Hindawi
Aquaculture Research
Volume 2023, Article ID 6680943, 15 pages
https://doi.org/10.1155/2023/6680943

Research Article

Partial Replacement of Fishmeal with Duckweed (*Spirodela polyrhiza*) in Feed for Two Carnivorous Fish Species, Eurasian Perch (*Perca fluviatilis*) and Rainbow Trout (*Oncorhynchus mykiss*)

T. Stadtlander,[1] **F. Tschudi**,[2] **A. Seitz**,[2] **M. Sigrist**,[2] **D. Refardt**,[2] **and F. Leiber**[1]

[1]*Research Institute of Organic Agriculture (FiBL), Frick 5070, Switzerland*
[2]*Zurich University of Applied Sciences (ZHAW), Wädenswil 8820, Switzerland*

Correspondence should be addressed to T. Stadtlander; timo.stadtlander@fibl.org

Received 16 January 2023; Revised 6 March 2023; Accepted 7 March 2023; Published 16 March 2023

Academic Editor: Houguo Xu

Two four-week feeding trials were conducted with fingerlings of Eurasian perch (*Perca fluviatilis*, 3.52 ± 0.08 g) and rainbow trout (*Oncorhynchus mykiss*, 1.49 ± 0.05 g) fed with graded levels of dried (DWD) and fermented (DWF) duckweed meal (*Spirodela polyrhiza*). The purpose of these two trials was to evaluate DWD and DWF as replacements for fishmeal. Fishmeal protein was substituted by 12%, 24%, and 35% of duckweed protein and compared to control diets containing 40% (for perch) and 35% (for rainbow trout) fishmeal and no duckweed. The performance of the fish (growth, feed conversion, and protein and lipid utilization) and their whole-body composition were evaluated and compared with the control. While even the lowest inclusion level, regardless of its form (dried or fermented), resulted in significantly reduced performance in Eurasian perch, rainbow trout were able to utilize feed containing duckweed meal considerably well. Compared to the control, at a 12% inclusion level, rainbow trout showed an equal or comparable percent weight gain (PWG; DWD: 377%, DWF: 373%), specific growth rate (SGR; DWD: 4.37%/day, DWF: 4.33%/day), feed conversion ratio (FCR; DWD: 1.11, DWF: 1.12), and protein productive value (PPV; DWD: 21.5%, DWF: 21.2%). Increasing the inclusion levels above 12% of both DWD and DWF resulted in reduced performance in rainbow trout, with the most pronounced effects observed in the DWD35 group. All experimental diets, including control, affected the whole body composition of perch, most notably reducing the lipid content compared to initial fish. Compared to initial, control and DWD rainbow trout increased whole-body protein, lipid, and ash contents. In conclusion, for rainbow trout, fermented and dried *S. polyrhiza* duckweed meal appears to be a promising feed ingredient when used at a maximum inclusion level of 12%, while for Eurasian perch, it should not be considered as a feed ingredient.

1. Introduction

Aquaculture is the leading global consumer of fishmeal, despite a significant decrease in its relative inclusion levels in fish and crustacean diets over the last decades [1, 2]. While the fishmeal inclusion level for most grow-out diets has decreased significantly over time, especially the diets for various carnivorous fish species' fry and fingerlings still contain high amounts of fishmeal with contents as high as 50% being found in practical diets. Replacing fishmeal in the diets of carnivorous fry and fingerlings with a more sustainable protein source that does not compete with human food [3] could improve aquaculture sustainability. Fishmeal is regarded as the gold standard for aquafeeds but is also strongly disputed as being unsustainable. Although producing fishmeal from the trimmings of fish caught for human consumption is playing an increasingly important role in fish feeds [4], the targeted fishmeal production is considered unsustainable and detrimental to natural marine ecosystems [5]. Furthermore, it has been estimated that around 90% of fish caught for reduction to fishmeal are food or prime food grade, and its use for aquaculture directly competes with human food consumption [6]. Plant-based protein sources, such as soybeans, canola/rapeseed, maize, and wheat, have been developed and now serve as the primary protein sources in aquaculture feeds [7].

Soy, the primary fishmeal substitute, has high external costs due to habitat loss, eutrophication, acidification, and pesticide and plant protection product uses in Brazil and Argentina [8]. Additionally, soy can be directly consumed by humans, and it contains several antinutritional factors (ANF) such as protease inhibitors, lectins, phytoestrogens, saponins, and phytic acid [9]. Including soybean meal or soy protein concentrate in salmonid diets significantly reduces their specific growth rates [10]. Carnivorous fishes, such as salmonids, sea bass, and sea bream, are among the world's largest consumers of fishmeal [11], making the search for finding sustainable substitutes for fishmeal an important issue in aquaculture [12]. In recent years, insect meals from different species, particularly those made from the black soldier fly (BSF, *Hermetia illucens*), have shown great promise as a fishmeal replacement [13]. However, especially in the European Union and Switzerland, their production is only permitted when feed grade substrates are used (EU 2021/1372). This practice may not be sustainable, as it places BSF feed in direct competition with feed for other livestock [14].

Another potential protein source for aquaculture feeds is duckweed or water lentil species. These aquatic floating plants, which belong to the family Lemnaceae, have a high protein content ranging between 30 and 45% of dry matter [15–18]. Duckweed species are also known for their high efficiency in nitrogen (N) and phosphorus (P) uptake, with the ability to remove up to 98% of N and P from their growth medium [18–22].

For this project, duckweed species *Spirodela polyrhiza* was utilized as feed for two important carnivorous aquaculture species in Switzerland, Eurasian perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*). These fishes have a trophic level of >4 (according to https://www.fishbase.org/, as of March 14, 2022) and were selected for this study for three main reasons: (i) their economic importance in Switzerland, (ii) their high trophic level and thus their high protein requirement, and (iii) the lack of prior research on the use of duckweed in the diet of perch and rainbow trout fingerlings. While both species are highly carnivorous freshwater fish with similar natural food preferences (e.g., both consume insect larvae, insects, and other invertebrates, and as they grow, they are both increasingly piscivorous), they differ in their culture techniques. Rainbow trout culture has been established globally for over a century and is mostly practiced in open flow-through and partly recirculated systems, while perch culture is relatively young and mostly conducted in indoor recirculating aquaculture systems (RAS). Additionally, rainbow trout are among the most extensively studied fishes, and thus their nutritional requirements are well known, whereas perch are not produced in large numbers, and their nutritional requirements have not been well researched [23]. While trout are well suited for cold to medium temperatures, with an optimal temperature of 12–14°C, perch exhibit the best growth rates in warmer waters, with an optimal temperature of around 22°C.

We conducted two separate experiments to investigate the use of dried and fermented duckweed as a fish meal protein replacement for fingerlings of both Eurasian perch and rainbow trout. In two similar trials, varying amounts of duckweed were included to replace 12, 24, and 35% of fishmeal protein in the experimental diets. Two different *S. polyrhiza* meals, one derived from dried (DWD) and one derived from fermented (DWF) duckweed, were used and evaluated for their effects on growth and whole body proximate composition, as well as feed, protein, and lipid utilization.

2. Materials and Methods

2.1. Duckweed Production. Duckweed (*Spirodela polyrhiza*, collection number 9346) was provided by the Landolt Duckweed Collection (Zurich, Switzerland). The fronds were initially grown in a climate chamber, as described by Stadtlander et al. [21]. They were then transferred to a static system with a circular tank with a volume of 1.7 m^3 before being mass produced in two 20 m^2 circular pools, each of which was placed in a greenhouse. The duckweed production was initiated with an ammonium-nitrogen (NH_4-N) concentration of 20 mg l^{-1} on a modified Hoagland medium (Table 1). When the concentration of NH_4-N fell below 0.1 mg l^{-1}, it was increased again to 20 mg·l^{-1} by supplying additional fertilizer. Fresh biomass was harvested and air-dried on a shaded bench inside the greenhouse to protect it from direct sunlight before being completely dried in a drying chamber. Alternatively, the fresh biomass was fermented using effective microorganisms EM 1 (prepared to EM A according to the manufacturer's specification; EM Schweiz AG, Switzerland) and *Pediococcus pentosaceus* (PP100-25, BIOAGRO S.r.l., Italy) as starter cultures. The fermented duckweed was then dried in the same way as the freshly harvested biomass. All dried biomasses were ground to a fine powder and refrigerated at 4°C until use in the fish feed.

