Theses of Doctoral (PhD) Dissertation

Optimalization of the European perch (*Perca fluviatilis*) Digestion and the Utilization of the Yellow mealworm in the Perch Nutrition

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1. INTRODUCTION

The world population is projected to reach 9 billion in 2037 and 10 billion in 2057 from the current 7.9 billion (WORLDOMETER, 2022), and this poses a new challenge to researchers (BORIDEAN ET AL., 2020b). To provide enough food for the increasing population, it was projected that the current food production will need to almost double; while resources needed for increased production such as land and water resources are becoming scarce (VAN HUIS ET AL., 2013). Hence, there is a need to find alternative and sustainable ways of growing food (DA ROSA MACHADO & THYS, 2019). World population increase and increase in consumer demand for protein will sustainably make the production of protein a challenge in the future (ZHAO ET AL., 2016).

An increase in demand for fishmeal and soy as a result of fast-growing aquaculture production and consequently increase in price has led to new research in the use of insect protein in aquaculture, poultry, and pig feed (VAN HUIS ET AL., 2013). To face the challenge of protein production there is need for an alternative protein source that requires fewer resources to produce effectively and efficiently, whether it is for direct consumption or livestock feed production. The aquaculture industry is especially in dire need of an alternative protein source to produce complete feed for commercial aquaculture species because of the fast growth in the industry. Other than the economics and sustainability issues, the aquaculture industry is under pressure from consumer that are now more conscious not just of the quality of fish they eat but also of the ethics and environmental impact the production of the fish has. The aquaculture industry depends on capture fisheries for fishmeal, and this raises the question; How ethical, economical, or sustainable is the use of fish to produce fish? Although the aquaculture industry uses soybean meal as an alternative protein source there is still a need for animal protein sources, especially in carnivore species that are adapted to consumption of animal sources in their natural habitat.

Edible insects are consumed in parts of the world like Africa, Asia, and South America, but their consumption is still not widely accepted in the Western world. Because of the low level of acceptance, care must be taken in using it as the protein source in fish production because the fish will eventually be consumed by humans.

The use of edible insects has however been encouraged by FAO, and this has helped improve the acceptability of edible insects. Edible insect importance and suitability as an alternative protein source can be linked to their high nutritional value, less production requirement, low environmental impact, and high feed conversion efficiency compared to other livestock. Although there are over 2000 edible insects documented there are only 7 species (yellow mealworm, lesser mealworm, black soldier fly, housefly, house cricket, banded cricket, and field cricket) approved for use in pet, fish, poultry, and swine feed in the EU (EC2021/1372).

The yellow mealworm (YM) is one of the most bred and traded of these seven insects in the EU, it is an omnivore species and can be raised on any substrate, they are now being reared on "former foods". They can convert waste to useful protein mass, but this practice is subject to regulations for the country or region. They have been used as fishmeal replacements in several fish species, but this is mainly for research purposes and has yielded good outcomes. YM has low saturated fatty acids and hence higher unsaturated fatty acid, studies have however shown that the growth rate and nutrient composition of insects can be influenced by their diet providing an opportunity to alter the nutrient composition of insects through their feed. Common duckweed *Spyrodela polyrhiza* is a fast-growing floating water plant with a high protein content of about 30% and it has been considered an innovative feedstuff in animal nutrition, especially for fish and poultry. There is still an ongoing search for high-value feed for the mass production of the yellow mealworm. The modification of the nutrient composition of the yellow mealworm based on the diet composition provides an opportunity to explore the effect of duckweed as a feeding substrate in yellow mealworm production.

The order Perciformes consists of about 240 species, they are found in fresh and brackish water mostly in the northern hemisphere. They are easily distinguished from the trout and salmon family by the absence of an adipose fin. The European perch is a Percidae and the closest relative are the pikeperch and the yellow perch. The European perch is a carnivore fish, they feed on zooplankton and insect larvae at the larval stage and as they grow, they begin to feed on insect crustaceans and eventually other fishes. This fish has the potential to become an important species in European aquaculture especially because of the quality of their flesh. The flesh of this fish is white, has a mild taste, and has no bones, overall, the quality of the flesh is top-shelf quality, and it commands a premium price. The European perch has a low feeding rate, and the table size is about 150g, when reared in a RAS it can reach this size in about 8 months. Currently, there is no specific feed developed or tailored to the nutritional a need of this species, hence there is need for one.

It has been established from literature that there is a need for an alternative protein source to supplement the current traditional protein source in feed formulation.

Edible insect is an innovative protein source in the feed industry, and we identify the opportunity to use yellow mealworm in the formulation of European perch feed since it is a new species with a lot of potential in European aquaculture. The feeding habit of this species makes it a good candidate for the use of yellow mealworms.

On the other hand, providing a nutritionally balanced feed to fish in the culture system is not enough for a good fish production cycle. The process of digestion also plays a key role in determining the success of feed, an understanding of the digestion process of the European perch species is therefore important. The knowledge of the feedstuff, European perch digestion, and the effect of rearing conditions on digestion is important for the effective optimization of the nutrition of this species.

1.1 Justification

The European perch is still currently undergoing domestication, and its contribution to European aquaculture is still very low. Most of the perch production comes from capture fisheries because it is limited by its slow feeding rate. There is a need for the production of a feed suited to the digestion process of the European perch and the protein source for this feed must be of very high quality and an animal protein source. In the wild, the European perch already consume up to 50% insects as part of their diet, this makes it a suitable species for insect meal-based feed. Producing insect meal-based European perch feed reduces the environmental impact of feed production and makes it more sustainable. The process of this research is approached from two major perspectives, we divided the topic into major parts the yellow mealworm and European perch. First, we explored YM as shown in *Figure 1*.

