# Production Performance, Nutrient Digestibility, Serum Biochemistry, Fillet Composition, Intestinal Microbiota and Environmental Impacts of European Perch (Perca Fluviatilis) Fed Defatted Mealworm (Tenebrio Molitor)

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# Abstract

**Background**: Yellow mealworm (*Tenebrio molitor*) larvae meal (TM), one of seven approved insect species used in aquafeeds, is a frequently investigated candidate for fish diets.

**Results:** This study aimed to investigate the effects of dietary defatted TM on production performance, serum biochemistry, nutrient digestibility, fillet traits, intestinal microbiota, and environmental impacts of perch (*Perca fluviatilis*). Four experimental diets, characterized by defatted TM inclusion levels of 0, 6.8, 13.5 and 20.3%, respectively, or 0, 25, 50, and 75% at the expense of fishmeal (TM0, TM25, TM50, and TM75, respectively) were fed to juvenile perch (bodyweight 20.81 ± 3.36 g, total length 117.7 ± 7.2 mm) (quadruplicated per diet) for 105 days. Inclusion levels of 6.8% or 25% fishmeal replacement by defatted TM did not show a significant effect on specific growth rate and feed conversion ratio ($P > 0.05$), while further levels of 13.5 and 20.3%, or 50 and 75% fishmeal replacement with defatted TM, respectively, displayed a significant delay in these indices compared to the control diet ($P < 0.001$). The aspartate aminotransferase activities in perch's serum increased with increasing dietary TM ($P = 0.044$). Nutrient digestibility of perch exhibited TM-dose dependent ($P < 0.05$). Dietary defatted TM did not lead to any significant changes in the fillet composition of perch ($P > 0.05$). Defatted TM did not modify diversity of fish gut microbiota (Chao1 index, $P = 0.742$; Shannon index, $P = 0.557$; and observed species, $P = 0.522$), but significantly reduced abundance of *Lactobacillus* ($P = 0.018$) and *Streptococcus* ($P = 0.013$) while fed TM75 relative to TM0. TM-containing diets generated a comparable amount of total solid waste and solid phosphorus waste with TM0, except TM25, whereas solid nitrogen waste significantly increased with elevated TM levels ($P < 0.001$). Perch fed TM25 was comparable with TM0 for global warming potential, acidification, and land use ($P > 0.05$), whereas TM50 and TM75 exerted heavier burdens on energy use, eutrophication, and water use than TM0 ($P < 0.001$). Fishmeal replacement by TM significantly reduced economic fish-in fish-out ($P < 0.001$).

**Conclusion**: The inclusion of 6.8% or 25% fishmeal replacement by defatted insect meal (*T. molitor*) in European perch diets resulted in comparable production performance but entailed heavier burdens associated with solid outputs waste and environmental impacts. The present study underlined the major bottleneck of a substantial inclusion of defatted insect meal (*T. molitor*) in fish diets associated with solid nitrogen waste and environmental consequences associated with one unit of farmed perch produced. Our multidisciplinary study suggested important aspects while formulating diets for fish, using insect meals regarding production performance and environmental issues.


# 1. Introduction

The increasing use of alternative aquafeed ingredients for fishmeal (FM) and fish oil is necessary to ensure the sustainability of the aquaculture sector [1]. Terrestrial plants have become the most common alternative for aquafeeds [2, 3]. However, the limitations associated with specific unfavorable nutritional components [4], and the environmental consequences of product intensification, especially the increasing demand for arable land - the immense pressures on the planet [5, 6], could hamper their expanding use in

aquafeeds. Fishery and aquaculture by-products, together with insect meal, represent the most excellent protein sources to satisfy the aquafeed demand in the coming years [7, 8]. The share of fish by-products in the global production of FM has increased over the last few years and is expected to reach 34% by 2030 (FAO, 2018). The supply of insect protein to humans and feed use has been forecasted to reach approximately 1.2 million tonnes by 2025 [8]. Insect meal has become a sustainable protein source for livestock and aquaculture production due to its favorable nutritional values [10], health benefits for the fed organisms [11], lower environmental impacts associated with land and water resource demand than that of plant proteins [12, 13], and positive effects on the aquatic environment than an FM-based diet [14].

Yellow mealworm (*Tenebrio molitor*) larvae meal, one of the seven approved insect species used in aquafeed (European Commission, Regulation 2017/893), is a frequent candidate for use in fish diets [15–17]. The success of TM inclusion, as a replacement of FM in aquatic animal diets, without any detrimental impact on growth performance and feed efficiency, has been documented for the top FM consumers, for example, 20.5–30.5% inclusion, or 100% FM replacement in shrimp (*Litopenaeus vannamei*) [18–19], 20–25% inclusion, or 67–100% FM replacement in rainbow trout (*Oncorhynchus mykiss*) [20–22]. Dietary TM has been reported to affect the nutrient digestibility and meat quality of fed organisms to a great extent [23]; however, it did not modulate the bacterial community in the intestine of rainbow trout (*O. mykiss*) [24] or gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) [25].

Although an aquafeed is a key contributor to environmental burdens (e.g., carbon footprint) and is the major source of waste output (e.g., total solid waste, solid nitrogen waste) of aquaculture system [26, 27], the impact of TM inclusion in aquatic animal feeds on such environmental indicators remains fragmentary [28]. Moreover, aquaculture has become a dominant consumer of FM and fish oil derived from forage fish since the 2000s [29]. Investigation into forage fish use to produce one unit of farmed fish, the fish-in fish-out (FIFO) [30], could be considered as an essential measure of sustainability [31], especially for the alternative ingredients that are increasingly being used in aquafeeds, such as insect meal [32]. Therefore, it is necessary to consider a broad spectrum of indicators whenever new aquafeed ingredients are introduced.

European perch (*Perca fluviatilis*) has received great interest as a promising candidate for intensive aquaculture [33]. Globally, the aquaculture production of perch is on the rise, reaching 700 tonnes in 2018 [34], and will become an established aquaculture sector in Europe, together with other percid fish species [35]. In nature, aquatic insects are essential food sources for the ontogeny of *P. fluviatilis* [36]. Therefore, the possibility of introducing TM into aquafeeds for European perch, with minimal adverse effects on growth performance and physiology traits, is expected. This study aimed at investigating the effects of dietary defatted *T. molitor* larvae meal, as a substitution for FM, on production performance, serum biochemistry, nutrient digestibility, meat quality, and intestinal microbiota of juvenile European perch. Moreover, the environmental impact indicators associated with dietary defatted TM were also highlighted.

# 2. Materials And Methods

# 2.1. Experimental facilities and procedures

The experimental facilities and procedures were described elsewhere [37]. Briefly, defatted mealworm *T. molitor* was obtained from a commercial source (NovoProtein, Fishag Edelhof GmbH, Wien, Austria). Four experimental diets, including one control (insect-free) diet (TM0), and three diets with defatted TM inclusion levels of 6.8, 13.5 and 20.3%, were studied as replacements of FM at 25, 50, and 75% (diets abbreviated as TM25, TM50, TM75, respectively) to provide approximately 47% crude protein, 15% crude lipid and 21Mj/kg energy. Yttrium oxide ($Y_2O_3$) was added (0.5%) as an inert marker for nutrient digestibility evaluation. All the diets were produced, using a twin-screw extruder, by a commercial aquafeed manufacturer (Exot Hobby s.r.o, Černá v Pošumaví, Czech Republic). The main ingredients, proximate composition, amino acid profile of the defatted TM and the experimental diets are presented in Tables 1, 2. Fatty acids profile were presented earlier [37].

Table 1
Ingredients (%, as it) and proximate composition of defatted *Tenebrio molitor* larvae meal (TM) and experimental diets [54]

| Ingredients (%) | Fishmeal | TM | Experimental diets | | | |
|---|---|---|---|---|---|---|
| | | | TM0 | TM25 | TM50 | TM75 |
| Soybean concentrate | | | 29.0 | 29.0 | 29.0 | 29.0 |
| Fishmeal | | | 27.1 | 20.3 | 13.5 | 6.8 |
| *Tenebrio molitor* | | | 0.0 | 6.8 | 13.5 | 20.3 |
| Soybean meal | | | 14.5 | 14.5 | 14.5 | 14.5 |
| Corn flour | | | 9.7 | 9.7 | 9.7 | 9.7 |
| Fish oil | | | 7.7 | 7.7 | 7.7 | 7.7 |
| Rapeseed oil | | | 5.8 | 5.8 | 5.8 | 5.8 |
| Methionine[a] | | | 0.8 | 0.8 | 0.8 | 0.8 |
| Lysine[b] | | | 0.5 | 0.5 | 0.5 | 0.5 |
| Valine[c] | | | 0.2 | 0.2 | 0.2 | 0.2 |
| L-Threonine[d] | | | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamins & minerals[e] | | | 0.8 | 0.8 | 0.8 | 0.8 |
| Additives[f] | | | 3.5 | 3.5 | 3.5 | 3.5 |
| Yttrium oxide ($Y_2O_3$) | | | 0.5 | 0.5 | 0.5 | 0.5 |
| **Proximate composition (as it)** | | | | | | |
| Dry matter (%) | 96.5 | 95.0 | 94.8 | 95.7 | 95.6 | 95.6 |
| Crude protein (%) | 71.2 | 71.1 | 47.5 | 48.7 | 47.4 | 47.2 |
| Crude lipid (%) | 7.9 | 8.5 | 16.3 | 13.9 | 15.6 | 17.0 |
| Ash (%) | 14.0 | 7.1 | 8.9 | 9.0 | 8.3 | 7.6 |
| Fibre (%) | 1.24 | 2.8 | 2.0 | 2.0 | 2.2 | 2.3 |
| Nitrogen-free extract (%)[g] | 1.3 | 5.5 | 19.5 | 21.8 | 22.3 | 21.6 |
| Gross energy (Mj/kg)[h] | 20.1 | 21.1 | 21.0 | 20.8 | 21.2 | 21.5 |
| Chitin[i] | - | 4.8 | - | 0.33 | 0.65 | 0.97 |

| Ingredients (%) | Fishmeal | TM | Experimental diets | | | |
|---|---|---|---|---|---|---|
| | | | TM0 | TM25 | TM50 | TM75 |
| [a] Adisseo, China | | | | | | |
| [b] Inner Mongolia Eppen Biotech Co., Ltd. | | | | | | |
| [c] Ajinomoto Animal Nutrition Europe. | | | | | | |
| [d] Ningxia Eppen Biotech, China. | | | | | | |
| [e] Aminovitan Sak, Trouw Nutrition Biofaktory s.r.o, Czech Republic. | | | | | | |
| [f] Feed limestone (0.5%); Pentasodium triphosphate (Fosfa a.s, Czech Republic). (0.5%) and binder (NutriBind, Adisseo, China) (2.5%). | | | | | | |
| [g] Nitrogen-free extracts (NFE) = dry matter - (crude protein + crude lipid + ash + fibre). | | | | | | |
| [h] Gross energy (MJ/kg) as gross energy content of protein (23.6 MJ/kg), lipid. (39.5 MJ/kg) and NFE (17.2 MJ/kg). | | | | | | |
| [i] Estimated from Basto et al. [15] for defatted TM. | | | | | | |