2.2. Feed Preparation. For both fish species, seven different diets, one control diet, and six test diets were prepared. Dried duckweed (DWD) meal and fermented duckweed (DWF) meal were used to replace 12, 24, and 35% of fishmeal protein in both species. The corresponding treatment groups were C (control, no duckweed), DWD12, DWD24, and DWD35, as well as DWF12, DWF24, and DWF35.

The proximate composition of feed ingredients is presented in Table 2. The nutritional content of the duckweed differed slightly for perch and rainbow trout due to the different harvest times of the duckweed batches. The diets were formulated according to Table 3 (perch) and Table 4 (rainbow trout) with some minor differences in formulation between species. The nutritional content was based on Fiogbé et al. [24] and Kestemont et al. [25] for perch and NRC [23] for rainbow trout. The diets were designed to be iso-nitrogenous, with a crude protein (CP) content of

Table 1: Modified Hoagland medium.

Compound	Concentration ($g \cdot l^{-1}$)
$MgSO_4 \cdot 7\ H_2O$	246
$Ca(NO_3)^2 \cdot 4\ H_2O$	236
$NH_4H_2PO_4$	136
KNO_3	101
H_3BO_3	2.86
$MnCl_2 \cdot 4\ H_2O$	1.82
$ZnSO_4 \cdot 7\ H_2O$	0.22
$Na_2MoO_4 \cdot 2\ H_2O$	0.09
$CuSO_4 \cdot 5\ H_2O$	0.09
$FeCl_3 \cdot 6\ H_2O$	0.484
EDTA iron(III) sodium salt	1.5

around 530 g kg^{-1} for both fish species, a crude lipid (CL) content of around 140 $g \cdot kg^{-1}$ for perch and 160 $g \cdot kg^{-1}$ for rainbow trout. The duckweed-free control diet was based on a high percentage of fishmeal (400 $g \cdot kg^{-1}$ for perch and 350 $g \cdot kg^{-1}$ for rainbow trout) and a poultry by-product meal (perch) or wheat gluten (rainbow trout) as protein sources. For preparation of the diet, all dry ingredients were thoroughly mixed using a handheld kitchen mixer. Lipid was then added and mixed again thoroughly until no lipid globules remained. Thereafter, water was added, and the dough was mixed and kneaded until the moisture was homogenously distributed. The dough was pelleted through a 2 mm dye in a meat grinder and dried at 40°C for 24 hours. The dry pellets were ground in a mechanical grain mill and sieved to produce final pellet fractions of 0.8–1.0 mm, 1.0–1.2 mm, and 1.2–1.6 mm. The feed was stored at −20°C when not in use.

2.3. Feeding Trial. The experiments were approved by the animal welfare committee of the canton of Aargau in Switzerland and the cantonal veterinarian authority under permission number AG 75722.

2.3.1. Eurasian Perch—Perca fluviatilis. A total of 780 perch fingerlings (3.52 ± 0.08 g, mean ± SD) were provided by Valperca (Charvonay, Switzerland), and they were acclimatized for one week in 55-l aquaria connected to a flow-through system. During the one-week acclimatization phase, the perch were fed the same feed that was used on the commercial farm (Biomar Inicio plus M; 1.1 mm, 57% CP, 15% CL, 10.4% CA, 0.4% crude fiber (CF)). Afterwards, 220 fish were subdivided into three similar groups, euthanized (150 $mg \cdot l^{-1}$ MS-222 buffered with 300 $mg \cdot l^{-1}$ sodium bicarbonate), and used for the initial determination of proximate composition. The remaining 560 fish were then placed into 28 10-l aquaria in groups of 20 fish each (initial stocking density approximately 7 $kg \cdot m^{-3}$), resulting in four replicates per diet that were randomly allocated. The aquaria were connected to a small recirculating system with a total volume of around 600 l and equipped with individual air stones. Water exchange was carried out daily in the early afternoon, with 200 l of the total water volume being replaced. Water quality (oxygen content and saturation, ammonium, nitrite, and pH) was determined twice per week in the morning. The average water temperature throughout the experiment was 21.9°C and ranged from 20.4 to 23.0°C, the average oxygen concentration was 6.52 mg l^{-1}, the average oxygen saturation was 77.9%, the average ammonium concentration was 0.27 mg l^{-1}, the average nitrite concentration was 0.32 mg l^{-1}, and the average pH was 8.05. During the whole experiment, a 12 h/12 h light/dark lighting regime was applied. Feed allowance was initially set to 5% [24] but was reduced to 3.5% of body weight per day. This was due to low acceptance of the experimental diets, in order to guarantee full consumption of all the feed that was provided. Feeding was carried out for six days a week with no feeding on Sundays to improve precision for the group weighings on Monday. A restricted feeding regime was chosen in order to guarantee equal feed consumption for all groups. Feeding was carried out by hand three times per day during the week and twice per day on Saturday with equally sized portions. At the start of the experiment and every Monday, the fish were group-weighed and the feed amount was adapted to the new group weights.

2.3.2. Rainbow Trout—Oncorhynchus mykiss. A total of 545 rainbow trout fingerlings (1.49 ± 0.05 g, mean ± SD) were purchased from a commercial trout farmer in Switzerland (Pisciculture de Vionnaz, Switzerland), and they were acclimatized for one week in 55-l aquaria connected to a flow-through system. During the acclimatization phase, the rainbow trout were fed the commercial feed that was used in the hatchery (Le Gouessant, Neo Supra, 1.1 mm, 58% CP, 13% CL, 9% CA, 0.5% CF). Afterwards, 120 fish were subdivided into 3 equal groups, euthanized (150 $mg \cdot l^{-1}$ MS-222 buffered with 300 $mg \cdot l^{-1}$ sodium bicarbonate), and used for the initial determination of proximate composition. The remaining 420 fish were then placed into 28 10-l aquaria in groups of 15 fish each (initial stocking density around 2.2 $kg \cdot m^{-3}$), resulting in four replicates per diet that were randomly allocated. The aquaria were connected to the same system that was described for the perch (above), and the feeding and weighing procedures, as well as the lighting conditions and water exchange were identical. Water quality (oxygen content and saturation, ammonium, nitrite, and pH) was determined twice per week in the morning. The average water temperature was 16.8°C and ranged from 15.5 to 17.3°C, the average oxygen concentration was 8.41 $mg \cdot l^{-1}$, the average oxygen saturation was 90%, the average ammonium concentration was 0.34 $mg \cdot l^{-1}$, the average nitrite concentration was 0.44 $mg \cdot l^{-1}$, and the average pH was 8.24. Feeding was carried out as was described for the perch but with a daily feed allowance of 5% of the body weight.

2.4. Sampling and Chemical Analysis. For both species, the general sampling schemes were mostly identical. After four weeks of experimental feeding, both experiments were terminated. All fish were euthanized as described above and group-weighed, and the largest and smallest two fish in each aquarium were individually weighed, and their fork length was measured. For the two largest fish in each aquarium, the

Table 2: Proximate composition of feed ingredients (on dry matter basis).

	DM ($g \cdot kg^{-1}$ FM)	CP ($g \cdot kg^{-1}$ DM)	CL ($g \cdot kg^{-1}$ DM)	CA ($g \cdot kg^{-1}$ DM)	NFE ($g \cdot kg^{-1}$ DM)	CF ($g \cdot kg^{-1}$ DM)	GE ($kJ \cdot g^{-1}$ DM)
Fishmeal	929	671	91.5	234	4.30	n.d	18.8
DWD perch	950	350	42.1	189.5	418.4	n.d	17.9
DWD trout	937	372	32.0	202	298	96.1	15.6
DWF perch	900	275	44.4	200	480.6	n.d	17.6
DWF trout	959	331	44.8	142	380	103	16.8
Wheat gluten	932	868	64.4	8.60	59.9	3.20	23.2
PBM	940	745	106	95.7	53.2	n.d	22.0
Wheat flour	887	149	21.4	16.9	813	22.5	20.4
Potato starch	810	0.00	0.00	1.60	998.4	0.00	19.9
Alpha-cellulose	950	0.00	0.00	0.00	0	100	20.0

FM: fresh matter, DM: dry matter, CP: crude protein, CL: crude lipid, CA: crude ash, NFE: nitrogen free extract, CF: crude fiber, GE: gross energy, DWD: dried duckweed, DWF: fermented duckweed, and PBM: poultry by-product meal.