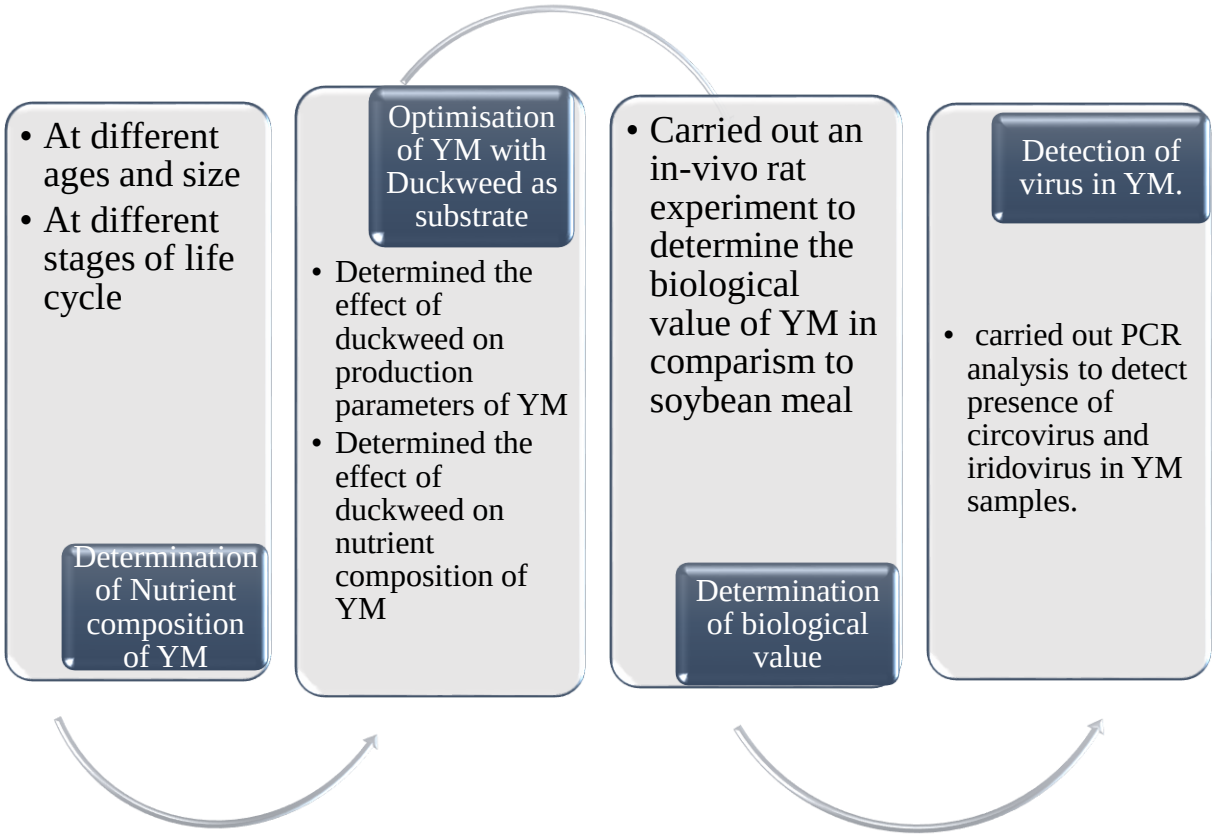

Figure 1: Workflow for mealworm experiment.

For the European perch, we explored different aspects of the digestion process, the European perch from the colder countries in the north are usually bigger because of this we analysed the enzyme activity of the European perch at different rearing temperatures lower than the reported 23 °C optimal temperature. The workflow for the experiments of the European perch is shown in *Figure 2*.

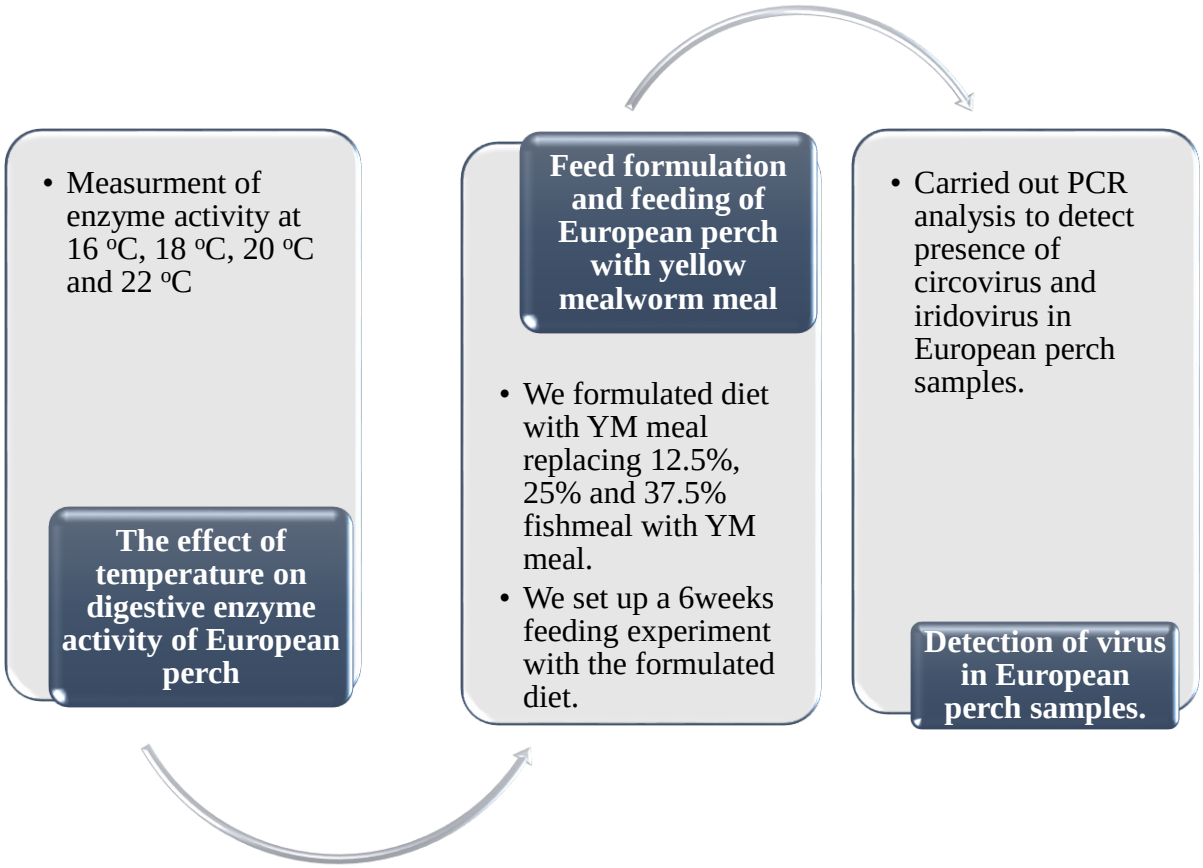

Figure 2: European perch Experiments workflow

1.2 Objectives

- To determine the biological value of yellow mealworm using an Animal model
- To determine the nutrient composition of yellow mealworms at different ages sizes and stages of life cycle.
- To optimize the production parameters and nutrient composition of yellow mealworm using Duckweed (*Spirodela polyrhiza*).
- To determine the effect of different rearing temperatures on the digestive enzyme activity of European perch.
- To determine the effect of replacing fishmeal with yellow mealworm meal on the production parameters of European perch.

2. MATERIALS AND METHOD

2.1. Yellow mealworm experiments.

2.1.1 Determination of biological value of the Yellow mealworm meal.

To determine the biological value of the yellow mealworm (YM) meal, we carried out an in-vivo rat experiment with Wistar rats, to compare the protein efficiency ratio (PER), net protein ratio (NPR), and true digestibility (TD) of YM meal with soybean meal. The goal of this experiment is to determine the suitability of YM meal as a protein source using soybean meal as a reference point. The experiment was carried out at the Food Science research institute ÉKI by carrying out the feeding trial for 10 days. The rats were weighed at the beginning of the experiments and divided into 5 rats/groups. Each group represents 1 treatment, and each treatment has 2 replicates.

After lyophilisation and grinding the yellow mealworm (YM) semi-synthetic diet was prepared. The rats were fed with an iso-energetic and iso-protein semi-synthetic diet containing 10% of protein. Two different amounts of mealworms were added to the test diets. In one group, 50% of the protein content, in the other group 25% of the protein content comes from YM, while the rest of the protein comes from the extracted soy.

In the soy control group, the protein consists of only extracted soybeans. The determination of metabolic and endogenous protein was determined by feeding a separate group a diet containing 4 percent egg protein (FMK).