Table 2
Amino acid profile (g/100g) of defatted yellow mealworm *Tenebrio molitor* larvae meal (TM) and experimental diets

| | Experimental diets | | | | |
|---|---|---|---|---|---|
| | TM | TM0 | TM25 | TM50 | TM75 |
| Indispensable amino acids (IAA) | | | | | |
| Methionine | 1.29 | 1.55 | 1.52 | 1.49 | 1.33 |
| Threonine | 2.69 | 1.70 | 1.79 | 1.61 | 2.00 |
| Valine | 2.95 | 2.02 | 2.02 | 1.88 | 1.99 |
| Isoleucine | 2.16 | 1.70 | 1.69 | 1.54 | 1.68 |
| Leucine | 5.32 | 3.21 | 3.25 | 3.07 | 3.43 |
| Phenylalanine | 2.31 | 1.79 | 1.84 | 1.70 | 1.83 |
| Histidine | 1.09 | 1.31 | 1.19 | 1.05 | 1.23 |
| Lysine | 4.21 | 3.30 | 3.25 | 2.73 | 3.23 |
| Arginine | 4.63 | 2.84 | 2.89 | 2.73 | 3.07 |
| Tryptophan | 0.34 | 0.19 | 0.21 | 0.22 | 0.28 |
| Dispensable amino acids (DAA) | | | | | |
| Cysteine | 1.53 | 0.48 | 0.59 | 0.64 | 0.69 |
| Aspartic acid | 4.83 | 4.83 | 4.43 | 4.09 | 4.41 |
| Serine | 4.27 | 1.99 | 2.20 | 2.09 | 2.39 |
| Glutamic acid | 10.68 | 7.13 | 7.30 | 6.78 | 7.71 |
| Proline | 0.42 | 0.16 | 0.20 | 0.19 | 0.26 |
| Glycine | 3.74 | 2.07 | 2.04 | 1.84 | 2.12 |
| Alanine | 3.41 | 2.16 | 2.10 | 1.93 | 2.11 |
| Tyrosine | 2.66 | 1.45 | 1.56 | 1.50 | 1.57 |
| Total amino acids | 58.54 | 39.89 | 40.05 | 37.08 | 41.34 |
| IAA/DAA | 0.86 | 0.97 | 0.96 | 0.95 | 0.95 |

Perch juveniles were obtained from a commercial hatchery (Anapartner s.r.o, Prague, Czech Republic) and transported, in oxygenated 1 $m^3$ tanks, to the Research Institute of Fish Culture and Hydrobiology. Fish

underwent two weeks of adaptation to the experimental facility and were fed a commercial diet (Aller Aqua, Christiansfeld, Denmark).

At the start of the experiment, eighty-two fish (bodyweight 20.81 ± 3.36 g, total length 117.7 ± 7.2 mm) were randomly assigned to each of sixteen black circular 180 L tanks (quadruplicated per diet) connected to a recirculating system (total volume 11,400 L). A water inflow of 6.5 L/min, combined with in-tube stone aeration, created a constant clockwise flow of 4.6 cm/s in each tank. The circular tanks enclosed funnel-like bottoms, which allowed to collect the faeces and any unconsumed feeds to be collected conventionally. The photoperiod (12h:12h, light : dark), light intensity 58.6 lux were set up throughout the experimental period, water temperature 22.44 ± 0.66°C, pH 7.00 ± 0.29, oxygen saturation 80.41 ± 8.02%, ammonia-N 0.28 ± 0.16 mg/L, and nitrite nitrogen < 0.45 mg/L parameters were maintained during the experiment.

Fish were fed five times daily, with an excessive amount, at 7.00, 9.00, 11.00, 13.00, and 15.00 using automatic feeders (EHEIM Twins, Deizisau, Germany) for 105 days. Any unconsumed feed was removed fifteen minutes after each feeding and dried to determine the feed intake. Fish mortality was recorded daily in each experimental tank.

# 2.2. Sample collection and calculations

# 2.2.1. Growth performance

The fish were inspected every three weeks and at the end of the experiment, following 24 h of feed deprivation, for the biometry (weight, total length) under a light anesthesia dose (50 mg/L) of tricaine methanesulfonate (MS222) (Sigma-Aldrich Chemicals, Missouri, USA) in order to minimize handling stress. The production performance indices, survival rate, and feed efficiency were calculated as follows:

Survival rate (%) = 100 × the final number of fish/the initial number of fish

Condition factor (CF) = 100 x (body weight (g)/total length$^3$ (cm))

Weight gain (WG, g) = ($W_f - W_i$), where $W_f$ is the final wet weight, and $W_i$ is the initial wet weight

Specific growth rate (SGR, %/day) = [(ln $W_f$ – ln $W_i$)/t] × 100, where t is the number of days

Feed conversion ratio (FCR) = total feed fed (g)/WG (g)

Protein efficiency ratio (PER) = WG (g)/dry protein intake (g)

Daily feeding rate (DFR, % body weight/day) = [total dry feed intake (g) × 100]/[t × (($W_i$ + $W_f$) × 0.5)]

# 2.2.2. Digestibility trial

Faeces from each tank were collected from the 42nd day of the experiment to evaluate the apparent nutrient digestibility of the experimental diets. At that time, the morphometrics of the fish fed the

experimental diets were 42.2 ± 1.9 g (weight) and 146.2 ± 0.2 mm (total length) for TM0; 41.6 ± 1.4 g and 143.6 ± 0.3 mm for TM25; 37.9 ± 1.3 g and 141.2 ± 0.2 mm for TM50; 33.0 ± 0.7 g and 135.6 ± 0.2 mm for TM75. The faeces were collected after the last feeding time (at 15.00) following unconsumed feed removal, by means of siphoning, and stored at -20°C until being analyzed. The apparent digestibility coefficients (ADC) of the dry matter, protein, lipid, ash, phosphorus, and fatty acids of the experimental diets were calculated according to the following equation [38]:

ADC of nutrient (ADC, %) = 100 – (100 × (%$Y_2O_3$ in diet/%$Y_2O_3$ in feces) × (% Nutrient in feces/% Nutrient in diet)).

## 2.2.3. Serum biochemistry

A random sample of 3 fish/tank (n = 12 fish/group) was taken at the end of the feeding trial (105th day), following 24 h of starvation, and sacrificed by an overdose of anesthesia (MS222, 125 mg/L). Blood samples (approximately 1 mL) were collected from the caudal vein and centrifuged at 3,000 × *g* at 4 $^{o}$C for 10 min. The separated serum was frozen at -80°C until further analysis.

## 2.2.4. Organo-somatic indices and proximate fillet composition

The liver, intestines, spleen, and viscera were removed immediately after blood sampling and weighed (and the length of intestine was measured) to calculate the organo-somatic indices. Skin-off fillets were sampled for fillet yield and stored at -20°C for further proximate composition analysis. The organo-somatic indices were calculated with the following formulae:

Hepatic somatic index (HSI, %) = 100 × liver weight (g)/body weight (g)

Visceral somatic index (VSI, %) = 100 × viscera weight (g)/body weight (g)

Spleen somatic index (SSI, %) = 100 × weight of spleen (g)/body weight (g)

Intestine somatic index (ISI, %) = 100 × weight of intestine (g)/body weight (g)

Mesenteric fat index (MSI, %) = 100 × (perivisceral fat weight/body weight)

Relative gut length (RGL) = intestinal length (mm)/fish total length (mm).

Fillet yield (FY, %) = 100 × ((right fillet weight (g) + left fillet weight(g))/body weight (g)

## 2.2.5. Microbiota

At the end of the feeding trial, two fish per tank (n = 8 fish/diet group) were randomly taken and euthanized by means of overdose anesthesia (MS222, 125 mg/L). To ensure all the sampled fish had digesta throughout the intestinal tract, the fish were deprived of feeds 12 h before the sampling time. The exterior of the fish was wiped with 70% ethanol before opening the abdomen, the whole intestine was

subsequently removed from the abdominal cavity from each fish and the digesta from the proximal to distal intestine was squeezed gently into a 1.5 mL sterile microtube and immediately stored at -80°C for further analysis.

## 2.2.6. Environmental impact indices

The total solid waste (TSW), solid nitrogen waste (SNW), and solid phosphorus waste (SPW) were calculated as described by Bureau and Hua [39].

TSW = feed consumed × (1 – ADC of dry matter)

SNW = feed consumed × Nitrogen content in diet × (1 – ADC of protein)

SPW = feed consumed × Phosphorus content in diet × (1 – ADC of phosphorus)

The simulated environmental impacts associated with one-kilogram farmed perch production were calculated as environmental impacts of the diet multiplied by the respective FCR. The environmental impacts of one kilogram diet were calculated using the life cycle assessment database generated by the Global Feed Lifecycle Institute [40] as described by Tran et al. [41]. Given that the environmental impact of ingredients in the GFLI database varies with location, average global values were used. The minerals, vitamins, additives and supplemented amino acids in the present study are classified as 'Total minerals, additives, vitamins, at plant/RER Mass S' in the GFLI database. Due to unavailable data on water use for the production of one kg of *T. molitor* meal, we used the value of 4.3 $m^3$ required for one kg fresh mealworm [42] with an assumption that the drying process of mealworm did not require additional water [43]. The six environmental impact categories comprise global warming potential (GWP, kg $CO_2$ equivalent (eq.)), energy use (kg oil eq.), acidification (kg $SO_2$ eq.), eutrophication (kg P eq.), land use ($m^2$ arable land (a.)) and water use ($m^3$). The impacts associated with feed production at the feed mill and fish farming phase were beyond the scope of the present study.