Table 3: Feed formulation for perch fry and feed proximate composition (g·kg^{-1} DM).

	Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35
Fish meal	400.0	352.0	304.0	260.0	352.0	304.0	260.0
PBM	319.8	319.8	319.8	319.8	319.8	319.8	319.8
DWD	0.0	89.8	179.6	261.9	0.0	0.0	0.0
DWF	0.0	0.0	0.0	0.0	114.3	228.5	333.3
Potato starch	147.0	115.0	80.0	50.0	95.0	45.0	0.9
α-cellulose	45.9	35.2	27.6	18.5	32.0	16.2	0.0
Fish oil	67.4	68.2	69.1	69.9	66.9	66.5	66.1
Vitamin premix	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mineral premix	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Proximate composition							
Dry matter (%)	92.0	92.8	93.4	93.6	91.9	92.5	92.9
Crude protein (% DM)	52.8	52.7	52.9	53.0	53.1	53.4	53.5
Crude lipids (% DM)	14.1	13.6	14.0	13.9	13.8	14.1	14.3
Crude ash (% DM)	12.5	13.0	13.7	14.3	13.1	13.1	13.3
Crude fiber (% DM)	2.93	3.34	2.89	3.31	3.26	3.24	3.12
NFE (% DM)	17.6	17.3	16.5	15.5	16.8	16.2	15.7

PBM: poultry by-product meal; NFE: nitrogen free extract (100–CP–CL–CA–CF).

Table 4: Feed formulation for rainbow trout fry and feed proximate composition (g·kg^{-1} DM).

	Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35
Fish meal	350.0	308.0	266.0	227.5	308.0	266.0	227.5
Wheat gluten	321.2	321.2	321.2	321.2	321.2	321.2	321.2
DWD	0	75.6	151.2	220.6	0	0	0
DWF	0	0	0	0	85.2	170.4	248.5
Wheat flour	77.0	77.0	77.0	77.0	77.0	77.0	77.0
α-cellulose	126.1	91.1	56.1	23.9	82.9	39.7	0
Sunflower oil	105.6	107.1	108.5	109.8	105.7	105.7	105.7
Vitamin premix	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mineral premix	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Proximate composition							
Dry matter (%)	95.1	94.4	94.2	93.5	94.7	94.3	94.6
Crude protein (% DM)	52.7	52.8	52.9	52.8	52.9	53.2	53.1
Crude lipids (% DM)	16.0	15.5	15.7	16.0	15.6	15.7	16.2
Crude ash (% DM)	9.57	10.1	10.6	11.1	9.61	9.86	10.0
Crude fiber (% DM)	8.52	6.67	5.31	3.64	6.65	4.35	2.33
NFE (% DM)	13.2	15.0	15.5	16.4	15.2	16.9	18.4

NFE: nitrogen free extract (100–CP–CL–CA–CF).

intestines were excised and weighed to determine the intestine-somatic index (ISI). The liver was subsequently excised and weighed to determine the hepatosomatic index (HSI). To estimate the amount of intestinal lipid in the two largest fish, a 4-step key was used. An estimation of the intestinal fat was made, it ranges from 0–25, 26–50, 51–75, and 75–100%. The lowest category (0–25%) indicated no or very little visible intestinal fat, while the highest category (75–100%) indicated that only fat was visible after opening the abdominal cavity. All fish, including those used for determination of ISI and HSI, as well as the fish sampled at the onset of both experiments, were stored in groups at −20°C until whole body analysis was completed.

For whole-body composition analysis, frozen fish were cut with garden scissors while still frozen, autoclaved for 30 minutes at 90°C, and homogenized (Ultra-Turrax T25, IKA Labortechnik, Staufen, Germany). The fish homogenate was frozen at −20°C and lyophilized (Alpha 1-4 LSC, Christ, Osterode am Harz, Germany). Lyophilized fish samples were ground using a coffee mill, and the dried powder was stored at −20°C until proximate composition analysis.

The experimental diets and homogenized fish were analyzed for dry matter (DM), crude protein (CP), crude lipids (CL), and crude ash (CA), and the diets were additionally analyzed for crude fiber (CF). In brief, DM was determined by drying samples for five hours at 105°C, CP was determined by the Dumas method ($CP = N \times 6.25$), CL was determined by the Soxhlet method, CA was determined by burning the samples for 5 hours at 550°C in a muffle furnace, and crude fiber was determined according to method 6.1.1 (Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten) [26]. To determine the CA content of the samples, digestion was performed using boiling sulphuric acid and caustic potash. The resulting residue was filtered, washed, dried, and weighed. The essential amino acid (EAA) determination for the different experimental diets was performed according to the method described by Bidlingmeyer et al. [27]. Feed samples were collected in the last week of both experiments and pooled per treatment and species. The samples were hydrolyzed using hydrochloric acid, and derivatization was carried out using a mix of ethanol, triethylamine, water, and phenyl isothyocyanate (7 : 1 : 1 : 1) at ambient temperature for 20 min. Amino acid determination was carried out with liquid chromatography and an aqueous buffer (0.14 M sodium acetate containing 0.15 ml·l^{-1} trimethylamine, pH 6.35) and 60% acetonitrile in water as solvents. The nitrogen-free extract was estimated by difference for feed (NFE = 100–CP–CA–CL–CF) and fish (without CF).

2.5. Calculations and Statistics. To evaluate the growth and feed utilization response of the fish, the following formulas were used:

Weight gain (WG, g): final body weight – initial body weight

Percent weight gain (PWG, %): (final body weight – initial body weight)/initial body weight·100

Specific growth rate (SGR, % day^{-1}): [(ln final body weight) – (ln initial body weight)]/days of experiment·100

Condition factor (*K*): [(final body weight)/(fork length^3)]·100

Intestine somatic index (ISI, %): (intestine weight/whole body weight)·100

Hepatosomatic index (HSI, %): (liver weight/whole body weight)·100

Feed conversion ratio (FCR): total dry feed intake/weight gain

Protein productive value (PPV; %): [(final fish protein content – initial fish protein content)/(total feed protein intake)]·100

Lipid productive value (LPV; %): [(final fish lipid content – initial fish lipid content)/(total feed lipid intake)]·100

Data from both experiments were statistically analyzed using SPSS version 24 (IBM Corporation, Armonk, USA), following Amelchanka et al. [28]. For that, the procedure "compare means > one-way analysis" was used to run a polynomial contrast analysis for all parameters to evaluate the effects of dried and fermented duckweed supplementation on perch and rainbow trout. The contrast analysis was conducted between the control and either the dried (DWD) or fermented (DWF) duckweed groups. For the whole body composition comparison, two contrast analyses were performed for each duckweed type. One analysis compared the whole body composition of the initial fish to that of the final fish, including the control, and the other contrast analysis compared the control and duckweed fed fish. A significance level of $p < 0.05$ was considered for all data which are presented as the mean value of four replicates ± standard deviation, if not stated otherwise.

3. Results

Analysis of diet proximate compositions revealed comparable nutrient levels between all diets for the same fish species (Tables 3 and 4). Essential amino acid composition and total EAA showed relatively little variation in the perch diets (Table 5), while the amounts of all EAAs in the rainbow trout diets tended to be lower with higher duckweed concentration and generally in the diets that contained fermented duckweed (DWF), with the most pronounced reduction in DWF35 (Table 6). While most EAA in the rainbow trout diets remained at or above the required level, lysine was below the required levels in all diets and methionine was below the required levels in the DWF diets.