The individual weight of experimental rats was measured at the beginning of the experiment, we ensure that among the average weight of each group, there was no significant difference at the start of the experiment (78.8 g). Feed and water were given ad-libitum and feed consumption was determined by weighing the remaining feed for each group at the end of each day. Individual weight was measured daily throughout the experiment to determine the daily weight gain. We calculated the total protein consumption per animal from the protein content of the feed and total feed consumption. We collected the faeces of test animals daily, weighed and determined the protein content and nitrogen content.

Statistical Analysis

Results were analysed with IBM SPSS 22.0. The homogeneity of the data was checked by Levene test. One-way analysis of variance (ANOVA) Tukey test ($P<0.05$) was used to evaluate the results.

2.1.2. Determination of the nutrient composition of Yellow mealworm at different ages and stages of life cycle.

The yellow mealworms (YM) were fed a mixture of semolina, flour, and oat flakes. Potatoes, carrots, and apples were used as water sources. All stock received uniform feed and there was no variation in the type and quantity of feed received. The feed is replaced every 2 weeks and the vegetables are replaced every 2 days to prevent mold.

The mealworms and darkling beetles were kept in a transparent container 12 and 24 inches respectively. The temperature was maintained at 20-26 °C. Eggs are collected from the darkling beetle box every 2 weeks and reared for 105 days. 3 different batches were maintained for this experiment.

Samples were collected at weeks 8, 10, and 12. The YM were separated by size into large-sized and small-sized groups by passing them through a sieve (mesh 10; diameter: 2 mm) at week 8. The ones that passed through were considered small, while the ones that remained in the sieve were the large ones. The large- and small-sized groups were maintained in separate containers. All samples were collected in three replicates; the sample collection was limited to three replicates because the temperature in the insect room rose during the summer months.

Throughout the sample collection 200 pcs of each group were counted and weighed to determine the average body weight. Samples of pre-moult YM, newly moulted YM, cuticles, and pupae were collected at week 12. We determined the body mass/surface area ratio by measuring the length and circumference of 50 mealworms from each group for calculation of the surface area. Samples were stored at - 20 °C until analysis.

Samples were analyzed to monitor changes in nutrient composition with age and size. The dry matter was determined in gravimetric measurement after drying (AOAC, 2000). The weight was determined as an average of two measurements. Crude protein content was determined according to the Kjeldahl method (AOAC, 2000). Total crude fat was determined gravimetrically after acid hydrolysis and solvent extraction (AOAC, 2000). A laboratory-scale Soxtec unit (Soxtec 2050, Foss Electric, Denmark) was used to extract the fat component from the sampled powders. Samples (5 ± 0.03 g each) corresponding to each sample were put into tarred cellulose thimbles which were then loaded in the Soxtec (the unit temperature at 135 ºC). Extractions were carried out in duplicates. After completion of the extraction process, sample cups were dried in a pre-heated oven at 100 °C for 10 min and transferred to a desiccator and cooled to ambient temperature.

Crude fibre was determined by Fibertec method (FOSS, Hilleroed, Denmark) according to ISO 6865:2000

Chitin was determined according to HAHN ET AL. (2018) as subtraction of the acid detergent lignin content (ADL) from the acid detergent fibre (ADF) that both measured by gravimetric method according to ISO 13906:2008 standard method.

Statistical Analysis

Results were analysed with IBM SPSS 22.0. The homogeneity of the data was checked by Levene test. One-way analysis of variance (ANOVA) Tukey test ($P<0.05$) was used to evaluate the results of growth. Mean values of nutrient composition were compared using univariate analysis of variance Tukey test ($P < 0.05$).

2.1.3.1. Optimization of Yellow mealworm nutrient composition using feeding substrate.

The purpose of this study was to determine the effect of feeding yellow mealworm (YM) with a substrate containing a mixture of semolina and duckweed at different percentages on the production parameters and nutrient composition of YM. Yellow mealworms used for this experiment were obtained from a commercial farm and transported to the Aquaculture Laboratory of the University of Debrecen. The experiment was set up in a biological chamber at the laboratory at 25 °C and 67% humidity.

Duckweed was harvested form the pond at the Aquaculture laboratory of the University of Debrecen, they were dried in a dehydrator and milled into fine powder.

Semolina was purchased from the store and the nutrient composition of both duckweed and semolina was determine.

A feeding substrate was prepared with different composition of duckweed and semolina.

There were 5 treatments in the experiment (S, S75D25, S50D50, S25D75, and D), with 4 replicates each. The experiment was set up in a transparent 0.15-liter box. 40 mealworms were stocked per box, biomass=2.6 g (Average body weight= 0.065).

The experiment lasted 6weeks and we measured the growth rate, FCR and checked the survival rate every week for the duration of the experiment. The larvae were then stored at -20°C until further analysis. The production parameters were calculated as follows;

$$\boldsymbol{Growth\ rate} = \frac{\boldsymbol{weight\ of\ YM\ at\ end\ of\ experiment}}{\boldsymbol{weight\ of\ YM\ at\ start\ of\ experiment}} \times \mathbf{100}$$

$$\boldsymbol{FCR} = \frac{\boldsymbol{Feed\ consumed}}{\boldsymbol{weight\ gain}}$$

$$\boldsymbol{survival\ rate} = \frac{\boldsymbol{number\ of\ yellow\ mealworm\ at\ end\ of\ experiment}}{\boldsymbol{number\ of\ yellow\ mealworm\ at\ start\ of\ experiment}} \times \mathbf{100}$$

At the end of the experiment the yellow mealworm (YM) was analysed for different nutrient compositions (DM, CP, CF, FB, ash and NFE), Dry material was determined in gravimetric measurement after drying (AOAC, 2000). The weight was determined as an average of two measurements. Crude protein content was determined according to the Kjeldahl method (AOAC, 2000).

Total crude fat was determined gravimetrically after acid hydrolysis and solvent extraction (AOAC, 2000). Crude fibre was determined by Fibertec method (FOSS, Hilleroed, Denmark) according to ISO 6865:2000.

Chitin was determined according to HAHN ET AL., 2018 as subtraction of the acid detergent lignin content (ADL) from the acid detergent fibre (ADF) that both measured by gravimetric method according to ISO 13906:2008 standard method. Crude ash was determined gravimetrically (AOAC, 2000). NFE was calculated by subtracting the sum of DM, CP, CF, fibre, and ash content from 100.

Statistical Analysis

Results from the calculation of the production parameter were plotted on a graph using Microsoft Excel 2016. Results were analysed with IBM SPSS 22.0 of nutrient composition. The homogeneity of the data was checked by Levene test. One-way analysis of variance (ANOVA) Tukey test ($P<0.05$) was used to evaluate the results.

2.2 European perch experiments.

The purpose of this experiment is to gain insight into the changes in enzyme activity of European perch based on the rearing temperature.

The choice of temperature used in this experiment was based on the information from literature that European perch captured from the colder countries attain a larger size compared to warmer countries. This, therefore, informed our choice of temperatures lower than the optimal temperature (23 °C) reported.