The economic fish-in fish-out (eFIFO) ratio, indicating the amount of fish used to produce one unit of farmed fish, was developed by Kok et al. [44]; it is based on an economic allocation commonly used in life cycle assessments. The formula used to calculate the eFIFO ratio is: eFIFO = FCR × ∑(Fi,j × EFi,j), where FCR is feed conversion ratio; Fi is the fraction of ingredient i in the feed (%); EFi is the embodied fish in ingredient i; i, FM or FO; j is the source of the ingredient. The value of embodied fish in ingredient was taken from a 2021 database [44].

# 2.3. Analytical methods

## 2.3.1. Chemical composition

The defatted insect meal (*T. molitor*), experimental diets, faeces, fillets, and whole-body fish were well homogenized and analyzed according to AOAC [45] for dry matter (934.01), crude ash (942.05) and crude fibre (985.29). Crude protein was determined, by means of the Kjeldahl method, using an automatic

Kjeldahl System (Buchi, Flawil, Switzerland). The lipid and fatty acid profiles were determined as described by Mráz and Pickova [46]. The phosphorus content in the insect meal, diets and faeces was determined using an inductively coupled plasma atomic emission spectrophotometer (ICPOES, Prodigy7, Leeman Labs, USA). Yttrium oxide ($Y_2O_3$) in the diets and faeces samples were analyzed using inductively coupled plasma emission spectrometry (ICPOES) following digestion with nitric acid at 180°C for 48 h. Amino acid profile of insect meal and diets was analyzed as described by Stejskal et al. [47].

### 2.3.2. Serum biochemistry

Serum samples were determined using an Architect c4000 automatic analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). The serum biochemical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, glucose, cholesterol, triglyceride, and alkaline phosphatase (ALP).

### 2.3.3. Gut microbiota

DNA extraction and 16S rRNA amplicon target sequencing were performed according to the following procedures:

Nucleic acid was extracted from the gut content (500 mg as the starting material). The total DNA was extracted from the samples using an RNeasy Power Microbiome KIT (Qiagen, Milan, Italy) according to the manufacturer's instructions. One microliter of RNase (Illumina Inc, San Diego, CA) was added to digest RNA in the DNA samples and incubated of 1 h at 37°C. DNA was quantified using the NanoDrop and standardized at 5 ng/μL.

DNA extracted directly from digesta samples was used to assess the microbiota by the amplification of the V3-V4 region of the 16S rRNA gene [48]. The PCR products were purified according to the Illumina metagenomic standard procedure (Illumina Inc, San Diego, CA). Sequencing was performed with a MiSeq Illumina instrument with V3 chemistry and 250 bp paired-end reads were generated according to the manufacturer's instructions.

## 2.4. Statistical analyses

The obtained data were checked for normal distribution (Shapiro-Wilks's test) and homogeneity of variances (Levene's test). All the statistical analyses were performed using the R Statistic Package, R Development Core Team 2009–2021. One-way ANOVA was used to test the differences, followed by Tukey's post-hoc test, when appropriate. Differences were regarded as significant at $P < 0.05$.

Paired-end reads were first joined by means of FLASH software (http://sourceforge.net/projects/flashpage) to default parameters for gut microbiota. The reads obtained after quality filtering (at Phred < Q20), using QIIME 2 software (v2018.11) [49] were analyzed by means of a recently described pipeline [50]. Picking the operational taxonomic units (OTUs) was performed at 97% of similarity, and taxonomy assignment was done by using the Greengenes16S rRNA gene database 2017 (http://greengenes.lbl.gov). The centroids sequence was then manually blasted to confirm the

taxonomic assignment. The OTU table obtained with QIIME was rarefied at the lowest number of sequences and the higher taxonomy resolution genus or family was displayed. The vegan package of R [51] was used to calculate the alpha diversity. The diversity indices and the OTU table were further analyzed using the pairwise comparisons from the Wilcoxon rank-sum test to assess any differences between the diets. A difference was considered significant at $P < 0.05$. Weighted UniFrac distance matrices and OTU tables were used to perform Adonis and Anosim statistical tests in the R environment.

# 3. Results

### *3. 1. Growth performance*

The inclusion of defatted *Tenebrio molitor* larvae meal had a significant effect ($P < 0.05$) on body weight and total length of juvenile European perch throughout biometry time-series (Fig. 1). Feeding perch with dietary TM did not affect survival rate ($P = 0.729$) and condition factor ($P = 0.479$) after 105-day trial, but, at substantial inclusion levels (TM50 and TM75), significantly compromised weight gain and SGR ($P < 0.001$), and increased FCR ($P < 0.001$) compared with TM0 (Table 3). The negative correlation with increasing TM level and PER ($P < 0.001$) was also detected (Table 3).

Table 3
Production performance, feed efficiency, organo-somatic indices, and serum biochemistry of European perch (*Perca fluviatilis*) fed experimental diets for 105 days

| Item | Experimental diets | | | | SEM[1] | *P*-value |
|---|---|---|---|---|---|---|
| | TM0 | TM25 | TM50 | TM75 | | |
| **Production parameters** | | | | | | |
| Survival (%) | 99.09 | 99.09 | 98.48 | 98.78 | 0.21 | 0.729 |
| CF | 1.53 | 1.54 | 1.52 | 1.46 | 0.02 | 0.497 |
| WG (g) | 57.56$^{a}$ | 57.14$^{a}$ | 46.48$^{b}$ | 30.59$^{c}$ | 2.90 | < 0.001 |
| SGR (%/day) | 1.26$^{a}$ | 1.26$^{a}$ | 1.12$^{b}$ | 0.86$^{c}$ | 0.04 | < 0.001 |
| FCR | 1.15$^{a}$ | 1.19$^{ab}$ | 1.33$^{b}$ | 1.77$^{c}$ | 0.07 | < 0.001 |
| PER | 1.85$^{a}$ | 1.72$^{ab}$ | 1.60$^{b}$ | 1.21$^{c}$ | 0.06 | < 0.001 |
| DFR (% BW/day) | 1.26$^{a}$ | 1.31$^{ab}$ | 1.33$^{ab}$ | 1.41$^{b}$ | 0.02 | 0.021 |
| Fillet yield (%) | 38.50 | 39.79 | 38.22 | 37.42 | 0.40 | 0.209 |
| **Somatic indies** | | | | | | |
| VSI (%) | 14.23 | 13.65 | 14.14 | 14.27 | 0.26 | 0.959 |
| HSI (%) | 1.53 | 1.53 | 1.65 | 1.58 | 0.05 | 0.868 |
| MFI (%) | 9.27 | 8.89 | 8.87 | 8.75 | 0.22 | 0.880 |
| ISI (%) | 0.75 | 0.73 | 0.84 | 0.87 | 0.03 | 0.420 |
| RGL | 0.58 | 0.61 | 0.60 | 0.56 | 0.01 | 0.139 |
| SSI (%) | 0.08 | 0.09 | 0.09 | 0.10 | 0.00 | 0.459 |
| Serum biochemistry | | | | | | |
| ALT (ukat/L) | 0.29 | 0.33 | 0.30 | 0.36 | 0.01 | 0.249 |
| AST (ukat/L) | 0.62$^{a}$ | 1.29$^{ab}$ | 1.48$^{ab}$ | 2.04$^{b}$ | 0.19 | 0.044 |
| Glucose (mmol/L) | 5.90 | 4.75 | 4.65 | 6.94 | 0.42 | 0.161 |

CF = condition factor; WG = weight gain; SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio; DFR = daily feeding rate; VSI = visceral somatic index; HSI = hepatic somatic index; MFI = mesenteric fat index; ISI = intestine somatic index; RGL = relative gut length; SSI = spleen somatic index; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase.

[1] Standard error of the mean (pooled). Means with the same superscript letter in a same row are not significantly different ($P > 0.05$).

| Item | Experimental diets | | | | SEM[1] | *P*-value |
|---|---|---|---|---|---|---|
| | TM0 | TM25 | TM50 | TM75 | | |
| Cholesterol (mmol/L) | 5.66 | 4.61 | 4.55 | 4.61 | 0.21 | 0.182 |
| Triglycerides (mmol/L) | 9.75 | 7.79 | 7.97 | 9.05 | 0.44 | 0.384 |
| Total protein (g/L) | 40.70 | 32.64 | 43.29 | 42.25 | 2.54 | 0.476 |
| ALP (ukat/L) | 0.43 | 0.37 | 0.40 | 0.49 | 0.02 | 0.256 |

CF = condition factor; WG = weight gain; SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio; DFR = daily feeding rate; VSI = visceral somatic index; HSI = hepatic somatic index; MFI = mesenteric fat index; ISI = intestine somatic index; RGL = relative gut length; SSI = spleen somatic index; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase.

[1] Standard error of the mean (pooled). Means with the same superscript letter in a same row are not significantly different ($P > 0.05$).

Feeding dietary defatted TM appeared to be satisfied by European perch in terms of palatability as indicated by DFR values, which were significantly higher in TM75 than TM0 ($P = 0.021$). Fish displayed an excellent response to TM25 as those growth indices were comparable with TM0 ($P > 0.05$). Fillet yield of perch, ranged 37.42–39.79%, was TM-inclusion independent ($P = 0.209$). A similar pattern was observed for all organo-somatic indies ($P > 0.05$) (Table 3).

Serum biochemistry indices of perch did not differ among diet groups ($P > 0.05$), except AST activity which was significantly higher in perch fed TM75 than did TM0 ($P = 0.044$) (Table 3).