3.1. Eurasian Perch—Perca fluviatilis. Feed acceptance for perch was low at the start of the experiment, and only after 2-3 days were all experimental diets accepted well by the fish. As this was the case with all diet types, this cannot be attributed to the inclusion of duckweed. Once feeds were accepted, they were consumed within two minutes of feeding with a tendency toward slower consumption in the higher concentrated duckweed diets. Perch reared on diets that contained duckweed had significantly reduced performance, including growth, feed conversion, and nutrient utilization (Table 7). The reduction in performance and feed and nutrient utilization was more pronounced in perch fed fermented duckweed compared to perch fed dried duckweed. The results show clearly that the performance reduction increased with increasing the duckweed inclusion level, independent of duckweed type (Table 7 and Figure 1). The performance reduction was already seen in perch fed with DWD12, which was the best performing duckweed diet. The PWG was only 86.5% compared to 111% in the control. The worst performing duckweed diet, DWF35, resulted in just 35.4% PWG. Similarly, SGR, FCR, PPV, and LPV were inferior in DWF35 (0.86% day^{-1}, 4.60, 6.03%, and 9.13%, respectively) compared to control (SGR: 2.13, FCR: 1.53, PPV: 20.0%, and LPV: 11.7%, respectively). Perch fed with the highest inclusion level of fermented duckweed also showed a marked increase in liver weights and a reduction in survival rates. The most obvious change in perch whole body composition between the initial fish and fish of all experimental groups including the control was a significant decrease in CL and an increase in CA (Table 8). When comparing the two duckweed types with the control, no significant trend was observed for moisture, CP, CL, or CA. Perch fed with DWF35 and DWD35 showed higher mortalities (21.2% and 15%, respectively) compared to the other duckweed groups and the control diets.

3.2. Rainbow Trout—Oncorhynchus mykiss. Immediately after the experiment started, the rainbow trout accepted all experimental feeds, including those with the highest inclusion levels of both duckweed types, and all feed was eaten within the first minute after feeding. Similarly to perch, rainbow trout performance, including growth, feed, and nutrient utilization was influenced by feeding both

Table 5: Essential amino acids in the different perch diets (% DM).

	Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35	Req
Arginine	3.18	3.11	3.36	3.06	3.35	3.08	2.96	1.8
Histidine	1.04	1.02	1.06	1.00	1.11	1.05	1.02	
Leucine	3.24	3.17	3.44	3.21	3.45	3.37	3.27	
Isoleucine	1.81	1.75	1.88	1.76	1.90	1.85	1.78	
Lysine	3.89	3.73	3.74	3.47	3.65	3.50	3.19	2.2
Methionine	0.91	0.84	0.83	**0.75**	0.91	0.84	0.84	0.8
Phenylalanine + tyrosine	2.79	2.73	2.83	2.73	2.95	2.97	2.92	
Threonine	1.75	1.70	1.89	1.68	1.82	1.72	1.66	1.2
Valine	2.32	2.27	2.49	2.31	2.49	2.41	2.34	
Sum	20.93	20.32	21.52	19.97	21.63	20.79	19.98	

Bold numbers indicate values below the required level according to the National Research Council for European sea-bass (*Dicentrarchus labrax*) (rightmost column). Requirements for Eurasian perch are not published.

Table 6: Essential amino acids in the different rainbow trout diets (% DM).

	Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35	Req
Arginine	2.10	2.24	2.17	2.13	1.88	1.89	1.73	1.5
Histidine	1.03	1.06	0.98	1.01	0.97	0.99	0.89	0.8
Leucine	3.33	3.41	3.18	3.25	3.08	3.19	2.96	1.5
Isoleucine	1.82	1.87	1.83	1.81	1.64	1.69	1.52	1.1
Lysine	**1.99**	**2.27**	**1.90**	**1.76**	**1.86**	**1.85**	**1.46**	2.4
Methionine	0.80	0.78	0.74	0.70	**0.61**	**0.64**	**0.57**	0.7
Phenylalanine + tyrosine	3.31	3.47	3.37	3.23	2.88	3.10	2.83	1.8
Threonine	1.43	1.47	1.37	1.41	1.21	1.27	1.13	1.1
Valine	2.16	2.23	2.17	2.17	1.97	2.03	1.89	1.2
Sum	17.97	18.8	17.71	17.47	16.1	16.65	14.98	

Bold numbers indicate values below the required level according to the National Research Council [23] (rightmost column).

Table 7: Dietary effects on growth, feed conversion, protein and lipid utilization, and body condition parameters in Eurasian perch. Values are averages of four replicates.

	Perch fed with								p value	
		Dried duckweed (DWD)			Fermented duckweed (DWF)					
	Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35	SEM	DWD	DWF
IBW (g)	3.53	3.46	3.55	3.51	3.50	3.51	3.55	0.02	—	—
FBW (g)	7.30	6.45	6.05	5.20	6.25	5.69	4.68	0.18	<0.001	<0.001
WG (g)	3.76	2.99	2.50	1.69	2.75	2.18	1.13	0.17	<0.001	<0.001
PWG (%)	111	86.5	75.0	50.2	80.6	64.4	35.4	5.09	<0.001	<0.001
SGR (% day^{-1})	2.13	1.78	1.59	1.16	1.68	1.39	0.86	0.09	<0.001	<0.001
FCR (g·g^{-1})	1.53	1.79	2.10	2.88	1.86	2.42	4.60	0.24	0.011	0.011
PPV (%)	20.0	16.7	12.9	11.5	15.3	11.4	6.03	0.88	<0.001	0.010
LPV (%)	11.7	1.91	5.16^{a}	−8.17	−1.45	−0.63	−9.13	1.36	0.033	<0.001
K	1.57	1.51	1.54	1.46	1.52	1.41	1.45	0.02	0.843	0.019
ISI (%)	7.67	7.55	8.12	7.70	8.10	6.92	8.13	0.12	0.575	0.691
HSI (%)	1.42	1.40	1.33	1.40	1.32	1.27	1.50	0.03	0.550	0.036
Visceral fat (%)	46.9	25.0	25.0	21.9	25.0	33.3	25.0	2.67	0.026	0.460
Survival (%)	93.8	93.8	85.0	92.5	95.0	92.5	78.8	1.86	0.422	0.023

IBW: initial body weight; FBW: final body weight; WG: weight gain; PWG: percentage weight gain; SGR: specific growth rate; FCR: feed conversion ratio; PPV: protein productive value; LPV: lipid productive value; *K*: condition factor; ISI: intestinal-somatic index; HSI: hepatosomatic index.

duckweed types. However, compared to perch, the effects were less pronounced, and the lowest inclusion levels of both duckweed types did not produce any effects when compared to the control diets, while the highest inclusion levels triggered the largest effects (Table 9 and Figure 1). Rainbow trout fed with either DWD35 or DWF35 showed the lowest growth (PWG: 296% and SGR: 3.93% day^{-1} for DWD35 and PWG: 324% and SGR: 4.10% day^{-1} for DWF35, respectively), highest FCR (1.26 for DWD35 and 1.20 for DWF35, respectively), lowest PPV (18.8% for DWD35 and 19.8% for DWF35, respectively) and LPV (21.3% for DWD35 and 22.3% for DWF35, respectively), and the lowest visceral fat reserves (47.9% for both DWD35 and DWF35). There was no significant effect on survival rates by feeding duckweed, regardless of the duckweed type. However, in diets with the highest inclusion levels of both DWD and