European perch were maintained in a 150-liters aquarium at the Fish Biology Laboratory on the University of Debrecen Agrar Campus. They were maintained at four different temperatures 16 °C ± 0.2, 18 °C ± 0.2, 20 °C ± 0.2, and 22 °C ± 0.2 which represented the treatment. The different temperatures were maintained using individual heaters in each aquarium (Tetra GmbH Herrenteich, 78 D-49324 Melle, Germany- HT 100). Each temperature had 4 replicates each with 25 fishes in each aquarium making a total of 100 fishes per treatment. Fish used were of uniform stock hence uniform age. Fish weight range from 45- 60 g. The experimental design was completely randomized.

Experimental fish were kept in the aquarium without feeding for 48 hours for acclimatization and emptying of the digestive tract before the commencement of feeding. The fish were fed with commercial feed containing 48% protein and 13% fat content. The fish were fed a total of 1.5% of their body mass for the experiment. The fish were fed twice, to ensure that the whole digestive tract was filled with feed and ensure that there will be detectable enzyme activity. At first feeding, the fish were fed 0.75% of their body mass, after 6 hours the fish were fed another 0.75% of their body mass.

The fish were collected from the rearing tank and euthanized. Each fish was weighed before dissecting and the weight of the whole digestive tract was also measured. Samples in each replicate were pooled. Samples were stored at -80 °C for transportation and -20 °C until processing into analytical samples.

Analysis was carried out at the Food Science research institute ÉKI. Digestive enzyme extracts were prepared from the gastrointestinal tract of *Perca fluviatilis* with an approximate weight of 60 g from each replicate. Before the preparation of enzyme extract, they were thawed on ice batteries, at approximately 4 °C. We separated the stomach and intestine apart from each other at the line of the pylorus. The stomach was cut longitudinally, then its content was washed out with 1 ml (in the case of each fish specimen) of distilled water adjusted with 0.1 M HCl (pH 2.5) (DE MELO OLIVEIRA ET AL., 2014) to prepare the crude enzyme extract.

The contents of the intestine were pushed out manually, and then the intestine was cut longitudinally and washed with 1 ml (in the case of each fish specimen) of the 50 mM Tris-HCl buffer containing 0.5 M NaCl and 20 mM CaCl (pH 7.8), recommended by FUCHISE ET AL. (2011). The obtained mixture of the stomach/or intestine content (includes digested food and

digestive fluids) and the applied buffer represents the crude digestive enzyme extracts maintaining the fish's physiological conditions.

The gained 25-25 gastric or intestinal enzyme extracts for each ambient temperature (16 °C, 18 °C, 20 °C, 22 °C) were pooled to have representative samples that can be used for further enzyme extracts preparation. The preparation of the crude enzyme extracts was performed on ice batteries as a precaution to keep the samples chilled, to avoid enzyme denaturation and autolysis (DE MELO OLIVEIRA ET AL., 2014).

The crude enzyme extracts were homogenized (30 sec) with Ultra-Turrax T25 (Janke & Kunkel Labortechnik) in beakers (DE MELO OLIVEIRA ET AL., 2014).

The respective gastric and intestine suspensions were mixed (BioSan Multi Bio RS-24) for 1 hour in a refrigerator at 4 °C for homogenizing the samples and to enhance proper extraction of the enzymes. The homogenized samples were centrifuged at 4100 rpm for 10 minutes at room temperature. Furthermore, the obtained supernatants were transferred into 2-2 ml Eppendorf tubes and then were centrifuged again for 15 min at 4 °C at 12000 rpm. This way, the cell debris, lipids, feed, and other materials could be discarded from the supernatant. The remaining feed residue was stored lyophilized (digested feed) and will be used to determine in vivo biological accessibility of protein, lipid, and carbohydrate. The supernatants, which contained the enzymes, were stored at -20 °C until enzyme activity measurements.

Protein measurement was done according to BRADFORD (1976) while pepsin, trypsin, total alkaline protease, lipase, and amylase activities were measured according to the protocol described by MINEKUS ET AL., (2014) with details in the electronic supplementary material (ESI). Pepsin and amylase activities were determined using the spectrophotometric stop reaction method, trypsin and total alkaline protease activities were determined using the continuous spectrophotometric rate determination method while lipase activity was determined according to the pH-stat reaction method.

Statistical Analysis.

Shapiro-Wilk Normality Test and Bartlett Test of Homogeneity of Variances in R (Team R Core 2021) was used to verify the normality and homogeneity of variances of our results.

ANOVA tests were applied (TEAM R CORE 2021) according to ALKARKHI AND ALQARAGHULI (2018) to find out whether the environmental temperature has a significant effect on pepsin, trypsin, total alkaline proteolytic, lipase and α-amylase activity, respectively.

Tukey Honest Significant Difference method in R (TEAM R CORE 2021) was used to determine whether there is a significant difference between the effect of 16 °C, 18 °C, 20 °C and 22 °C regarding pepsin, trypsin, total alkaline proteolytic, lipase and α-amylase activity.

2.2.2 European perch feeding experiment with Yellow mealworm meal diets.

This experiment was carried out to determine the effect of replacing fishmeal with YM meal at different percentages on the production parameters of European perch.
European perch with average body weight 40g was used for the experiment, the fishes were reared from larvae stage at the Aquaculture Laboratory of the University of Debrecen. The experiment was carried out in a RAS system, 40 pieces of fish were stocked in each 350liter circular tank. The water parameters were temperature: 20±0.8 °C DO: 7.5±1.4 mg/l, PH 6.7±0.4, ammonia 0.02±0.003mg/l.
The experiment was carried out for a period of 6weeks, sampling was done every week for the duration of the experiment. The fish were feed 1.5% body weight in 2times daily feeding. They were fed in the morning and in the evening.
Four experimental diets were prepared for this experiment. The control diet did not contain YM meal while the other 3 experimental diets contained 12.5%, 25% and 37.5% YM meal respectively. Live YM was purchased from a commercial farm, it was dehydrated at 43 °C and milled. The different feed ingredients were homogenised using a mixer and the mixture was made into 3 mm pellets using a pelletizer after which it was dried in the dryer.

Statistical Analysis.

Results were analysed with IBM SPSS 22.0. The homogeneity of the data was checked by Levene test. One-way analysis of variance (ANOVA) Tukey test ($P<0.05$) was used to evaluate the results.

3. RESULTS

3.1 Yellow mealworm experiments.

The growth of test animals is expressed in our result as the average daily weight and average weight gain of test animals in the different groups.

The result of the biological value determination is shown through the PER, NPR, and TD values. It is important for us to determine the biological value to know the nutritive value, quality, and bioavailability of the protein in yellow mealworm meal as there have been suggestions that although insects have high protein content the nutritive value could be low due to the presence of chitin. We determined the NPR as a backup for the PER as it is recommended (MITCHELL ET AL., 1989).

Protein Efficiency Ratio (PER)

Figure 1. shows the PER value for the test diet, the tendency observed was that the PER increase with the increase in inclusion level of yellow mealworm meal. However, there was no statistical difference ($p<0.05$) between the PER of all 3 test diets.