## 3. 2. Apparent digestibility of experimental diets

As depicted in Table 4, the nutrient digestibility of European perch was significantly affected by increasing dietary defatted TM ($P < 0.05$), except ash ($P = 0.05$). Digestibility of fatty acids were significantly influenced by dietary defatted TM ($P < 0.05$), except C14:0 ($P = 0.19$) and C16:1 ($P = 0.39$).

## 3. 3. Proximate composition perch fillet

Feeding defatted TM levels did not alter the proximate composition and fatty acid profile of the perch fillets ($P > 0.05$) (Table 5). DHA, ranged from 14.95% (TM50) to 18.18% (TM75) of the total fatty acids, was the second-largest constitute in perch fillets following oleic acid, and unaffected by administration of defatted TM ($P = 0.48$). The EPA share was meager (2.47–3.08 % of the total fatty acids) and was consistent among diet groups ($P = 0.13$) (Table 5).

## 3. 4. Microbiota analysis

After sequencing and quality filtering, 334,095 reads were obtained and used for further analysis with an average value of 12,661 reads/sample. Analysis of the rarefactions and estimated sample coverage indicated a satisfactory coverage of all samples (median coverage value of 98%). No significant difference in alpha diversity indices of Shannon ($P$ = 0.557), observed OTUs ($P$ = 0.523), and Chao1 ($P$ = 0.741) (Fig. 2).

The bacterial community in the perch intestine was mainly dominated by phyla *Firmicutes*, *Actinobacteria* and *Fusobacteria* (Fig. 3A). The microbiota composition at the genus level (Fig. 3B) was mainly represented by *Clostridium* (52%, 87%, 50 and 63% in TM0, TM25, TM50 and TM75, respectively), *Mycobacterium* (33, 4, 31 and 9%), *Lactobacillus* (4, 3, 1 and 1%) and *Cetobacterium* (1, 0.1, 9 and 22%). A minor OTUs fraction (below 0.2%) was composed of *Enterobacteriaceae*, *Streptococcus*, *Candidatus*, *Chlamydia*, *Clavibacter*, *Bacillus*, *Parachlamydiaceae,* and *Solirubrobacterales* (Fig. 3B).

The principal component analysis based on OTUs abundance showed no clear separation across diet groups (Fig. 4).

A significant change in microbial composition as a result of the inclusion of defatted TM was observed. By considering the significant difference in the OTUs among diets (Fig. 5), the inclusion of 20.3% or 75% FM replacement by defatted TM significantly reduced the abundance of *Lactobacillus* ($P$ = 0.02) and *Streptococcus* ($P$ = 0.038) genera compared with the control group.

## 3. 5. Solid waste output and environmental impacts

Dietary defatted TM significantly affect environmental impacts associated with TSW ($P$ < 0.001), SNW ($P$ < 0.001) and SPW ($P$ < 0.001) (Table 6). TM75 showed a significant reduction in TSW ($P$ = 0.031), whereas feeding perch with TM25 inverted the pattern ($P$ = 0.005) compared with the control diet. SPW was not different among TM0, TM50 and TM75 ($P$ > 0.05), but was significantly higher in TM25 ($P$ < 0.001). Dietary defatted TM significantly increased SNW (< 0.001) (Table 6).

The eFIFO, ranged 0.99–1.45, was significantly reduced with increasing levels of defatted TM ($P$ < 0.001). Among the TM-containing groups, the ratio was less than one for the TM75 diet, whereas those of TM25, and TM50 were greater than 1 (1.19 and 1.07, respectively) (Table 6).

As far as the environmental impacts associated with one kg farmed perch production are concerned, TM25 was comparable with TM0 for the global warming potential, acidification, and land use ($P$ > 0.05). TM50 and TM75 exerted heavier burdens than the control diet on all the impact categories ($P$ < 0.05).

**Table 4** Nutrient digestibility (%) of European perch (*Perca fluviatilis*) fed experimental diets for 105 days

| Nutrients | Experimental diets | | | | SEM[1] | *P*-value |
|---|---|---|---|---|---|---|
| | TM0 | TM25 | TM50 | TM75 | | |
| Dry matter | 78.95$^{a}$ | 77.01$^{c}$ | 77.58$^{b}$ | 76.52$^{d}$ | 0.28 | <0.001 |
| Crude protein | 92.72$^{a}$ | 90.99$^{b}$ | 90.42$^{b}$ | 87.48$^{c}$ | 0.58 | <0.001 |
| Crude lipid | 92.62$^{a}$ | 91.96$^{a}$ | 92.95$^{a}$ | 89.14$^{b}$ | 0.49 | <0.001 |
| Phosphorus | 45.93$^{a}$ | 36.26$^{b}$ | 38.24$^{b}$ | 37.76$^{b}$ | 1.46 | 0.041 |
| Ash | 41.98 | 38.14 | 40.11 | 38.30 | 0.61 | 0.058 |
| Fatty acids | | | | | | |
| C14:0 | 98.13 | 97.91 | 98.05 | 97.97 | 0.04 | 0.192 |
| C16:0 | 96.81$^{a}$ | 96.09$^{c}$ | 96.10$^{bc}$ | 96.33$^{c}$ | 0.10 | 0.002 |
| C16:1 | 98.53 | 98.53 | 98.62 | 98.40 | 0.04 | 0.390 |
| C18:1n9 | 97.90$^{ab}$ | 97.79$^{bc}$ | 97.93$^{a}$ | 97.71$^{c}$ | 0.03 | <0.001 |
| C18:2n6 | 98.40$^{a}$ | 98.10$^{b}$ | 98.21$^{ab}$ | 98.21$^{ab}$ | 0.04 | <0.001 |
| C18:3n3 | 98.90$^{a}$ | 98.70$^{b}$ | 98.84$^{ab}$ | 98.15$^{c}$ | 0.09 | <0.001 |
| C20:1n9 | 97.05$^{a}$ | 96.81$^{a}$ | 95.68$^{b}$ | 77.40$^{c}$ | 2.50 | <0.001 |
| SFA | 96.71$^{a}$ | 95.91$^{b}$ | 95.89$^{b}$ | 96.13$^{b}$ | 0.11 | <0.001 |
| MUFA | 97.76$^{b}$ | 97.77$^{b}$ | 97.88$^{a}$ | 97.60$^{c}$ | 0.03 | <0.001 |
| PUFA | 98.34$^{a}$ | 97.97$^{b}$ | 98.07$^{b}$ | 98.08$^{b}$ | 0.04 | <0.001 |
| n3 | 98.99$^{a}$ | 98.70$^{c}$ | 98.83$^{b}$ | 98.65$^{c}$ | 0.04 | <0.001 |
| n6 | 98.43$^{a}$ | 98.14$^{b}$ | 98.22$^{b}$ | 98.21$^{b}$ | 0.04 | <0.001 |
| DHA | 98.93$^{a}$ | 98.49$^{b}$ | 98.61$^{b}$ | 98.91$^{a}$ | 0.06 | <0.001 |
| EPA | 99.38$^{a}$ | 99.16$^{b}$ | 99.24$^{b}$ | 99.37$^{a}$ | 0.03 | <0.001 |

EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[1] Standard error of the mean (pooled). Means with the same superscript letter in a same row are not significantly different ($P > 0.05$).

**Table 5** Fillet composition (% as wet basis) and fatty acid profile (% of total FA) of European perch (*Perca fluviatilis*) fed experimental diets

| | Experimental diets | | | | SEM[1] | *P*-value |
|---|---|---|---|---|---|---|
| | TM0 | TM25 | TM50 | TM75 | | |
| Moisture | 79.04 | 77.70 | 77.93 | 76.99 | 0.35 | 0.239 |
| Crude protein | 22.25 | 22.74 | 22.55 | 22.0 | 0.25 | 0.784 |
| Crude lipid | 1.45 | 1.30 | 1.21 | 1.28 | 0.04 | 0.193 |
| Ash | 1.11 | 1.11 | 1.12 | 1.11 | 0.01 | 0.99 |
| Fatty acid profile[2] | | | | | | |
| C14:0 | 1.59 | 1.62 | 1.37 | 1.53 | 0.05 | 0.378 |
| C16:0 | 16.97 | 18.35 | 15.94 | 17.20 | 0.55 | 0.527 |
| C16:1 | 4.54 | 5.09 | 4.50 | 4.87 | 0.18 | 0.647 |
| C18:0 | 2.51 | 2.68 | 2.37 | 2.73 | 0.10 | 0.585 |
| C18:1n9 | 32.58 | 31.44 | 27.39 | 29.69 | 1.16 | 0.499 |
| C18:2n6 | 12.34 | 12.07 | 10.58 | 12.24 | 0.43 | 0.477 |
| C18:3n3 | 2.84 | 2.66 | 2.15 | 2.37 | 0.11 | 0.144 |
| C20:1n9 | 1.56 | 1.42 | 1.18 | 1.36 | 0.05 | 0.055 |
| C20:5n3 (EPA) | 3.06 | 3.08 | 2.47 | 3.03 | 0.12 | 0.13 |
| C22:6n3 (DHA) | 17.00 | 16.92 | 14.95 | 18.18 | 0.71 | 0.483 |
| ΣSFA | 22.34 | 23.87 | 20.76 | 22.67 | 0.71 | 0.531 |
| ΣMUFA | 39.97 | 38.92 | 34.34 | 38.66 | 1.33 | 0.496 |
| ΣPUFA | 37.61 | 37.13 | 44.83 | 38.47 | 1.98 | 0.525 |

[1] Standard error of the mean (pooled).

[2] Fatty acids with less than 1% total fatty acids in experimental diets (C10:0, C12:0, C13:0, C14:1, C15:0, C15:1, C17:0, C17:1, C16:3, C18:1n9 trans, C18:1n7, C18:2n6 trans, C18:3n6, C20:0, C21:0, C20:3n6, C20:3n3, C20:4n6, C22:0, C24:0, C24:1n9, C22:5n6) were not presented in the table but included in fatty acids group calculation. EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Means with the same superscript letter in a same row are not significantly different ($P > 0.05$).