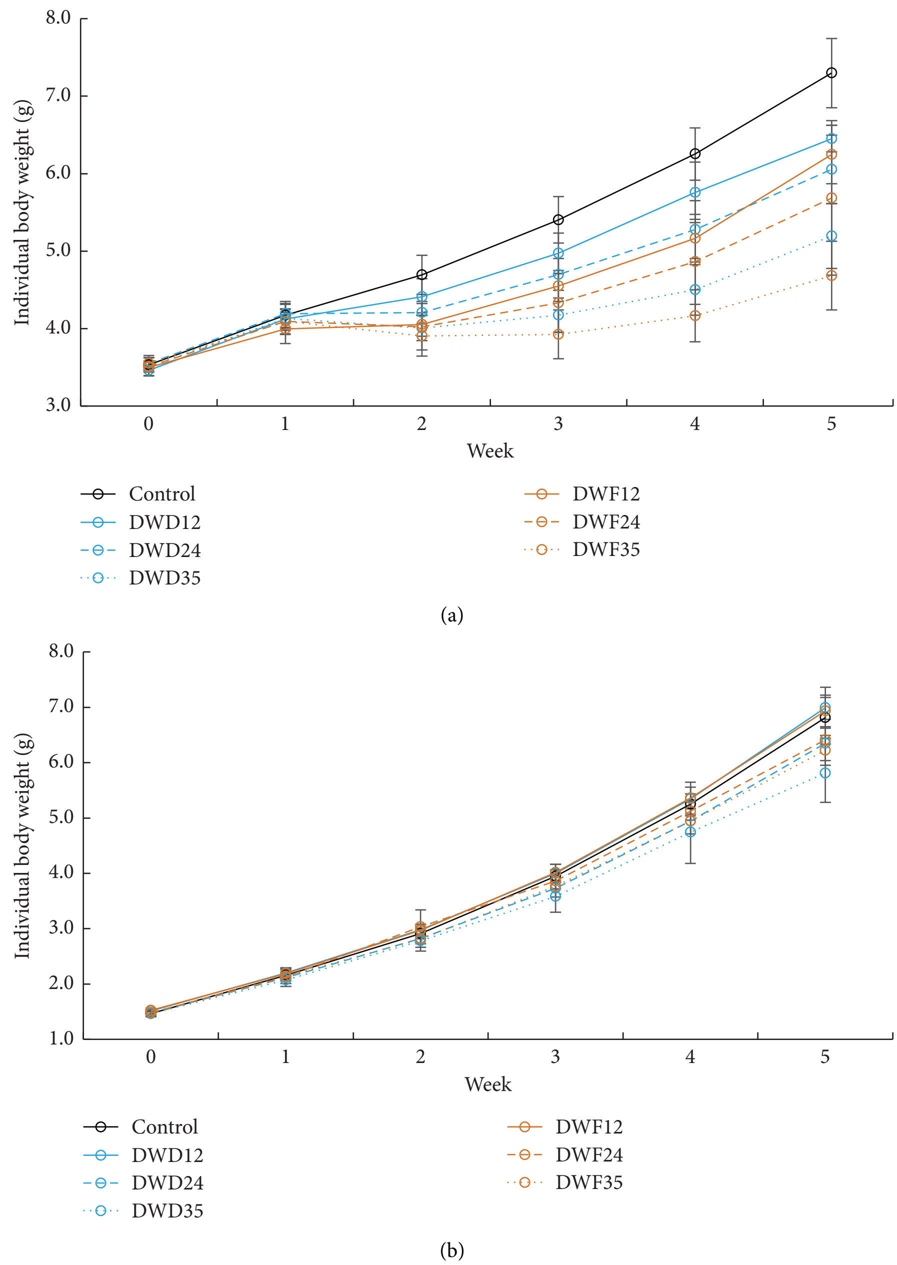

Figure 1: Growth of Eurasian perch (a) and rainbow trout (b) fingerlings fed with control diet (black, solid) and diets including dried (turquoise) and fermented (orange) duckweed to replace different proportions of fish meal protein (12%: solid, 24%: dashed, and 35%: dotted). Values are mean ± SD ($n = 4$).

DWF, the survival rates were slightly reduced. Compared to the initial fish, moisture was reduced in all experimental groups including the control, while CP was only higher in the control and DWD12 groups (Table 10). Crude lipids increased in all experimental groups, although contrast analysis only showed a significant effect for DWD-fed rainbow trout. Comparing the effects of the diets on whole body composition, the most remarkable effect was a decreasing CP content with an increased duckweed composition, while no clear effects for CL and CA could be determined (Table 10).

4. Discussion

Duckweed is a promising plant-based protein source for fish feeds, and its application as a feed component in diets for herbivorous or omnivorous fish such as carp or tilapia has been studied repeatedly, testing different duckweed species and concentrations [29–35]. However, according to our knowledge, duckweed has not been tested as fishmeal substitute for Eurasian perch, and only two studies have reported on the utilization of duckweed in rainbow trout. When rainbow trout fry received feed with only smaller

Table 8: Initial and final whole-body composition of Eurasian perch ($N = 4$).

	Initial fish	Final fish fed with								p value*	
		Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35	SEM	DWD	DWF
Moisture (%)	73.5	74.2	74.7	72.5	74.2	75.6	74.3	75.7	0.33	0.908 (0.577)	0.023 (0.967)
Crude protein (g·100 g^{-1} FM	16.4	17.2	17.0	17.7	16.1	16.4	17.2	16.1	0.17	0.802 (0.238)	0.417 (0.154)
Crude lipids (g·100 g^{-1} FM)	6.18	4.50	3.65	4.45	2.94	3.36	3.94	3.45	0.19	<0.001 (0.507)	<0.001 (0.457)
Crude ash (g·100 g^{-1} FM)	3.56	4.00	4.10	4.60	4.29	3.98	4.35	4.37	0.065	0.001 (0.208)	<0.001 (0.009)

*Contrast analysis between initial and final fish = p value; contrast analysis between control and duckweed fed fish = (p value).

Table 9: Dietary effects on growth, feed conversion, protein and lipid utilization, and body condition parameters in rainbow trout ($N = 4$).

	Rainbow trout fed with								p value	
		Dried duckweed (DWD)			Fermented duckweed (DWF)					
	Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35	SEM	DWD	DWF
IBW (g)	1.47	1.51	1.47	1.47	1.53	1.48	1.48	0.009	—	—
FBW (g)	6.81	6.99	6.35	5.81	6.93	6.41	6.22	0.097	0.001	0.033
WG (g)	5.35	5.48	4.88	4.34	5.41	4.93	4.74	0.092	0.001	0.023
PWG (%)	364	377	333	296	373	337	324	6.61	<0.001	0.033
SGR (% day^{-1})	4.39	4.37	4.17	3.93	4.33	4.18	4.10	0.037	<0.001	0.012
FCR (g·g^{-1})	1.11	1.11	1.16	1.26	1.12	1.19	1.20	0.012	<0.001	0.023
PPV (%)	21.4	21.5	20.4	18.8	21.2	20.0	19.8	0.22	<0.001	0.016
LPV (%)	23.7	23.8	22.8	21.3	23.5	22.4	22.3	0.20	<0.001	0.020
K	1.27	1.23	1.24	1.18	1.25	1.27	1.20	0.011	0.051	0.277
ISI (%)	16.0	16.0	15.9	16.4	16.6	16.7	17.7	0.16	0.578	0.008
HSI (%)	2.43	2.42	2.42	2.31	2.53	2.55	2.60	0.035	0.595	0.162
Visceral fat (%)	66.7	58.3	50.0	47.9	60.4	52.1	47.9	1.84	0.003	0.004
Survival (%)	100	100	100	95.0	100	100	96.7	0.60	0.058	0.391

IBW: initial body weight; FBW: final body weight; WG: weight gain; PWG: percentage weight gain; SGR: specific growth rate; FCR: feed conversion ratio; PPV: protein productive value; LPV: lipid productive value; *K*: condition factor; ISI: intestinal-somatic index; HSI: hepatosomatic index.

proportions of fishmeal being replaced by duckweed (1/16 and 1/8), only slightly reduced feed conversion ratios were observed that did not differ between the duckweed inclusion levels [21]. The current study reports significantly differing performances between how the two carnivorous species Eurasian perch and rainbow trout utilize dried and fermented duckweed *S. polyrhiza*.

4.1. Perch (P. fluviatilis) Trial. The initial low feed acceptance of the experimental diets in perch confirms observations in previous experiments by us (not published). Adaptation to new feeds, especially when they have different physical properties (e.g., floating versus sinking), needs more time in perch compared to other fishes.

When fed with diets containing dried and fermented duckweed, perch exhibited a significantly increased FCR irrespective of the level of duckweed inclusion and, especially at higher levels of duckweed inclusion, a reduction of growth. This effect was stronger with feed containing fermented duckweed (DWF) than with feed containing dried duckweed (DWD). Growth curves showed a clear pattern, with control-fed fish performing best, followed by DWD12-fed perch, and, finally, with perch fed either DWD35 or DWF35 performing worst (Figure 1). The low growth performance of perch fed with duckweed was also reflected in a significantly decreased condition factor *K* of perch fed with increasing levels of DWF but not in those fed with DWD, where *K* was not influenced by DWD levels. Survival rates were significantly reduced in fish fed with the highest concentrations of both duckweed types (DWD35 and DWF35). This was confirmed by daily visual observations that revealed higher intraspecific aggression of larger and more dominant individuals towards smaller and subordinate individuals. All mortalities occurred either due to fish being killed during periods when no observation took place or due to being severely injured and euthanized by us according to Swiss animal welfare regulations. No apparent nutrient deficiency signs were observed, and perch fingerling behavior was similar to earlier experiments where the strongest fish showed intensive agonistic behavior towards weak and smaller conspecifics. However, considering the highest mortalities occurred in both DWD35 and DWF35, the diets with the highest duckweed content, a connection with nutrient content, availability, or digestibility, appears reasonable. The most pronounced effect of the experimental diets on body composition was the significant decrease in whole-body lipids in all treatments when compared to the fish at the onset of the experiment. The experimental diet was formulated according to the nutritional requirements of perch (*P. fluviatilis*) [24, 25]. In this study, the lipid content of the feed was set to 14%, which was based on the observation that even though a content of 18% led to the highest growth in perch fry, it also led to increased lipid droplets in the hepatocytes [25].