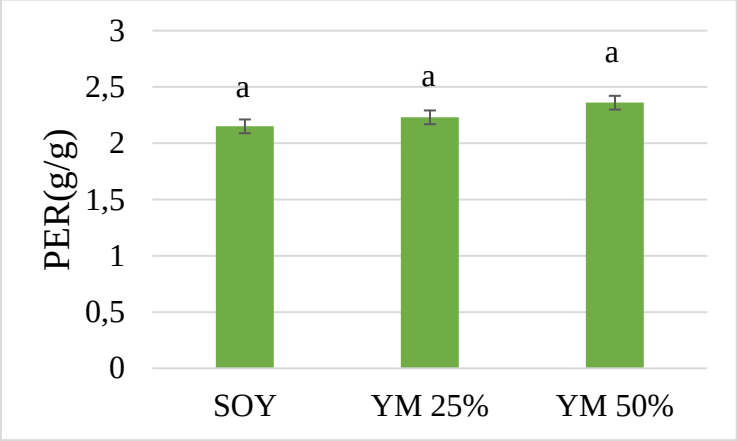

Figure 1: Protein efficiency ratio of wistar rat fed experimental diets.

Soy: 100% soy diet

YM 25%: 25% yellow mealworm meal diet

YM 50%: 50% yellow mealworm meal diet

Net protein ratio (NPR)

Figure 2. shows the NPR of the test groups, the tendency observed was that the NPR increase with the increase in inclusion level of yellow mealworm meal There was no statistical difference ($p<0.05$) between the test groups.

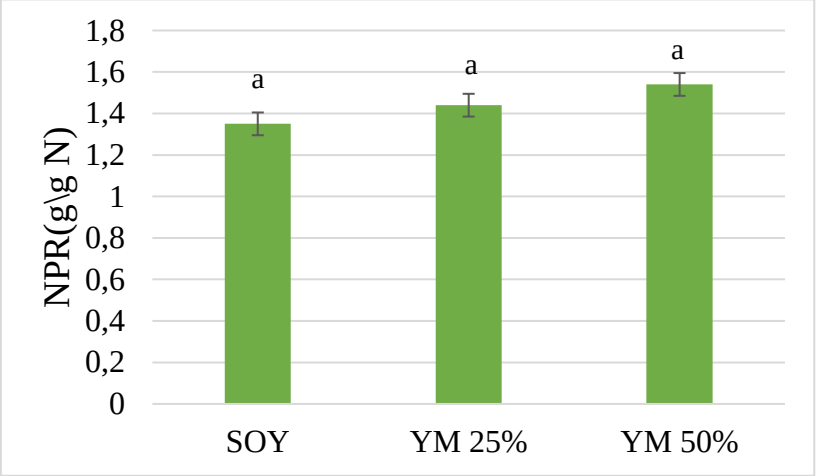

Figure 2: Net Protein Ratio of wistar rat fed experimental diets.

Soy: 100% soy diet

YM 25%: 25% yellow mealworm meal diet

YM 50%: 50% yellow mealworm meal diet

True digestibility

Figure 3. shows the true digestibility of Wistar rat groups fed different experimental diets containing soy, YM 25%, and YM 50% as protein sources. There was no trend observed between the TD of the different groups. There was no statistical difference ($p<0.05$) in the TD of all test diets.

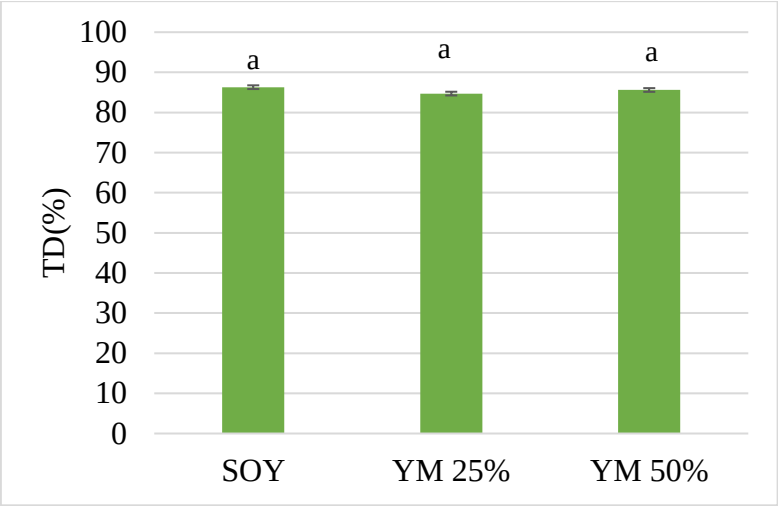

Figure 3: TD of Wistar rats feed experimental diets.

Soy: 100% soy diet

YM 25%: 25% yellow mealworm meal diet

YM 50%: 50% yellow mealworm meal diet

Discussion

The rats fed diets with YM meal diets had the best liveweight of all the treatments. The lowest daily weight was observed in FMK (control) as they were only provided protein for maintenance. The average weight of gain test diets was significantly higher than FMK (control), YM 50% had the highest weight gain. Although YM 50% had the highest average weight gain there was no significant difference ($p<0.05$) among the groups (soy, YM 25%, and YM 50%). This shows that the YM 25% and YM 50% had no adverse effect on the growth of the test animals, and it is comparable to soy as a protein source.

When we considered the biological value of the test diets using the PER and NPR the result followed the same trend YM 50% had the highest PER and NPR while soy had the lowest. All the diets were however statistically the same, showing that the protein quality of the diet with 50% replacement of soy with YM meal produced feed with the same protein quality.

The TD of all 3 diets did not differ statistically soy had the highest TD, the result did not follow a particular trend like PER and NPR. These results show that replacing 25% and 50% soy with yellow mealworm meal in the feed kept the quality of protein in the feed and growth response of the test animals the same as when 100% soy was used as the protein source.

This information provides us with the basis for the formulation of our experimental diet with the aim of including YM meal as a protein source for the European perch. This guided our decision on the percentage inclusion of YM meals during the diet formulation.

3.1.2. Determination of the nutrient composition of yellow mealworm at different ages and stages of life cycle.

Surface area/body mass ratio, length and width of Yellow mealworm at week 12.

Table 1. displays the length, width, and surface area/body mass ratio (SA:BM) of yellow mealworms at week 12. There was no statistically significant difference between the ratios of large- and small-sized worms. Although there was no significant difference between the width values, the average length of the large-sized mealworm stock was statistically higher than that of the small-sized stock.

Table 1:

Average surface area (SA)/ body mass (BM) ratio, length, and width of yellow mealworm at week 12.

	Large	**Small**
SA:BM	1:55±9[a]	1:62±24[a]
Length (cm)	2.7±0.20[a]	1.9±0.10[b]
Width (cm)	0.4±0.05[a]	0.3±0.05[a]

Means in the same row with different superscript differ significantly ($p < 0.05$). SA: surface area BM: body mass

Nutrient composition of yellow mealworm at different ages and sizes (%).

With age, both the large- and small-sized mealworms' dry matter, fat, and chitin contents declined while their protein contents increased (*Table 2*). The sizes rather than the ages show significant variations.

Independent of age, the dry matter contents of the large yellow mealworms were consistently and significantly larger than those of the small-sized groups; nevertheless, the chitin and NFE contents of the small-sized groups were of significantly higher value. In all ages, the large-sized mealworm consistently has a larger ash content than the small size. In all samples, there was no discernible trend in the NFE.

Table 2:

Average nutrient composition of Yellow mealworm at different ages and sizes (%).