**Table 6** Solid waste output and environmental impacts associated with 1 kg production of European perch (*Perca fluviatilis*) fed experimental diets for 105 days

| | Experimental diets | | | | SEM[1] | *P*-value |
|---|---|---|---|---|---|---|
| | TM0 | TM25 | TM50 | TM75 | | |
| **Solid waste output** | | | | | | |
| TSW (g) | 1115$^{b}$ | 1268$^{a}$ | 1104$^{b}$ | 1004$^{c}$ | 52 | 0.0002 |
| SNW (g) | 385$^{c}$ | 497$^{ab}$ | 471$^{b}$ | 535$^{a}$ | 30 | 0.0001 |
| SPW (g) | 2860$^{b}$ | 3514$^{a}$ | 3038$^{b}$ | 2660$^{b}$ | 178 | 0.0008 |
| **Environmental impacts associated with 1 kg perch production** | | | | | | |
| GWP (kg $CO_2$ eq.) | 3.00$^{c}$ | 3.24$^{c}$ | 4.01$^{b}$ | 5.58$^{a}$ | 0.27 | <0.001 |
| Energy use (kg oil eq.) | 0.35$^{d}$ | 2.45$^{c}$ | 5.29$^{b}$ | 10.19$^{a}$ | 0.96 | <0.001 |
| Acidification (kg $SO_2$ eq.) | 10.71$^{c}$ | 11.94$^{c}$ | 15.17$^{b}$ | 21.65$^{a}$ | 1.11 | <0.001 |
| Eutrophication (kg P eq.) | 0.36$^{d}$ | 1.54$^{c}$ | 3.14$^{b}$ | 5.94$^{a}$ | 0.54 | <0.001 |
| Land use ($m^2a$) | 2.40$^{c}$ | 2.76$^{c}$ | 3.60$^{b}$ | 5.23$^{a}$ | 0.29 | <0.001 |
| Water use ($m^3$) | 0.03$^{d}$ | 0.37$^{c}$ | 0.82$^{b}$ | 1.61$^{a}$ | 0.15 | <0.001 |
| **Economic fish-in fish-out** | | | | | | |
| eFIFO | 1.45$^{a}$ | 1.19$^{b}$ | 1.07$^{bc}$ | 0.99$^{c}$ | 0.05 | <0.001 |

[1] Standard error of the mean (pooled).

TSW = total solid waste; SNW = solid nitrogen waste; SPW = solid phosphorus waste; GWP = global warming potential, eFIFO = economic fish-in fish-out.

# 4. Discussion

Insect meal has been considered the most promising raw material for the supply of protein sources in aquafeeds for the coming decades [7, 8]. A wide range of aquatic animals has been investigated for the possibility of including insect meals in their feeds [17, 23, 52]. European perch (*P. fluviatilis*) is a potential candidate for aquaculture diversification in Europe, and the intensive aquaculture of this species is taking off with increasing production volume over the last decades, reaching approximately 700 tonnes in 2018 [34]. The potential use of insect meal as an alternative protein source for perch was investigated by Stejskal et al. [32], indicating that a 40% inclusion level of black soldier fly (*H. illucens*) was suitable for

perch aquafeeds. Our study investigated another insect meal frequently used in aquafeed research, yellow mealworm (*T. molitor*) for European perch, and the outputs could offer an additional protein source for the continuously-growing percid aquaculture sector [35].

# 4.1. Production performance, somatic indices and serum biochemistry

In the present study, the condition factor (1.46–1.53) remained consistent among treatment groups and was slightly higher than the 1.15–1.22 reported by Stejskal et al. [32] for perch fed dietary black soldier fly (*H. illucens*). The survival rate of fish was high (> 98%) in all treatments after a 105-day feeding trial. The experimental diets were well accepted by European perch as indicated by DFR, which was significantly higher in the TM-based diets than in the control group. Stejskal et al. [32] also reported a comparable feeding rate for perch fed dietary defatted *H. illucens.* Similar findings were observed for rainbow trout (*O. mykiss*) [21], red seabream (*Pargus major*) [53]. On the contrary, Gasco et al. [16] reported a significant reduction in feed intake for European sea bass (*D. labrax*) fed increasing full-fat *T. molitor* levels. These differences could be attributed to the different processing forms of the consumed insect meal, as defatted insect meal has been reported to improve the palatability of catfish (*Clarias gariepinus*) [54].

In our study, feeding perch with TM25 showed a consistent growth performance compared to the control diet, whereas higher replacement levels had detrimental effects. This phenomenon could be linked to the presence of chitin, which has been shown to affect the growth rate of fed organisms [16, 55]. The compromising performance mechanism consists of a lowering of the energy availability and a reduction of the nutrient digestibility of fish [56]. Defatted TM contained 4.63% chitin [15], and increasing dietary TM, therefore, corresponded to increasing the chitin levels in TM-containing diets (Table 1). Consequently, a reduction in nutrient digestibility of perch fed these diets relative was observed, compared to the control diet (Table 5). The limited ability of fish to utilize chitin as energy hampers fish growth when substantial FM replacements with insect meal (*H. illucens*) are introduced [55]. Another nutritional factor that may impair fish performance is linked to a fatty acid deficiency [52]. The declining EPA and DHA observed in our study as dietary defatted TM increased [37] could evidence a growth delay. Although taurine amino acid was not measured in our study, it is known to compromise fish growth when included at low availability [57]. Basto et al. [15] found that defatted TM contains a lower content of this sulfonic acid than the full-fat form. As a result, increasing inclusion levels of defatted TM, accompanied by a reduction in taurine levels, could have hampered the performance of perch fed TM50 and TM75 in our study.

Stejskal et al. [32] reported that inclusion levels of up to 40% (or 42% FM replacement) of defatted *H. illucens* in diets showed no adverse effect on the growth performance of perch compared to the insect-free diet. It is evidenced that dietary *H. illucens* is preferable to *T. molitor* for perch, in terms of growth rate. A study on European seabass (*D. labrax*) fed a diet with 30% FM replacement with TM and *H. illucens* meal also showed a superior growth and feed efficiency of the latter insect species compared to the former [58].

The present study has found that organ-somatic indices were TM-dose independent, which is in agreement with the previous publications in which dietary TM was fed to blackspot seabream (*Pagellus bogaraveo*) [59], mandarin fish (*Siniperca scherzeri*) [60]. The HSI index, ranged 1.53–1.65, was consistent with that of Stejskal et al. [32], who reported an HSI of 1.21–1.76 perch fed dietary *H. illucens*. However, VSI in our study, which varied between 13.65 and 14.27, was higher than that reported for perch, which was 8.79–10.56 [61]; 7.30–9.81 [62]; 2.79–3.06 [32]. This discrepancy could be attributed to the differences in the fish size, the dietary lipid content, and mesenteric fat among trials. Our data on MFI (8.75–9.27) were comparable with those reported in perch [63]. The SSI value is also in agreement with one reported earlier [32]. The RGL in the present study, ranged from 0.56 to 0.61, is in agreement with that of carnivores (0.5–2.4) (Kramer and Bryant, 1995). Our RGL data did not differ among the experimental diets, which is in contrast with recent findings that have pointed out the significant effect of dietary insect meal (*T. molitor*) on RGL of trout (*O. mykiss*) [65] and seabream (*S. aurata*) [66]. Despite the adaptive plasticity of gut length to different food sources, perch might experience an initial reduction in their body conditions when consumed diet is changed [67]. This may be a reasonable explanation for the phenomenon observed in our study whereby the body weight and length of perch significantly reduced with increasing inclusion of TM while maintaining their RGL.

The fillet yield in our study (37.42–39.79%) was slightly higher than the 34.87–36.68% reported for perch fed commercial feeds [68], but unaffected by dietary defatted TM, a result that is consent with other publications, reporting the independence of fillet yield of fish is independent of the insect meal dose [65, 66, 69].

Our study revealed that the serum biochemistry was unaffected by dietary insect meal (*T. molitor*), except for AST. The AST activities could be a proxy of stress-induced tissue damage [70]. Song et al. [71] reported that a low level of FM replacement by TM could induce liver damage as indicated by a significant increase in AST activities compared to the control group, thereby indirectly impairing the growth performance of gentian grouper (*Epinephelus lanceolatus* × *E. fuscoguttatus*). In our study, perch fed TM75 showed a significantly lower growth rate than the other groups, which could be linked to stressors. Similarly, Iaconisi et al. (2017) evidenced a stress status of sea bream (*P. bogaraveo*) fed dietary TM. This pattern seemed to be too mild in the present study to induce severe mortality.

# 4.2. Nutrient digestibility

In this study, dietary defatted TM significantly affected nutrient digestibility of European perch, except for ash. All experimental diets resulted in high digestibility values for protein and lipid, whereas lower results were observed for ash and phosphorus (Table 5). In general, chitin in TM-containing diets could be responsible for the different levels of digestibility of perch. This substance could hinder nutrient digestibility by interfering with the digestive enzyme activities of other nutrients [55]. Although many fish can produce the chitin-degrading enzyme, chitinase [56], it seems null in other fish species [72, 73]. The chitinase enzyme is presented in perch and is mainly excreted from the pancreas and, to a lesser extent, produced by intestinal bacteria [74]. However, the animals' capacity to digest chitin remains particularly low and tends to decline with increasing chitin levels [75, 76]. High fibre and chitin contents in TM-

containing diets (Table 1) could reduce the digesta transit time in the gastrointestinal tract, as confirmed in humans, chickens [77] and fish [75], thereby reducing the exposure time of food to digestive enzymes.

In the present study, the observed declining lipid digestibility due to dietary insect meal (*T. molitor*) was consistent with previous findings [55, 66, 78, 79]. Chitin has been reported to bind with lipid and bile in fish and mammals [55, 80], thereby reducing lipid digestibility. Feeding chitin-containing diet has been reported to numerically lower lipase activities in meagre (*Argyrosomus regius*) compared to the chitin-free diet [72]. These effects seem to be too mild to impair digestibility of TM25 and TM50 but do for TM75 in our study. The high fatty acid digestibility observed in our study is in agreement with those reported for Atlantic salmon (*S. salar*) [79, 81].

