. **Control**: fish fed with mealworm larvae; **Co**: fish fed with cobalt-enriched mealworm larvae; **Mn**: fish fed with manganese-enriched mealworm larvae; **CoMn**: fish fed with cobalt and manganese-enriched mealworm larvae, mean values within rows with different letters are significantly different ($p < 0.05$).

Whole-body cobalt concentrations in fish were totally correlated with the results of the fillet samples. The amount of cobalt enriched in the body of the perch was significantly higher in the Co treatment (1527.8 ± 717.7 µg/kg) compared to the treatment enriched with both trace elements (CoMn = 722.3 ± 266.6 µg/kg), while the control group and the Mn treatment did not differ statistically at the end of the experiment.

The results for manganese content of fish were similar to those for cobalt. Significantly higher levels of manganese were measured in fish fillet in the treatments specifically enriched with this trace element (Mn = 1691 ± 103.56 µg/kg; CoMn = 1058.5 ± 370.5 µg/kg) compared to the control (807.8 ± 153.8 µg/kg) and Co (852.7 ± 179.2 µg/kg). The results of the whole body manganese enrichment measurements showed a total correlation with the trace element content of the fillet samples, similar to the cobalt enriched treatments.

4. NEW SCIENTIFIC RESULTS OF THE THESIS

1. Based on the results of the experiments, I found that for juvenile European perch, a black bottomed, light grey sidewall tank has a positive effect on production parameters (body weight gain, specific growth rate) during intensive rearing, and my results were confirmed by antioxidant parameters (cortisol, glucose, catalase, vitamin C, MDA, GR, GSH, GSSG) in addition to production parameters.

2. Based on the results of my experiments on illumination and water turbidity, I found that moderate illumination (118.0 ± 24.4 lux) has a positive effect on individual body weight and specific growth rate during juvenile perch rearing. Water turbidity did not have a positive effect on production parameters, only at these light conditions.

3. Based on the results of my studies with juvenile perch, I found that at the same feed rations (feed composition: crude protein 53%, crude fat 18%, intensity 5%), feeding frequency had no effect on production parameters when feeding was carried out between 2 - 3 - 4 times per day.

4. I have found that Yellow mealworm larvae in live form can be incorporated into the diet of juvenile perch. The results indicate that the insect larvae can replace dry feeds up to 75% in dry matter equivalence without deterioration of production parameters.

5. It was found that the Yellow mealworm larvae, when enriched with cobalt chloride and manganese chloride in the substrate, efficiently accumulate the two trace elements after only 7 days. The cobalt content of the larvae was significantly higher after 14 days of enrichment than after 7 days of supplementation, while the manganese content of the larvae was not statistically different between the investigated two times.

6. I found that feeding Yellow mealworm larvae enriched in combination with cobalt and manganese has a positive effect on the production parameters (specific body weight, feed conversion, specific growth rate) of market-size perch. Based on the analysis of whole body and fillet samples of fish, I showed that perch can accumulate large amounts of both trace elements from insect larvae indirectly.

5. APPLICABILITY OF THE RESULTS

European perch is a native predatory fish in Hungary, but it does not play a significant role in aquaculture production. The aim of this PhD thesis is to support the introduction of perch into the production and market, partly by developing a feeding protocol based on the use of Yellow mealworm larvae, an alternative protein source of animal origin.

The other main objective of the research was to improve perch farming technology. Based on the results of my experiments in differently darkened tanks, I found that a dark bottomed, light sided tank has a clear positive effect on the stress status of the fish. Adapting the colour of the tank to the needs of each species and age class is an important factor of intensive rearing, however, there are no research in the literature that has investigated the relationship between tank colour and antioxidant parameters, and my results may contribute to the development of intensive rearing technology for perch. The experiences gained with tank colour, lighting and water turbidity may also provide producers with useful information for the design and development of the RAS system.

An important pillar of the results of the doctoral research is that the Yellow mealworm larvae can be incorporated into fish feed, which is supported by the positive results obtained in feeding trials. The positive results of feeding experiments with mealworm larvae may contribute to the development of insect-based feeding, and in the long term the research may contribute to the reduction of fishmeal consumption.

An important practical experience is that mealworm larvae can be effectively enriched with cobalt and manganese. Based on the favourable results obtained, experiments on the incorporation of other trace elements and bioactive substances, as well as on the enrichment of various live food organisms, could be initiated in the future.

In the final stage of perch rearing, cobalt and manganese were also present in the fish fillets as a result of feeding the enriched live mealworm larvae, therefore the accumulation of the trace element is not only in the organs. This is also highly beneficial from a human health, so it can be stated that some of the results of the doctoral research may even contribute to the promotion and increase of fish consumption in Hungary.