	Week 8		Week 10		Week 12	
	Large	**Small**	**Large**	**Small**	**Large**	**Small**
DM	38.60± 0.30[a]	36.23±0.40[b]	38.53±1.60[a]	36.00±1.22[b]	37.50±1.13[a]	35.63±1.25[b]
Crude Protein	43.70±0.50[a]	43.00±0.70[a]	45.23±2.57[a]	44.20±2.06[a]	45.13±2.38[a]	44.73±1.34[a]
Crude Fat	39.60±0.53[a]	39.33±0.77[a]	38.10±2.00[a]	37.93±1.74[a]	37.80±2.23[a]	37.90±1.71[a]
Fibre	11.53±0.61[a]	11.00±0.10[b]	11.83±0.15[a]	10.93±0.25[b]	12.13±0.25[a]	10.90±0.26[b]
Chitin	21.93±0.56[b]	23.53±0.50[a]	21.40±0.61[b]	23.10±0.55[a]	20.87±0.58[b]	22.50±0.56[a]
Crude Ash	3.65±0.08[a]	3.62±0.05[a]	3.64±0.05[a]	3.62±0.05[a]	3.64±0.01[a]	3.60±0.06[a]
NFE	1.52±0.03[b]	3.05± 0.10[a]	1.2±0.01[b]	3.32±0.09[a]	1.3± 0.13[b]	2.87±0.02[a]

Means in the same row with different superscripts differ significantly ($p < 0.05$).

When examining the DM, CP, CF, fibre, chitin ash content and NFE contents, the nutrient composition of mealworms showed no significant difference among the age groups. The 36.5% reported by YI ET AL. (2013) was within the range of the DM content we observed, though the DM content recorded was slightly greater than the 32.2% reported by JONES ET AL. (1972). The NFE we observed was lower than what was reported by the same study, while the ash content we measured was higher than what was reported by the studies of FINKE (2002). Contrary to what GHOSH ET AL. (2017) stated, the ash content measured in our study was lower while the NFE was higher. Contrary to DM, fibre, chitin, and NFE, which differed significantly, the CP and CF values of the various size groups did not show significant differences. The large-sized group had higher levels of DM and fibre than the small-sized ones, while the smaller sizes had higher levels of chitin content and NFE. This can be explained by the fact that smaller individuals have a higher surface area to body mass ratio than larger individuals, who have a lower surface area to body mass ratio. This indicates that the exoskeleton makes up a larger percentage of the smaller size than the larger size. Increasing the average body weight during harvest offers the chance to boost DM and decrease chitin content. The ash content was lower than 4.9% and 4.0%, respectively but higher than the 2.4% (GHOSH ET AL., 2017).

3.1.3 Optimization Yellow mealworm nutrient composition using feeding substrate.

This chapter the effect of inclusion of duckweed in the feeding substrate of European perch can be shown. First, we show the effect of the different composition on production parameters and then the modifications that can be seen in the nutrient composition. It is important that from the following result that the effect of duckweed on the production parameters need to be considered together in order to draw accurate conclusion.

Figure 4. shows the average body weight of yellow mealworms in the different treatments. S75D25 had the highest average body weight, followed by S50D50. There was no statistical difference between the average body weight of yellow mealworms fed diet S and S25D75.

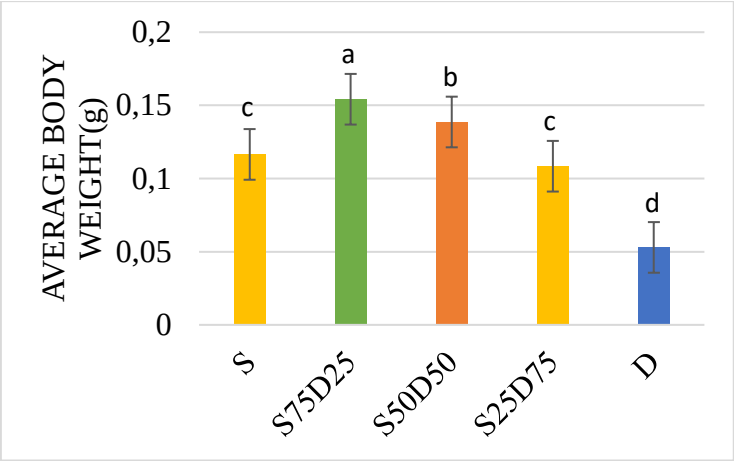

Figure 4: Average body weight of yellow mealworm fed different inclusion level of duckweed.

S: 100% semolina

S75D25: 75% semolina + 25% duckweed

S50D50: 50% semolina +50% duckweed

S25D75: 25% semolina + 75% duckweed

D: 100% duckweed

Figure 5. shows the FCR of yellow mealworms reared on different substrates, there was no statistically significant difference in the FCR observed in yellow mealworms fed diets S, S75D25, and S50D50.

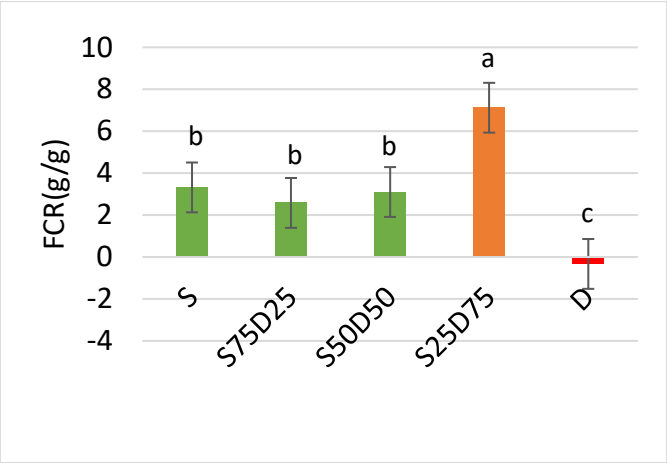

Figure 5: FCR values of yellow mealworm fed different duckweed inclusion level.

S: 100% semolina

S75D25: 75% semolina + 25% duckweed

S50D50: 50% semolina +50% duckweed

S25D75: 25% semolina + 75% duckweed

D: 100% duckweed

Table 3 shows the nutrient composition of the yellow mealworm, the CP, CF, FB, and ash were significantly affected by every 25% inclusion of duckweed. The chitin did not show a trend and the NFE was the same for all treatments.

Table 3:

Average nutrient composition of yellow mealworm fed different duckweed inclusion level (%).

	S	S75D25	S50D50	S25D75	D
Dry Matter	38.3±0.26^{b}	39±0.36^{a}	37.3±0.10^{c}	37.3±0.02^{c}	37.7±0.17^{d}
Crude protein	45.7±0.18^{e}	50.8±0.22^{d}	55.8±0.18^{c}	61.1±0.18^{b}	69±0.22^{a}
Crude fat	43.7±0.22^{a}	37.9±0.28^{b}	30.5±0.27^{c}	24.2±0.14^{d}	14.8±0.20^{e}
Crude fiber	7.4±0.22^{e}	7.9±0.18^{d}	9.4±0.18^{c}	10±0.18^{b}	11.1±0.18^{a}
Crude ash	2.8±0.18^{e}	3.4±0.14^{d}	4.2±0.18^{c}	4.6±0.12^{b}	5.0±0.23^{a}
Chitin	21.5±0.17^{a}	20.0±0.12^{b}	21.5±0.28^{a}	21.8±0.20^{a}	20.8±0.12^{b}

Means in the same row with different superscripts differ significantly ($p< 0.05$).