Wang et al. [14] reported phosphorus digestibility of tilapia (*Oreochromis niloticus*) fed dietary insect meal (*Musca domestica*) ranged from 89.21 to 92.41% and found the independence to *M. domestica* inclusion. The phosphorus digestibility of whiteleg shrimp (*L. vannamei*), ranged from 31.4 to 76.4%, was insect meal (*Bombyx mori*)-dose-dependent [82]. Basto et al. [15] reported that the phosphorus digestibility of seabass (*D. labrax*) fed diets, in which 20% protein diets were replaced by *T. molitor, H. illucens* and locust meal, were in the 57.2–63.9% range. Our results (36.26–45.93%) were affected to a great extent by the dietary defatted TM. The stomach pH [83] and dietary calcium to phosphorous ratio [82] are known to influence phosphorus digestibility of fish. Moreover, the capacity of fish to utilize phosphorous or bind phytate-phosphorus has been shown to vary a great deal from carnivores to omnivores [84].

# 4.3. Fillet composition

The composition of perch fillets in the present study was similar to that of published data on perch fed commercial feeds [68]. The TM-dose independence of fillet traits was also in agreement with previous studies on seabream (*P. bogaraveo*) [59] and rainbow trout (*O. mykiss*) [65]. The particularly low lipid content of fillet (1.20–1.45%) was similar to previous studies on wild and farmed perch [62, 85–88].

Fillets of perch farmed in an intensive aquaculture system have been considered a valuable source of fatty acids, such as linoleic acid (LA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [88]. Our DHA and LA values were relatively higher than those found for wild and farmed perch in earlier studies, whereas EPA remained lower [88, 89]. A more bio-conversion of DHA than EPA, and peroxisomal β-oxidation synthesis of the former from the latter through intermediate product C24:6n3 [62] could explain the discrepancies in the proportion of those FAs in the fillets in our study. We also observed a high LA in the fillets, which was consistent with the result of Xu and Kestemont [86], who reported the ready accumulation of this fatty acid into fish tissues as a stored lipid. The low proportion of linolenic acid (C18:3n3) in the fillets as well as high proportions of DHA and EPA, relative to the respective diets, indicated the high capacity of elongation and desaturation of C18:3n3 in perch, which was confirmed the previous findings [63, 86]. Our study indicated that palmitic acid (15.94–18.35%) and oleic acid (27.39–32.58%) were the predominant saturated and monounsaturated FAs, respectively, in fish fillets. A similar finding was reported for perch {88, 89] and other farmed fish [90].

# 4.4. Gut microbiota

In accordance with previous works on brown trout (*Salmo trutta*) [91], rainbow trout (*O. mykiss*) [24], seabream (*S. aurata*) and European sea bass (*D. labrax*) [25] fed dietary TM, our results showed the consistency of the bacterial diversity and richness of perch regardless of the diet groups. On the other hand, Antonopoulou et al. [25] reported that administration of TM significantly altered the microbiota community of trout (*O. mykiss*). The digestive tract of fish involves multi-physiological functions, which provide abundant and nutritional substrates for microorganisms [92]. Therefore, the discrepancies among studies could be linked to fish physiology and nutritional availability of fish gut, which could be responsible for the dietary treatment effects on the microbiota population [93].

The most prevalent bacteria in intestine of perch fed experimental diets belonged to *Firmicutes, Actinobacteria, and Fusobacteria* phylums. The first two phylums were found abundant in rainbow trout (*O. mykiss*) fed dietary insect meal (*H. illucens*) [94] and also in mealworm larvae (*T. molitor*) [95]. It has been suggested that these bacteria are of insect meal origin [94]. Our sequencing data were aligned with the intestinal microbiota composition of perch and freshwater fish, which are dominated by *Clostridium* genus [96, 97]. This shows that *Clostridium* spp. is a core species in the intestine of European perch.

Contradictory results on the effect of dietary TM on the intestinal population of *Lactobacillus* spp. are present in the literature. Mikołajczak et al. [91] highlighted a significant decrease in the concentration when fed *S. trutta* with 10% hydrolyzed TM administration compared with a FM-based diet. An opposite result was reported for *O. mykiss* [24], where an 0.2-fold increase was observed in a TM-diet, compared to a FM-based one [98]. Józefiak et al. [98] found TM-dose independence pertaining to the count of this lactic acid-produced bacteria in Siberian sturgeon (*Acipenser baerii*). Such discrepancy could be ascribed to the nutrition status in the intestine of tested fish, as it has been well known that *Lactobacillus* group requires nutritious substrates to thrive [99]. A reduction in the *Lactobacillus* genus in intestine of perch fed TM75 in present study could be linked to unfavorable status of perch intestine associated with the deficiency of certain amino acids, fatty acid (DHA, EPA), and with the presence of chitin.

The *Clostridium* and *Lactobacillus* genera, which are among the prevalent species in this study, have been used as probiotics for fish [100]. Therefore, our results suggest the potential application of beneficial microorganisms isolated from the intestine of perch fed insect meal (*T. molitor*).

Bacteria from the *Mycobacterium* genus were also found predominant in perch fed diet treatments. Moutinho et al. [101] conducted a feeding trial on seabream (*S. aurata*) fed dietary meat bone meal as a replacement for FM and reported the existence of these bacteria in the intestine of specimens. *Mycobacterium* spp. are commonly known as the causative agent of mycobacteriosis syndromes in aquaculture species [102] and have a zoonotic potential [103]. Although many of *Mycobacteria* spp. were found to be present in the aquaculture systems in Czech Republic, the clinical pathogen, *M. marinum*, for humans and fish was absent [104]. The high survival rate and absence of pathogenic syndromes of

perch during the 105-day feeding trial could confirm the benignity of these microorganisms in our systems.

Previous studies on Perciformes fish, pikeperch (*S. lucioperca*), largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*), reported the relatively high abundance of *Cetobacterium* genus and suggested the critical role of this genus in fish digestion [105, 106]. The sequencing results detected a prevalence of this genus across four diet treatments and, although the absence of any statistical difference, perch fed TM50 and TM75 tended to proliferate *Cetobacteria* relative to control and TM25 diets. The replacement of 30% of FM by soybean meal was reported to significantly increased the abundance of *Cetobacterium* spp. in largemouth bass [107]. These authors also suggested that the inclusion of plant ingredients in the diets of carnivorous fish could enhance the *Cetobacterium* genus's community, which is responsible for the production of cobalamin, fermented proteins and carbohydrates.

Although established at a low relative abundance (< 0.2%), we found a significant reduction in the population of *Streptococcus* genus in TM75 compared to TM0 diets. These bacteria were found to be present at a low abundance in the digestive tract of *S. salar* (0.6% of the culturable bacterial community) [108], *O. mykiss* (< 0.01%) [109], and to be affected by dietary treatment [110]. Dietary fatty acids were confirmed to alter growth of intestinal bacteria, and linoleic acid, in particular, was shown to inhibit the growth of *Lactobacillus* spp. in the intestine of Arctic charr (*S. alpinus*) [111]. Gram-positive bacteria species were sensitive to dietary fatty acids, and a decrease in *Streptococcus* and *Lactobacillus* communities in TM75 group could be attributed to a significantly higher linoleic acid content in this diet than in the control group [37].

# 4.5. Environmental impacts

In the present study, we investigated the environmental consequence of dietary insect meal (*T. molitor*) in perch aquafeeds, concerning solid waste output, environmental impact associated with one kg of perch produced, and eFIFO, which has been considered as an important proxy for environmental sustainability of aquaculture sector [26, 27, 44].

The dietary defatted TM in the present study did generally not affect solid waste outputs associated with phosphorus waste, except for TM25, although increased nitrogen waste was observed, compared to the FM diet. Therefore, replacement of FM with defatted TM in perch diets could be an essential way of ensuring environmental benefit associated with the waste output, which has remained a critical concern for the public [112]. The digestibility of diet has been considered the most critical issue driving the waste output of aquaculture practices [127]. As previously mentioned, the presence of chitin is the factor that impairs nutrient digestibility of perch the most. Removing chitin components from insect meals [52], and supplementing enzymes [16] and probiotics containing chitinase-producing bacteria could be an effective way of improving digestibility of insect-containing diets for fed fish. Developing a suitable processing technique for aquafeeds could be considered for digestibility efficiency [113], which has recently been achieved for extruding feeds containing insect meals [55, 114].

As far as the environmental impacts associated with 1kg of farmed perch production is concerned, our simulated data indicated consistency of TM25, compared to the control diet, regarding global warming potential, acidification, and land use, but increased impacts pertaining to energy use, eutrophication, and water use. These environmental consequences were mostly influenced by the proportion of insect meal (*T. molitor*) vs. fish meal and FCR in/of experimental diets. The higher environmental impact associated with insect meal production than FM [12, 115] and higher FCR in TM diets and FM diet (Table 3) in our study could be responsible for the aforementioned findings. Le Féon et al. [28] confirmed greater impacts of acidification, eutrophication, GWP, land use, and energy use of TM-containing than insect-free feed for one kg trout produced. Stejskal et al. [32] documented a reduction of water use associated with insect meal (*H. illucens*) compared to FM feeds for perch, whereas GWP, land use and energy use increased. However, Smárason et al. [116] compared *H. illucens*- and FM-based feed associated with seven impact categories, and reported benefits of insect meal inclusion on abiotic depletion, acidification, eutrophication, GWP, human toxicity potential, and marine aquatic ecotoxicity potential, but a negative impact on energy use.

The present study revealed that increasing inclusion levels of insect meal (*T. molitor*) in perch feeds significantly reduced eFIFO, indicating fewer marine forage fish required per unit fish produced. A substantial replacement of FM with defatted TM at 75% reduced eFIFO to as low as 1, whereby the production of perch is a net producer of fish that is aligned with the current trends of most aquaculture species [44]. The observed reduction in eFIFO is consistent with data of Stejskal et al. [32], reporting a significant decrease in the FIFO ratio when dietary *H. illucens* increased. Our eFIFO data could be important information for percid aquaculture in order to move towards an established aquaculture sector in Europe [35].