The significantly reduced whole body lipids and the low, sometimes negative lipid productive values (LPV) suggest that, independently of treatment, CL and energy or the physiological availability, i.e., the digestibility, was too low in the formulated perch diets. Up to now, no study has evaluated the effects of fishmeal replacement by any plant protein on *P. fluviatilis*. For the closely related North American yellow perch (*P. flavescens*), a fish meal replacement of 50% and 100% by untreated soybean meal led to significantly reduced performance, while fish meal replacement by an ANF-reduced soybean variant in the same concentrations did not show similar strong effects [36].

4.2. Rainbow Trout (O. mykiss) Trial. Trout readily accepted the experimental diets, which confirmed previous observations with experimental diets containing insect meal or duckweed [21, 37].

The results clearly show that rainbow trout utilized feed containing duckweed better than perch, and the new diet did not compromise growth, FCR, or nutrient utilization at low to moderate levels of duckweed inclusion. Only at high inclusion levels was survival reduced. Like in perch, this was

Table 10: Initial and final whole-body composition of rainbow trout ($N = 4$).

	Initial fish	Final fish fed with								p value*	
		Control	DWD12	DWD24	DWD35	DWF12	DWF24	DWF35	SEM	DWD	DWF
Moisture (%)	79.6	71.4	70.6	74.4	76.0	73.2	74.0	76.6	0.67	0.001 (0.033)	0.005 (0.045)
Crude protein ($g \cdot 100\,g^{-1}$ FM	13.6	15.3	15.5	13.3	12.3	13.9	13.5	12.0	0.32	0.022 (0.011)	0.051 (0.014)
Crude lipids ($g \cdot 100\,g^{-1}$ FM)	4.89	7.95	8.43	6.47	6.24	7.71	7.45	6.61	0.32	0.007 (0.122)	0.092 (0.449)
Crude ash ($g \cdot 100\,g^{-1}$ FM)	1.54	1.86	2.16	2.07	2.08	1.84	1.90	1.73	0.047	0.031 (0.168)	0.074 (0.603)

*Contrast analysis between initial and final fish = p value; contrast analysis between control and duckweed fed fish = (p value).

confirmed by daily observations which revealed intraspecific aggression of larger and dominant individuals towards the smallest individuals in the corresponding tanks. Condition factors *K*, ISI, and HSI were, except for ISI in trout fed DWF35, not significantly influenced by duckweed, which renders a treatment-related mortality unlikely. Both types of duckweed, fermented and dried, had a significant effect on PPV, which was lowest in the diets with the highest inclusion levels (DWD35 and DWF35), while the lowest inclusion levels (DWD12 and DWF12) showed similar PPVs to the control group. The same picture was observed for the lipid productive value (LPV), in which increasing duckweed levels resulted in decreasing LPVs for both duckweed types, although this effect was somewhat less pronounced in DWF groups.

As rainbow trout is among the commercially most important freshwater fishes, several studies reporting on replacing fishmeal protein with plant-based proteins have been published. These include, among others, soybean, lupine, rapeseed/canola, sunflower seeds, and pumpkin seeds [38–42]. As in perch, however, almost no studies exist that tested duckweed as a fishmeal replacement in rainbow trout fry or fingerlings or even in other salmonids. One study included 6.25 and 12.5% concentrations of duckweed (*S. polyrhiza*) in rainbow trout fry feed [21], but the investigation did not strictly exchange fishmeal for duckweed on protein basis. There was only a small but significant increase in feed conversion, but no decrease in SGR was observed (a tendency was observed, though). Most interestingly, both groups of duckweed-fed trout showed similar performance, independent of dietary duckweed concentration [21]. One study reported the effects of partial fishmeal and soybean substitution by duckweed (*Lemna minor*) on grow-out rainbow trout. Only in diets with the highest inclusion level (280 g kg^{-1}) did they find a severe reduction in performance, resulting in the conclusion that up to 20% of fishmeal and soybean protein could be substituted by duckweed [43].

4.3. Duckweed as a Fishmeal Replacement. The success of fishmeal replacement depends on several factors, the more important ones being the concentration of fishmeal in the control diet, its relative replacement level, and the quality of the plant ingredient to which the protein concentration, amino acid composition, and the amount of ANFs contribute. While several ANFs are known for typical plant-based proteins such as soybean or canola/rapeseed [9], only few ANFs such as oxalic acid have been reported for *Lemna gibba* and *L. minor* [44, 45] and cyanides, tannins, and phytic acid for *S. polyrhiza* [46]. This does not exclude the potential presence of other ANFs such as protease inhibitors, thus warranting further study concerning the presence and concentrations of ANFs in different duckweed species.

The amino acid compositions reported for different duckweed species are usually comparable to those of soybean or lupine [47]. The EAA profiles for both feeds showed comparatively little differences. The most striking of these was a generally higher EAA concentration in the perch diets and a higher lysine content in the perch feeds compared to the rainbow trout feeds. This difference is likely caused by the difference in the secondary protein source in perch feeds (poultry by-product meal) compared to rainbow trout feed (wheat gluten), although this contradicts the usually higher lysine content in wheat gluten meal compared to poultry by-product meals [23]. While for Eurasian perch, according to the authors' knowledge, no EAA requirements have been published up to date, the EAA requirements for rainbow trout are mostly known. A deficit in lysine is reported to result in reduced growth and feed efficiency but could also cause certain health issues such as caudal fin erosion in trout [23]. Similarly, a deficiency of methionine leads to reduced growth and feed efficiency. Therefore, it could be assumed that, especially in diets containing higher levels of DWF, a lysine and methionine deficiency might have contributed to the reduced performance in rainbow trout. However, while rainbow trout performed rather well despite a presumed lysine and methionine deficiency in all diets, including the control, perch showed very poor performance, although no EAA deficiency appeared to exist and EAA concentrations were generally higher compared to rainbow trout. Although the true EAA requirements are not known for perch, the decreasing growth performance with increasing dietary DWD or DWF inclusion levels points to other factors significantly influencing and contributing to the poor performance in perch.

Up to now, different duckweed species have been used with varying success as a feed component or fishmeal replacement in several fish species. Most of the studies have been conducted in fish of the lower trophic levels belonging to the families of cichlids [31–33] and cyprinids [29, 30, 48]. The responses and performance of the different fish species depended strongly on the experimental setup and duckweed inclusion levels, as well as on duckweed protein content and quality. Feeding *Labeo rohita* with increasing levels of raw and fermented duckweed as a fishmeal replacement in formulated feeds resulted in similar or improved performance compared to the control diet [29]. The authors furthermore reported an apparent protein digestibility for dried and fermented duckweed of between 82.3 and 94.4%. For Nile tilapia (*Oreochromis niloticus*), duckweed protein digestibility was reported to range between 75.9 and 79%, depending on the inclusion level of duckweed, and the digestibility decreased with higher inclusion levels [32]. The authors also reported that dried and fresh duckweed inclusion of 20 or 40% led to a reduction of growth by 5–13%. Nevertheless, the authors highlighted that neither the reduction in growth and feed conversion, nor in the digestibility, was significant enough to discourage the use of duckweed in tilapia feeds, especially in places where fishmeal-based diets are scarce or too expensive. Replacing up to 30% of fishmeal protein by *S. polyrhiza* did not show any significant negative effects on performance traits when fed to fingerling Nile tilapia for 56 days [33]. Similarly, de Matos et al. [31] did not find any significant reduction in growth or increase in FCR after feeding *L. valdiviana* pellets (39.9% CP) to red tilapia fry (*O. mossambicus* × *O. niloticus*) for 83 days compared to commercial compound feed (53.3%