S: 100% semolina

S75D25: 75% semolina + 25% duckweed

S50D50: 50% semolina +50% duckweed

S25D75: 25% semolina + 75% duckweed

D: 100% duckweed

Discussion

The highest average body weight was achieved with the S75D25 substrate composition, also the average body weight of the S50D50 was higher than that of S but below 50% inclusion of semolina, the average body weight was lower than that obtained from semolina. This is understandable because the yellow mealworm is a pest of stored grains and flour (BORDIEAN ET AL., 2020a), which are rich in carbohydrates. Semolina is rich in starch, which requires less energy to digest into absorbable nutrients.

The duckweed has a negative FCR and this was because this substrate contained only duckweed and the mealworm was not able to effectively utilize substrate and convert it to absorbable nutrients and eventually body mass. This can be seen when the average body weight data is considered together with the FCR, the mealworms fed the duckweed substrate did not increase in body weight rather the body weight reduced from what was measured at the beginning of the experiment. The FCR 2.58 obtained in S75D25 was higher than the 1.57 to 2.08 obtained in the studies of BORDIEAN ET AL. (2020b), in mealworms fed chicken feed and wheat bran but lower than the 4.42 obtained in mealworms fed willow leaf sunflower.

The FCR obtained in semolina and S50D50 was also lower than those obtained from mealworms fed willow leaf sunflower. The FCR of S75D25 (2.58) was similar to the FCR in chicken (expressed in Kg of feed/Kg of live weight) but the FCR of semolina and S50D50 was higher. All FCR in our experiment was lower than FCR in swine (4.5) and cattle (10) (BORDIEAN ET AL., 2020b).

The CP, FB, and ash content followed the same trend increasing with every 25%increase in duckweed inclusion while the CF decrease with every 25% increase in the duckweed composition in the substrate. The protein content of the D100% was the best, but this substrate had the worse production parameters. Considering both the production parameters and nutrient composition, the combination of 25% duckweed and 75% semolina was the most suitable substrate to significantly improve the production parameters and Nutrient composition. These results support what has been reported in other studies that the nutrient composition of yellow mealworm can be enhanced by high nutritional value feedstuff (BORDIEAN ET AL., 2020b).

3.2. Nutrition experiments with European perch.

3.2.1. The effect of temperature on digestive enzyme activity of fish

Figure 10. shows the changes in enzyme activities at different rearing temperatures the enzyme activity increased at 18 °C, peaked at 20 °C and declined at 22 °C. One of the main factors that determine protein bioaccessibility is digestive enzyme activity. As the ambient temperature influences enzyme activity, we investigated the effect of water temperature (16 °C, 18 °C, 20 °C and 22 °C) on digestive enzyme activity of fish.

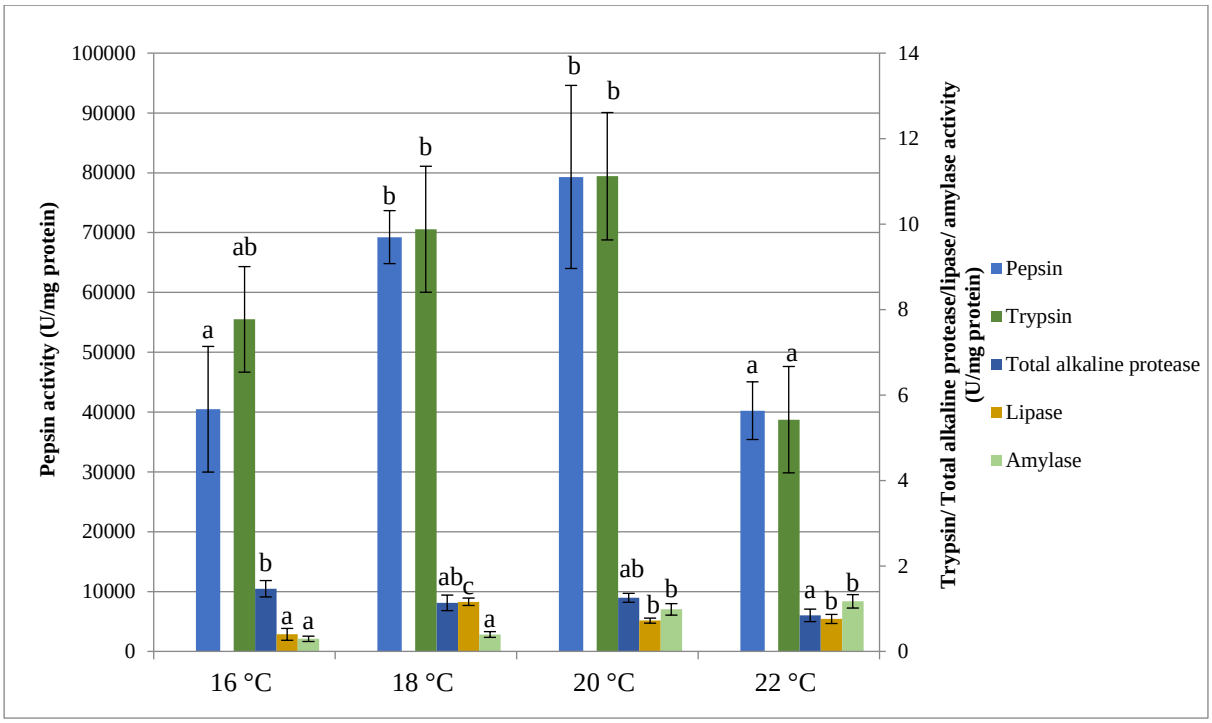

Figure 10: Digestive enzyme activity measurements of perch (*Perca fluviatilis*) kept at different water temperature.

Different letters designate significant difference (TukeyHSD method; $p<0.05$). The left axis of this figure represents pepsin activity, while the activities of the other enzymes are shown on the right axis.

Discussion

Our hypothesis that even a slight change in water temperature (between 16 °C and 22 °C) can significantly influence digestive activity was proven to be right. Still, the maximal activity (in this temperature range) was not the same for all enzymes. When choosing the optimal temperature, we must take into consideration the activity of all measured digestive enzymes (pepsin, total alkaline proteolytic, trypsin, lipase, α-amylase). Summarizing our results, we can say, that the ambient temperature affected all investigated digestive enzyme activities significantly. Pepsin and trypsin activities were the highest at 18 °C and 20 °C. Total alkaline proteolytic activity was significantly higher at 16 °C than at 22 °C, but it did not differ significantly from the activity at 18 °C and 20 °C. According to these results, 18 °C and 20 °C are the most favourable for proteolysis.

Lipase activity was the highest at 18 °C, while 20 °C and 22 °C were more favourable to amylase activity. Thus, if the preponderant part of the feed is made of lipids, we recommend keeping the stock at 18 °C. From a technological point of view, 18 °C is more advantageous, because it is more stable, and has a better O_2 storing capacity because oxygen has greater solubility in colder water (HARVEY ET AL., 2011).