6. REFERENCES

1. *Alanärä, A.* - *Strand, Å.*: 2015. The Energy Requirements of Percid Fish in Culture. [In: Biology and Culture of Percid Fishes: Principles and Practices; Szerk. Kestemont, P., Dabrowski, K., Summerfelt, R.C.] Springer: Dordrecht, The Netherlands; Heidelberg, Germany; New York, NY, USA; London, UK.353–368.
2. *De Souza-Vilela, J.* - *Andrew, N. R.* - *Ruhnke, I.*: 2019. Insect protein in animal nutrition. *Animal Production Science.* 59. 11: 2029-2036.
3. *Jentoft, S.* - *Oxnevad, S.* - *Aastveit, A. H.* - *Andersen, O.*: 2006. Effects of Tank Wall Color and Up-welling Water Flow on Growth and Survival of Eurasian Perch Larvae (*Perca fluviatilis*). *Journal of the World Aquaculture Society*. 37. 3: 313–317.
4. *Kim, S. W.* - *Less, J. F.* - *Wang, L.* - *Yan, T.* - *Kiron, V.* - *Kaushik, S. J.* - *Lei, X. G.*: 2019. Meeting global feed protein demand: challenge, opportunity, and strategy. *Annual Review of Animal Biosciences*. 7. 221-243.
5. *MA-HAL* (2022): Jelentés a Szövetség működésének 2021. évi eredményeiről. Budapest, 2022.
6. *Palińska-Żarska, K.* - *Żarski, D.* - *Krejszeff, S.* - *Nowosad, J.* - *Biłas, M.* - *Kucharczyk, D.*: 2013. Tank wall color affects swimbladder inflation in Eurasian perch, *Perca fluviatilis L.*, under controlled conditions. *Communications in Agricultural and Applied Biological Sciences*. 78. 338–341.
7. *Strand, Å.* - *Alanärä, A.* - *Staffan, F.* - *Magnhagen, C.*: 2007. Effects of tank colour and light intensity on feed intake, growth rate and energy expenditure of juvenile Eurasian perch, *Perca fluviatilis* L. *Aquaculture*. 272. 1-4: 312-318.
8. *Tamazouzt, L.* - *Chatain, B.* - *Fontaine, P.*: 2000. Tank wall colour and light level affect growth and survival of Eurasian perch larvae (*Perca fluviatilis* L.). *Aquaculture.* 182. 85–90.
9. *Tidwell, J. H.* - *Allan, G. L.*: 2001. Fish as food: aquaculture's contribution. *EMBO Reports*. 2. 11: 958-963.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/255/2023.PL
Subject: PhD Publication List

Candidate: Áron Molnár
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10070852

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (3)

1. **Molnár, Á.**, Dajka, B., Fehér, M.: Does the feeding frequency influence the growth performance of European perch juveniles (Perca fluviatilis) during intensive rearing?
Agrártud. Közl. 1, 123-128, 2022. ISSN: 1587-1282.
DOI: http://dx.doi.org/10.34101/actaagrar/1/10705

2. **Molnár, Á.**, Toviho, O. A., Fehér, M.: Investigation of the production parameters, nutrient and mineral composition of mealworm (Tenebrio molitor) larvae grown on different substrates.
Agrártud. Közl. 1, 129-133, 2022. ISSN: 1587-1282.
DOI: http://dx.doi.org/10.34101/actaagrar/1/10418

3. **Molnár, Á.**, Kovács, L., Homoki, D., Minya, D., Fehér, M.: Examining the production parameters of European perch (Perca fluviatilis) juveniles under different lighting conditions.
Agrártud. Közl. 1, 149-153, 2021. ISSN: 1587-1282.
DOI: http://dx.doi.org/10.34101/actaagrar/1/8494

Foreign language scientific articles in international journals (1)

4. **Molnár, Á.**, Homoki, D., Bársony, P., Kertész, A., Gálné Remenyik, J., Pesti-Asbóth, G., Fehér, M.: The Effects of Contrast between Dark- and Light-Coloured Tanks on the Growth Performance and Antioxidant Parameters of Juvenile European Perch (Perca fluviatilis).
Water. 14 (6), 1-10, 2022. EISSN: 2073-4441.
DOI: http://dx.doi.org/10.3390/w14060969
IF: 3.53 (2021)

Hungarian conference proceedings (1)

5. **Molnár, Á.**, Homoki, D., Lelesz, J. É., Kovács, L., Bársony, P., Fehér, M.: A mesterséges takarmány kiváltásának lehetőségei élő lisztbogár lárva (Tenebrio molitor) etetésével a csapósügér (Perca fluviatilis) intenzív nevelése során.
In: XXVII. Ifjúsági Tudományos Fórum Keszthely : Konferenciakötet. Szerk.: Bene Szabolcs, Magyar Agrár- és Élettudományi Egyetem Georgikon Campus, Keszthely, 27-33, 2021.
ISBN: 9786156338044