# 5. Conclusion

The present study highlighted the possibility of using defatted insect meal (*T. molitor*) in the diets of European perch (*P. fluviatilis*), an emerging, potential aquaculture candidate in Europe. It is recommended, for future aquaculture of this species, including as low as 6.8% or 25% FM replacement by defatted yellow mealworm, which could benefit the sector with respect to growth performance and environmental consequences. Although a substantial replacement of FM by defatted TM did not show promising outcomes for all the aspects considered in the present study, in particular concerning the waste output perspective, this replacement could reduce the total solid load, phosphorus waste, and economic fish-in fish-out in the aquaculture of European perch. Our study also underlined the major bottleneck of a substantial inclusion of defatted insect meal (*T. molitor*) in fish diets, as nitrogen waste and environmental consequences associated with one unit of farmed perch produced.

# Declarations

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## Author contributions

Vlastimil Stejskal, Laura Gasco: Conceptualization, Methodology, Funding acquisition, Supervision; Hung Quang Tran: Data curation, Writing - Original Draft, Writing - Review & Editing; Markéta Prokešová, Mahyar Zare, Jan Matoušek, Pavel Šablatura: Data curation; Ilario Ferrocino: Formal analysis, Writing - Original Draft. All authors have read and approved the final manuscript.

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## Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

## *Ethics approval and consent to participate*

The experimental procedures were conformed with the European Community Directive (No. 2010/63/EU) and were authorized by the Czech Ministry of Health (No. MSMT-6744/2018-2) regarding the protection of animals used for scientific purposes.

## *Consent for publication*

Not applicable

## *Competing interests*

The authors declare that they have no competing interests

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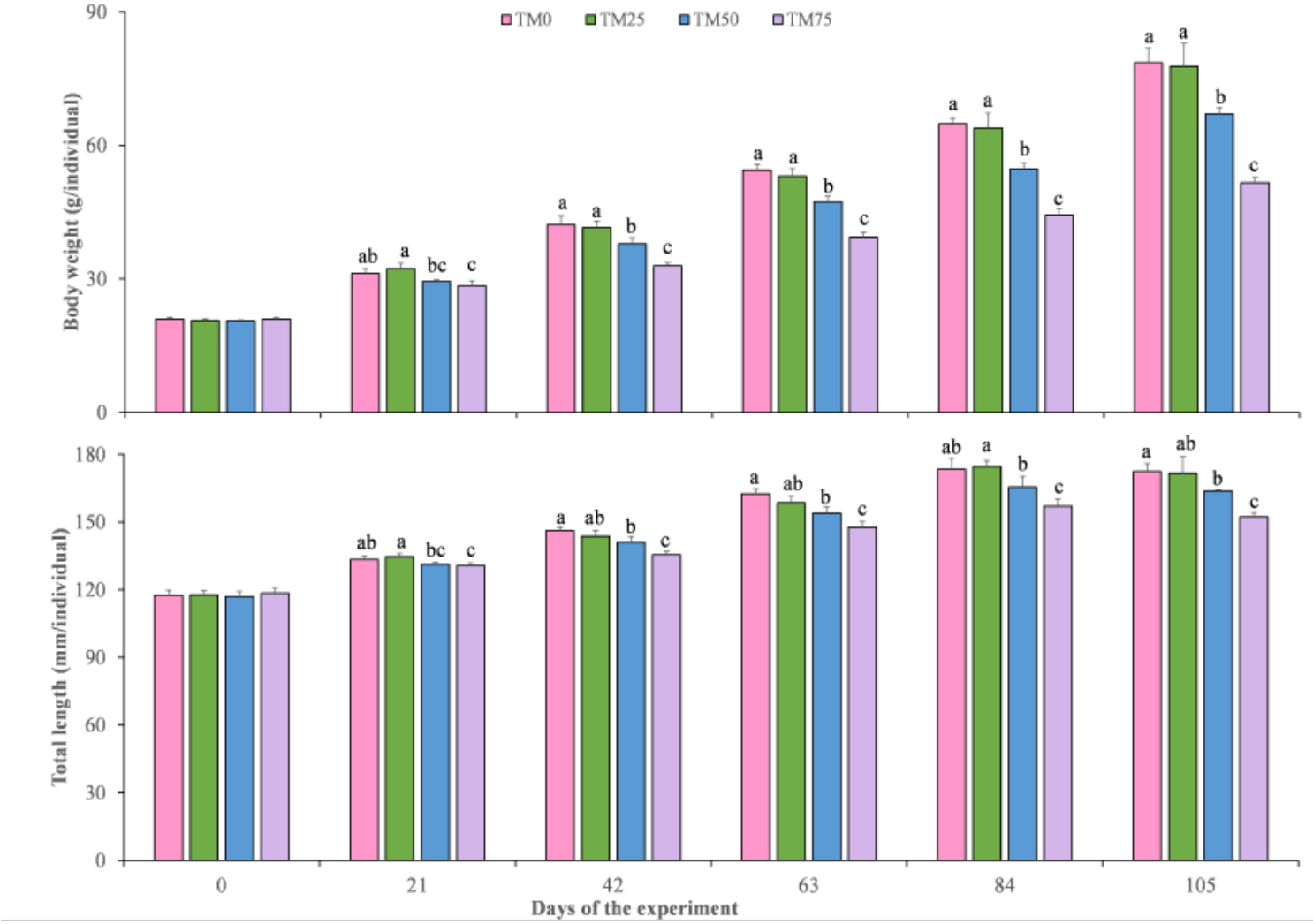
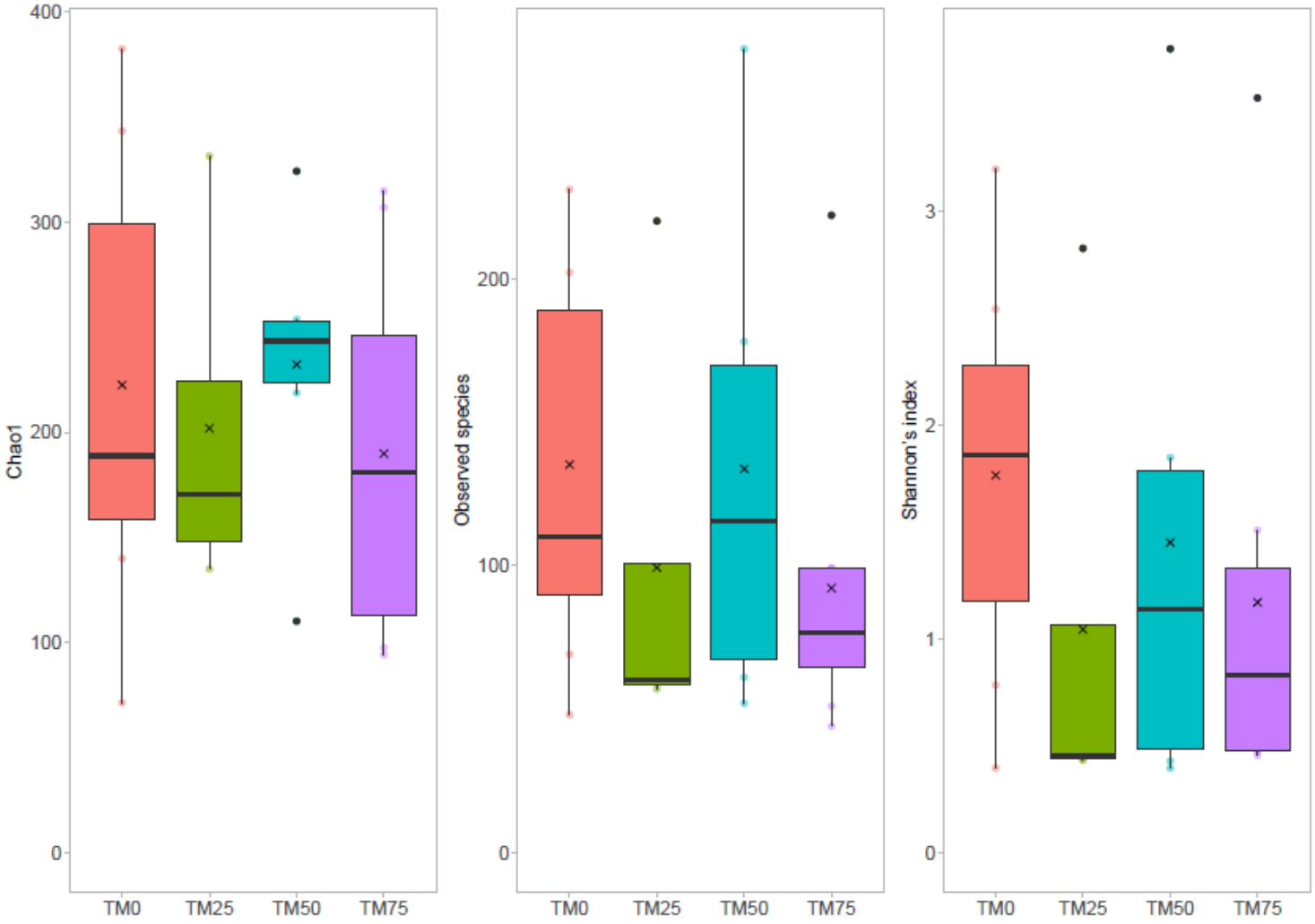
# Figures