According to our results 18 °C and 20 °C seemed to be the most appropriate for maximal protease (pepsin, total alkaline proteolytic, trypsin) activity. The temperature dependence of trypsin activity at 16 °C and 20 °C, is not reflected in the tendency of total alkaline proteolytic activity (at pH 8.5) because the latter is made of several components.

We proved that rearing temperature (16 °C, 18 °C, 20 °C and 22 °C) influences digestive enzyme activity significantly. This fact should be also taken into consideration in perch industry management practices and during the evaluation of feed components with a static in vitro digestion model.

3.2.2. European perch feeding experiment with yellow mealworm meal diets.

The results shown in this chapter how the partial replacement of fishmeal with YM meal at different inclusion levels impacted the weight gain FCR and survival rate.

Table 4. show the weight gain, FCR, and survival rate of European perch fed with YM, there was no significant difference between all production parameters of all treatments compared to the control.

Table 4:

Average weight gain, FCR and survival rate of European perch.

	CONTROL	**YM 12.5%**	**YM 25%**	**YM 37.5%**
IBW	39.75 ± 0.76^{b}	40.98 ± 0.76^{ab}	41.39 ± 0.76^{a}	39.99 ± 0.76^{ab}
Weight gain(g)	16.1±0.53	16.2±0.41	16.3±0.53	16.2±0.56
FCR(g/g)	1.41±0.05	1.46±0.03	1.47±0.04	1.42±0.04
Survival rate (%)	99.4±1.4	99.7±0.6	99.8±0.3	99.5±1.1

Means in the same row with different superscripts differ significantly ($p< 0.05$).

IBW: initial body weight

Discussion

There was no significant difference between all production parameters when 12.5%, 25%, and 37.5% of fishmeal was replaced with YM meal except for the IBW but this did not reflect on the weight gain and FCR. This shows that replacing up to 37.5% of fishmeal had no effect on the growth of European perch, hence reducing the quantity of fishmeal required in producing feed. The more sustainable protein source YM meal can therefore be included in European perch feed, improving the sustainability of the protein source.

A similar study was carried out to check the growth performance of European perch fed the experimental diet with 25%, 50%, and 75% replacement of fishmeal with defatted YM meal. The results showed that there was no significant difference in the growth performance between the control diet and 25% defatted YM meal which was similar to the result observed in our study. At 50% and 75% inclusion of defatted YM meal had detrimental effects on the growth performance. This study associated the declining growth performance with the increasing chitin content with the increasing inclusion rate of defatted YM meal, which leads to reduced nutrient digestibility. The survival rate observed in our study was similar to the >98% reported in this study as well (TRAN ET AL., 2022).

Another study reported the effect of partial replacement of fishmeal (20%, 40% & 60%) with partially defatted BSF in European perch feed. The results from the studies show that growth performance was not affected at 20% and 40% inclusion which is consistent with the result from our study, but there was reduced growth performance at 60% inclusion. There is a unanimous conclusion to be drawn from the results of this study and that of STEJSKAL ET AL., (2020a) and TRAN ET AL., (2022) that insect meal inclusion rate above 50% has a detrimental effect on the growth performance of European perch.

The FCR obtained in the current study at 25% (1.47) is higher than the 1.19 obtained in the study of TRAN ET AL., 2022 at a 25% inclusion rate, this could be because defatted YM meal was used as opposed to the full-fat YM meal used in this current study.

4. NEW SCIENTIFIC RESULTS

1. The protein efficiency ratio, net protein ratio and true digestibility (biological value) of feed containing 100% soy as a protein source is statistically the same as the same feed when 25% and 50% of the soy is replaced by yellow mealworm meal in an in-vivo rat experiment. The protein efficiency ratio is 2.15g/g, 2.23g/g and 2.36g/g for 100% soy, 25% yellow mealworm and 50% yellow mealworm diet respectively. The net protein ratio is 1.35g/g, 1.44g/g and 1.54g/g for 100% soy, 25% yellow mealworm and 50% yellow mealworm diet respectively. The true digestibility is 86.3%, 84.7% and 85.6% for 100% soy, 25% yellow mealworm and 50% yellow mealworm diet respectively.
2. Yellow mealworm chitin content is affected by the size. The larger sized larvae had a chitin content of 21.93%, 21.40%, and 20.87% at week 8, week 10 and week 12 respectively and this significantly lower than the chitin content of the smaller sized larvae 23.53%, 23.10% and 22.50% at week 8, week 10 and week12 respectively.
3. Our result shows 25% duckweed and 75% semolina as a feeding substrate of yellow mealworm can increase the average body weight by 25% after 6weeks compared to a feeding substrate made up of 100% semolina. Similarly, 50% duckweed and 50% semolina substrate can improve the average body weight of yellow mealworm by 16.7 percent compared to 100% semolina.
4. 100% duckweed as a feeding substrate for yellow mealworm increased Crude Protein by 48.57 % while it reduced Crude Fat by 66.67%, the yellow mealworm larvae however did not grow on this feeding substrate.
5. Yellow mealworm meal can replace fishmeal in the diet of European perch up to 37.5% without any adverse effect on growth, feed conversion ratio and survival rate.
6. Our data shows that highest pepsin, and lipase activity can be achieved at temperature between 18 °c and 20 °c indicating an optimal digestion at this temperature. When rearing temperature was increased from 16 °c to 18 °c pepsin, and lipase activity increased by 71.06%, and 190% respectively. When the temperature was increased from 18 °c to 20 °c the pepsin increased by 14.53%.

5. PRACTICAL RESULTS

1. The rat in-vivo experiment provides information on the quality of the yellow mealworm protein and how it compares to soybean meal as a protein source, this provides feed producers with information that can serve as a guide when using yellow mealworm meal in feed formulation.
2. Results from analysis of YM nutrient composition can provide insight to commercial insect farmers that there is an opportunity to explore diets that can shorten the life cycle of YM as a result of faster and better growth without loss of quality in terms of nutrient composition. It also provides information to farmers regarding moult and premoult larvae, it informs farmers that it is futile to synchronise moulting in YM larvae as this does not affect the chitin content of the larvae.
3. Results from the optimization of YM nutrient composition and production parameters can be used by insect farmers to achieve faster, better growth while also improving the nutrient composition of YM by using duckweed as part of the feeding substrate.
4. The results obtained from the enzyme activity at different rearing temperatures can help European perch farmers reconsider the optimal rearing temperature in order to maximize protein digestion and ultimately effective utilization of feed, thereby reducing the cost of production. These results can also help farmers reduce the energy cost by reducing rearing temperature from 23 ℃ to 20 ℃.

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Candidate: Odunayo Abigeal Toviho
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10070972

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (2)

1. **Toviho, O. A.**, Kovács, L., Bársony, P.: Insect-based protein nutrition in the aquaculture sector: potential, current situation and challenges.
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3. **Toviho, O. A.**, Moutia, I., Pusztahelyi, T., Bársony, P.: Effect of Duckweed (Spirodela polyrhiza)-Supplemented Semolina on the Production Parameters and Nutrient Composition of Yellow Mealworm (Tenebrio molitor).
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IF: 1.748

Total IF of journals (all publications): 8,948
Total IF of journals (publications related to the dissertation): 7,2

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