CP). Nevertheless, almost no studies exist evaluating duckweed as a fishmeal substitute in diets for carnivorous fishes other than rainbow trout. One study tested fermented duckweed (*Lemna minor*) in diets for juvenile barramundi (*Lates calcarifer*) in dietary concentrations of 15, 25, 35, and 45%. They found that barramundi fed with up to 35% fermented *L. minor* meal performed equal to control fish with barramundi fed 25% fermented duckweed meal even performing slightly better [49]. Generally, it is probably safe to assume that digestibility and nutrient availability are higher in fish of lower trophic levels that are more omnivorous or herbivorous compared to perch and rainbow trout. The results obtained in this study for Eurasian perch point towards a low utilization efficiency of duckweed for, up to now, unknown reasons, although the generally low lipid utilization in perch diets appears to be an important factor. Rainbow trout, on the other hand, appears to be reasonably able to utilize low to medium duckweed inclusion levels, independent of duckweed type, dried or fermented. For perch, neither DWD nor DWF seem to be an interesting feed ingredient, although the performance was somewhat worse in DWF-fed fish compared to DWD-fed fish. For rainbow trout, however, both DWD and DWF could be used in concentrations equaling those in DWD12 and DWF12. Higher concentrations start to reduce performance. Contrary to perch, the lowest performing group in rainbow trout was the one with the highest dried duckweed concentration, DWD35, although the differences to control were comparatively low. One factor contributing to the general performance of fish feeds is the feed manufacturing process. Our diets were cold extruded, which is not nearly as efficient in improving digestibility and nutrient availability compared to sophisticated extrusion processes [50, 51]. State-of-the-art feed production will likely cause beneficial effects to both fish species, which will allow higher inclusion levels.

5. Conclusion

The observed effects differ remarkably between the two carnivorous fish species Eurasian perch (*P. fluviatilis*) and rainbow trout (*O. mykiss*). While both species are at a trophic level of 4 or above and are considered to prey on similar organisms, they reacted differently to a graded inclusion of dried and fermented duckweed in their diets. In general, perch could not utilize duckweed well, independent of the duckweed type or inclusion level, and exhibited both reduced growth and an increased feed conversion ratio. On the contrary, trout were able to utilize both dried and fermented duckweed up to an inclusion level of 12% fishmeal protein replacement without significant growth reduction or increased feed conversion and up to 24% with only slight performance reductions. This study suggests that duckweed is an interesting future protein source for rainbow trout but not for Eurasian perch. Future work should focus on practical trials using extruded diets, determination of duckweed digestibility in trout or other salmonids, and presence and concentrations of important ANFs.

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

Both reported fish feeding trials were conducted according to the Swiss Animal Protection Regulation and were approved by the Cantonal Veterinarian Office (canton Aargau) and the Animal Welfare Board under registration number AG-75722.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Timo Stadtlander performed conceptualization and study design, material, data collection, and analysis including statistics, writing of the first draft manuscript, funding acquisition, and project administration. Fridolin Tschudi performed conceptualization of the study, reviewing and editing of the draft manuscript, funding acquisition, and project administration. Andreas Seitz performed production of dried and fermented duckweed and reviewing and editing of the draft manuscript. Mathias Sigrist performed production of dried and fermented duckweed and reviewing and editing of the draft manuscript. Dominik Refardt performed reviewing and editing of the draft manuscript and project administration. Florian Leiber performed conceptualization of the study, statistical consultation, reviewing and editing of the draft manuscript, funding acquisition, and project administration.

Acknowledgments

The authors would like to thank the Swiss Federal Office of Agriculture (FOAG) for the financial support of this study (grant no. 627000897). Furthermore, the authors would like to thank Percitech AG, Chavornay, Switzerland, for freely providing the Eurasian perch for this experiment. For their dedicated help in the laboratory during and after the experiments, the authors would also like to thank René Abbühl and Basil Trueb. Finally, the authors thank Jason Parry for language polishing.

References

[1] A. G. J. Tacon and M. Metian, "Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects," *Aquaculture*, vol. 285, no. 1-4, pp. 146–158, 2008.

[2] A. G. J. Tacon and M. Metian, "Feed matters: satisfying the feed demand of aquaculture," *Reviews in Fisheries Science & Aquaculture*, vol. 23, pp. 1–10, 2015.

[3] C. Schader, A. Muller, N. E.-H. Scialabba et al., "Impacts of feeding less food-competing feedstuffs on global food system sustainability," *Journal of the Royal Society Interface*, vol. 12, Article ID 20150891, 2015.

[4] R. L. Naylor, R. W. Hardy, A. H. Buschmann et al., "A 20-year retrospective review of global aquaculture," *Nature*, vol. 591, no. 7851, pp. 551–563, 2021.

[5] T. Cashion, F. Le Manach, D. Zeller, and D. Pauly, "Most fish destined for fishmeal production are food-grade fish," *Fish and Fisheries*, vol. 18, no. 5, pp. 837–844, 2017a.

[6] T. Cashion, P. Tyedmers, and R. W. R. Parker, "Global reduction fisheries and their products in the context of sustainable limits," *Fish and Fisheries*, vol. 18, no. 6, pp. 1026–1037, 2017b.

[7] M. Troell, R. L. Naylor, M. Metian et al., "Does aquaculture add resilience to the global food system?" *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 37, pp. 13257–13263, 2014.

[8] A. Boerema, A. Peeters, S. Swolfs et al., "Soybean trade: balancing environmental and socio-economic impacts of an intercontinental market," *PLoS One*, vol. 11, no. 5, Article ID e0155222, 2016.

[9] G. Francis, H. P. S. Makkar, and K. Becker, "Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish," *Aquaculture*, vol. 199, no. 3-4, pp. 197–227, 2001.

[10] S. A. Collins, M. Øverland, A. Skrede, and M. D. Drew, "Effect of plant protein sources on growth rate in salmonids: meta-analysis of dietary inclusion of soybean, pea and canola/rapeseed meals and protein concentrates," *Aquaculture*, vol. 400-401, pp. 85–100, 2013.

[11] S. L. Woodgate, A. H. L. Wan, F. Hartnett, R. G. Wilkinson, and S. J. Davies, "The utilisation of European processed animal proteins as safe, sustainable and circular ingredients for global aquafeeds," *Reviews in Aquaculture*, vol. 14, no. 3, pp. 1572–1596, 2022.

[12] S. Naseem, S. U. Bhat, A. Gani, and F. A. Bhat, "Perspectives on utilization of macrophytes as feed ingredient for fish in future aquaculture," *Reviews in Aquaculture*, vol. 13, no. 1, pp. 282–300, 2021.

[13] K. Mohan, D. K. Rajan, T. Muralisankar, A. R. Ganesan, P. Sathishkumar, and N. Revathi, "Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: a review of past and future needs," *Aquaculture*, vol. 553, Article ID 738095, 2022.

[14] C. Sandrock, S. Leupi, J. Wohlfahrt et al., "Genotype-by-diet interactions for larval performance and body composition traits in the black soldier fly, *Hermetia illucens*," *Insects*, vol. 13, no. 5, p. 424, 2022.

[15] I. G. Mbagwu and H. A. Adeniji, "The nutritional content of duckweed (*Lemna paucicostata* hegelm.) in the Kainji Lake area, Nigeria," *Aquatic Botany*, vol. 29, no. 4, pp. 357–366, 1988.

[16] T. Stadtlander, J. Bandy, D. Rosskothen et al., "Dilution rates of cattle slurry affect ammonia uptake and protein production of duckweed grown in recirculating systems," *Journal of Cleaner Production*, vol. 357, Article ID 131916, 2022.

[17] J. Xu, J. J. Cheng, and A.-M. Stomp, "Growing *Spirodela polyrrhiza* in swine wastewater for the production of animal feed and fuel ethanol: a pilot study," *Clean: Soil, Air, Water*, vol. 40, no. 7, pp. 760–765, 2012.

[18] Y. Zhao, Y. Fang, Y. Jin et al., "Potential of duckweed in the conversion of wastewater nutrients to valuable biomass: a pilot-scale comparison with water hyacinth," *Bioresource Technology*, vol. 163, pp. 82–91, 2014.

[19] E. I. Iatrou, A. S. Stasinakis, and M. Aloupi, "Cultivating duckweed *Lemna minor* in urine and treated domestic wastewater for simultaneous biomass production and removal of nutrients and antimicrobials," *Ecological Engineering*, vol. 84, pp. 632–639, 2015.

[20] R. A. Mohedano, R. H. R. Costa, F. A. Tavares, and P. Belli Filho, "High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds," *Bioresource Technology*, vol. 112, pp. 98–104, 2012.