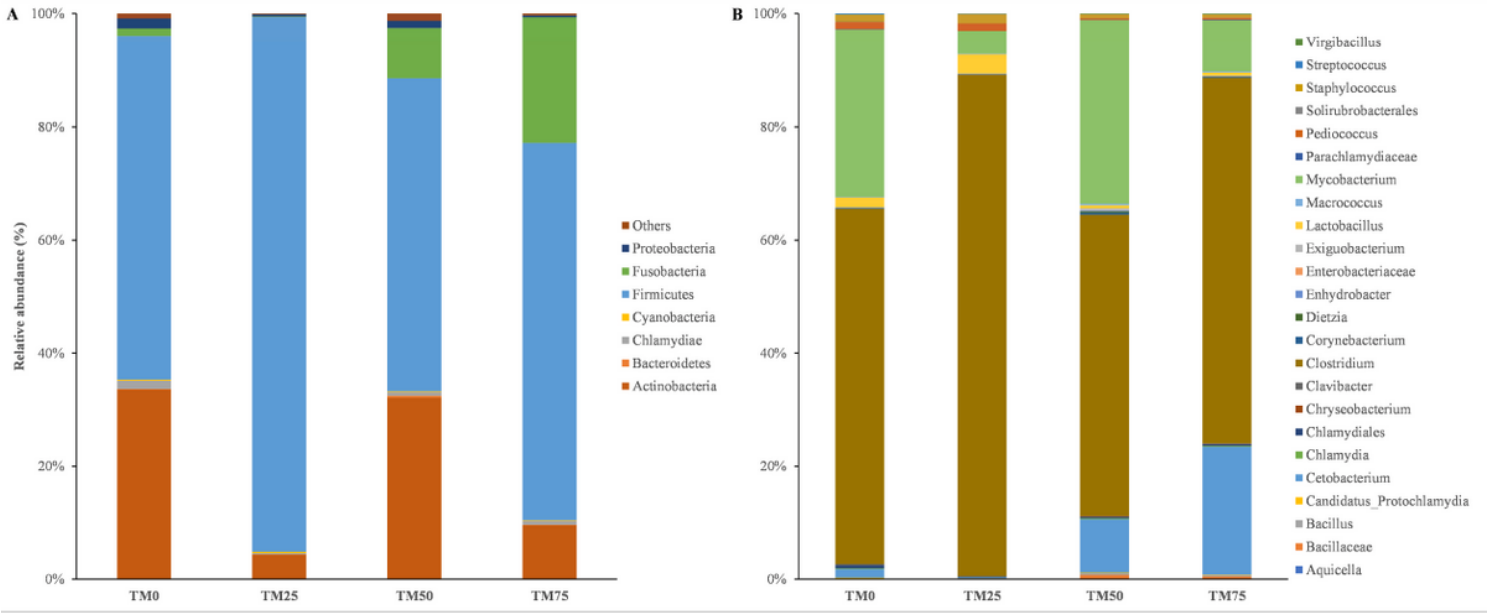

## Figure 1

Body weight and total length of European perch (Perca fluviatilis) fed dietary yellow mealworm Tenebrio molitor larvae meal throughout sampling time-series



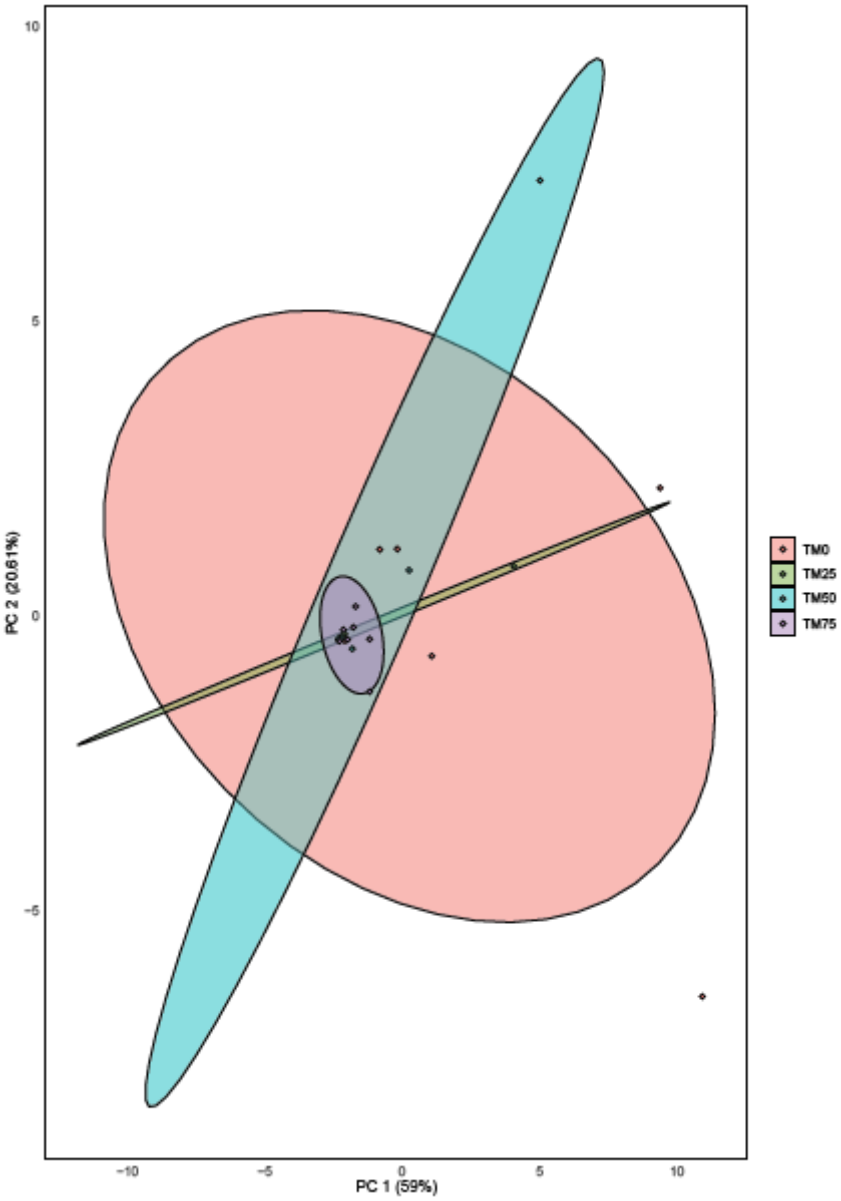

## Figure 2

Alpha diversity measures of the intestinal microbiota of European perch fed experimental diets. The black ‘x’ in the boxes represents the mean value, the horizontal line within the boxes represents the median separating interquartile range (upper quartile and lower quartile). The coloured circles and black circles represent observed data and outliers beyond the whiskers, respectively



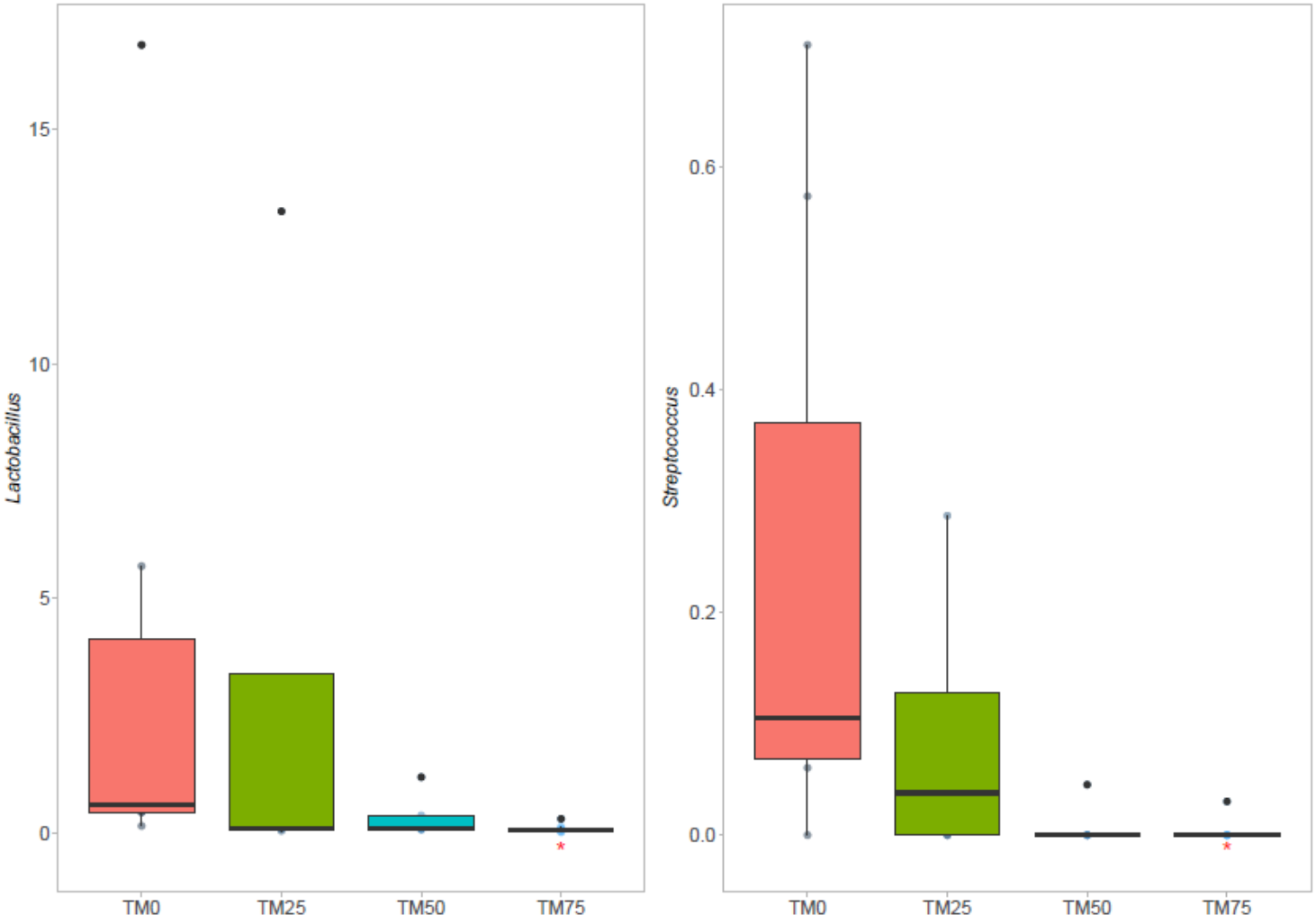

## Figure 3

Mean relative abundance (%) of the bacterial community at phyla (A) and genus (B) levels in the intestine of European perch fed the experimental diets




## Figure 4

Principal Coordinate Analysis (PCoA) in intestinal bacteria of European perch fed experimental diets




## Figure 5

Boxplots showing the relative abundance of the Streptococcus and Lactobacillus genera in the intestine of European fed experimental diets. The red asterisks indicate a significant difference from the control group