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Hungarian abstracts (2)

6. **Molnár, Á.**, Homoki, D., Kertész, A., Bársony, P., Fehér, M.: Rovarlárvával történő takarmányozás lehetőségei a csapósügér (Perca fluviatilis) intenzív nevelése során.
In: Halászatfejlesztés 38. Szerk.: Fazekas Gyöngyvér, Gyalog Gergő, Nagyné, Biró Janka, Bozánné Dr. Békefi Emese, Dr. Halasi - Kovács Béla, Szarvas, 25-27, 2021, (ISSN 0230-8312) ISBN: 9789632699615

7. **Molnár, Á.**, Homoki, D., Bársony, P., Stündl, L., Gálné Remenyik, J., Fehér, M.: A medenceszín hatása a csapósügér (Perca fluviatilis) termelési és élettani paramétereire.
Halászatfejlesztés. 37, 16-17, 2020. ISSN: 1219-4816.

List of other publications

Hungarian scientific articles in Hungarian journals (1)

8. Kertész, A., Bereczki, G., Dajka, B., **Molnár, Á.**, Bársony, P., Fehér, M.: A különböző fényspektrumok hatása az intenzíven nevelt harcsa (Silurus glanis) termelési és élettani paramétereire.
Halászatfejlesztés. 39, 41-43, 2022. ISSN: 1219-4816.

Foreign language scientific articles in Hungarian journals (2)

9. Kovács, L., Minya, D., Homoki, D., Toviho, O. A., **Molnár, Á.**, Fehér, M., Stündl, L., Bársony, P.: Comparision of growth of mature all-female and mixed-sex Common carp (Cyprinus carpio L.) stocks in RAS.
Agrártud. Közl. 1 (1), 65-68, 2020. ISSN: 1587-1282.
DOI: http://dx.doi.org/10.34101/actaagrar/1/3748

10. Homoki, D., Minya, D., Kovács, L., **Molnár, Á.**, Balogh, K., Bársony, P., Fehér, M., Kövics, G., Stündl, L.: Comparison of the technological background of aquaponic systems.
Agrártud. Közl. 1, 47-52, 2020. ISSN: 1587-1282.
DOI: http://dx.doi.org/10.34101/actaagrar/1/4511

Foreign language scientific articles in international journals (2)

11. Herman, P., Fehér, M., **Molnár, Á.**, Harangi, S., Sajtos, Z., Stündl, L., Fábián, I., Baranyai, E.: Iron and Manganese Retention of Juvenile Zebrafish (Danio rerio) Exposed to Contaminated Dietary Zooplankton (Daphnia pulex)-a Model Experiment.
Biol. Trace Elem. Res. 199 (2), 732-743, 2021. ISSN: 0163-4984.
DOI: http://dx.doi.org/10.1007/s12011-020-02190-z
IF: 4.081

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

12. Sajtos, Z., Fehér, M., **Molnár, Á.**, Stündl, L., Naszályi Nagy, L., Martins, J. C., Harangi, S., Magyar, I., Fehér, K., Baranyai, E.: The retention of Zr from potential therapeutic silica-zirconia core-shell nanoparticles in aquatic organisms.
Environmental Nanotechnology, Monitoring & Management. 16, 1-10, 2021. ISSN: 2215-1532.
DOI: http://dx.doi.org/10.1016/j.enmm.2021.100572

Hungarian abstracts (2)

13. Homoki, D., Kovács, L., Minya, D., **Molnár, Á.**, Fehér, M., Kövics, G., Stündl, L.: Különböző bazsalikom (Ocimum basilicum L.) fajták akvapóniás vizsgálata.
In: A XLIV. Halászati Tudományos Tanácskozás kiadványa = Proceedings of the 44th Scientific Conference on Fisheries & Aquaculture. Szerk.: Nagyné Biró Judit, NAIK HAKI, Szarvas, 31-33, 2020, (Halászatfejlesztés, ISSN 1219-4816 ; 37) ISBN: 9789637120428

14. Kovács, L., Minya, D., Homoki, D., **Molnár, Á.**, Fehér, M., Stündl, L., Bársony, P.: Monoszex ikrás pontyállományok (Cyprinus carpio L.) teljesítményvizsgálata.
In: XLIII. Halászati Tudományos Tanácskozás, Nemzeti Agrárkutatási és Innovációs Központ Halászati Kutatóintézet, Szarvas, 102-104, 2019, (ISSN 0230-8312)

Total IF of journals (all publications): 7,611
Total IF of journals (publications related to the dissertation): 3,53

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

15 June, 2023