[21] T. Stadtlander, S. Förster, D. Rosskothen, and F. Leiber, "Slurry-grown duckweed (*Spirodela polyrhiza*) as a means to recycle nitrogen into feed for rainbow trout fry," *Journal of Cleaner Production*, vol. 228, pp. 86–93, 2019.

[22] J. Xu and G. Shen, "Growing duckweed in swine wastewater for nutrient recovery and biomass production," *Bioresource Technology*, vol. 102, no. 2, pp. 848–853, 2011.

[23] National Research Council, *Nutrient Requirements of Fish and Shrimp*, National Academies Press, Washington, D.C, 2011.

[24] E. D. Fiogbé, P. Kestemont, C. Mélard, and J. C. Micha, "The effects of dietary crude protein on growth of the Eurasian perch *Perca fluviatilis*," *Aquaculture*, vol. 144, no. 1-3, pp. 239–249, 1996.

[25] P. Kestemont, E. Vandeloise, C. Mélard, P. Fontaine, and P. B. Brown, "Growth and nutritional status of Eurasian perch *Perca fluviatilis* fed graded levels of dietary lipids with or without added ethoxyquin," *Aquaculture*, vol. 203, no. 1-2, pp. 85–99, 2001.

[26] Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, "Methode 6.1.1. Bestimmung der Rohfaser," in *Das VDLUFA-Methodenbuch, Band III, Die Chemische Untersuchung von Futtermitteln*, VDLUFA Verlag Darmstadt, Germany, 1976.

[27] B. A. Bidlingmeyer, S. A. Cohen, and T. L. Tarvin, "Rapid analysis of amino acids using pre-column derivatization," *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 336, no. 1, pp. 93–104, 1984.

[28] S. L. Amelchanka, M. Kreuzer, and F. Leiber, "Utility of buckwheat (*Fagopyrum esculentum* Moench) as feed: effects of forage and grain on in vitro ruminal fermentation and performance of dairy cows," *Animal Feed Science and Technology*, vol. 155, no. 2-4, pp. 111–121, 2010.

[29] A. Bairagi, K. Sarkar Ghosh, S. K. Sen, and A. K. Ray, "Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita* Ham.) fingerlings after fermentation with a fish intestinal bacterium," *Bioresource Technology*, vol. 85, no. 1, pp. 17–24, 2002.

[30] Y. Cui, X. Liu, S. Wang, and S. Chen, "Growth and energy budget in young grass carp, *Ctenopharyngodon idella* Val., fed plant and animal diets," *Journal of Fish Biology*, vol. 41, no. 2, pp. 231–238, 1992.

[31] F. T. de Matos, F. R. Lapolli, R. A. Mohedano, D. M. Fracalossi, G. W. Bueno, and R. Roubach, "Duckweed bioconversion and fish production in treated domestic wastewater," *Journal of Applied Aquaculture*, vol. 26, no. 1, pp. 49–59, 2014.

[32] S. A. El-Shafai, F. A. El-Gohary, J. A. J. Verreth, J. W. Schrama, and H. J. Gijzen, "Apparent digestibility coefficient of duckweed (*Lemna minor*), fresh and dry for Nile tilapia (*Oreochromis niloticus* L.)," *Aquaculture Research*, vol. 35, no. 6, pp. 574–586, 2004.

[33] E. A. Fasakin, A. M. Balogun, and B. E. Fasuru, "Use of duckweed, *Spirodela polyrrhiza* L. Schleiden, as a protein

feedstuff in practical diets for tilapia, *Oreochromis niloticus* L," *Aquaculture Research*, vol. 30, no. 5, pp. 313–318, 1999.

[34] M. S. Hassan and P. Edwards, "Evaluation of duckweed (*Lemna perpusilla* and *Spirodela polyrrhiza*) as feed for Nile tilapia (*Oreochromis niloticus*)," *Aquaculture*, vol. 104, no. 3-4, pp. 315–326, 1992.

[35] E. Yılmaz, İ. Akyurt, and G. Günal, "Use of duckweed, *Lemna minor*, as a protein feedstuff in practical diets for common carp, *Cyprinus carpio*, fry," *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 4, pp. 105–109, 2004.

[36] V. Kumar, H.-P. Wang, R. S. Lalgudi, B. Mcgraw, R. Cain, and K. A. Rosentrater, "Processed soybean meal as an alternative protein source for yellow perch (*Perca flavescens*) feed," *Aquaculture Nutrition*, vol. 25, no. 4, pp. 917–931, 2019.

[37] T. Stadtlander, A. Stamer, A. Buser, J. Wohlfahrt, F. Leiber, and C. Sandrock, "*Hermetia illucens* meal as fish meal replacement for rainbow trout on farm," *Journal of Insects as Food and Feed*, vol. 3, pp. 165–175, 2017.

[38] B. Glencross, N. Rutherford, and W. Hawkins, "A comparison of the growth performance of rainbow trout (*Oncorhynchus mykiss*) when fed soybean, narrow-leaf or yellow lupin meals in extruded diets: a comparison of lupin kernel and soy meals in trout," *Aquaculture Nutrition*, vol. 17, no. 2, pp. e317–e325, 2011.

[39] A. M. Greiling, R. Reiter, and M. Rodehutscord, "Utilization of unprocessed and fibre-reduced oilseed cakes of rapeseed and sunflower seed in rainbow trout (*Oncorhynchus mykiss* W.) nutrition-Evaluation of apparent digestibility and growth performance," *Aquaculture Nutrition*, vol. 24, no. 3, pp. 1133–1143, 2018.

[40] A. M. Greiling, C. Schwarz, M. Gierus, and M. Rodehutscord, "Pumpkin seed cake as a fishmeal substitute in fish nutrition: effects on growth performance, morphological traits and fillet colour of two freshwater salmonids and two catfish species," *Archives of Animal Nutrition*, vol. 72, no. 3, pp. 239–259, 2018.

[41] B. Mwachireya, D. Higgs, Higgs, and Dosanjh, "Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout *Oncorhynchus mykiss* (Walbaum) held in fresh water: digestibility of canola protein products in rainbow trout," *Aquaculture Nutrition*, vol. 5, no. 2, pp. 73–82, 1999.

[42] T. Ostaszewska, K. Dabrowski, M. E. Palacios, M. Olejniczak, and M. Wieczorek, "Growth and morphological changes in the digestive tract of rainbow trout (*Oncorhynchus mykiss*) and pacu (*Piaractus mesopotamicus*) due to casein replacement with soybean proteins," *Aquaculture*, vol. 245, no. 1-4, pp. 273–286, 2005.

[43] E. Fiordelmondo, S. Ceschin, G. E. Magi et al., "Effects of partial substitution of conventional protein sources with duckweed (*Lemna minor*) meal in the feeding of rainbow trout (*Oncorhynchus mykiss*) on growth performances and the quality product," *Plants*, vol. 11, no. 9, p. 1220, 2022.

[44] V. R. Franceschi, "Oxalic acid metabolism and calcium oxalate formation in *Lemna minor* L," *Plant, Cell and Environment*, vol. 10, no. 5, pp. 397–406, 1987.

[45] R. F. Nuss and F. A. Loewus, "Further studies on oxalic acid biosynthesis in oxalate-accumulating plants," *Plant Physiology*, vol. 61, no. 4, pp. 590–592, 1978.

[46] E. A. Fasakin, "Nutrient quality of leaf protein concentrates produced from water fern (*Azolla africana* Desv) and duckweed (*Spirodela polyrrhiza* L. Schleiden)," *Bioresource Technology*, vol. 69, no. 2, pp. 185–187, 1999.

[47] K.-J. Appenroth, K. S. Sree, V. Böhm et al., "Nutritional value of duckweeds (Lemnaceae) as human food," *Food Chemistry*, vol. 217, pp. 266–273, 2017.

[48] K. Naskar, A. C. Banerjee, N. M. Chakraborty, and A. Ghosh, "Yield of *Wolffia arrhiza* (L.) Horkel ex Wimmer from cement cisterns with different sewage concentrations, and its efficacy as a carp feed," *Aquaculture*, vol. 51, no. 3-4, pp. 211–216, 1986.

[49] A. G. Mustofa, W. S. Ardianasyah, and H. I. Mulyati, "Use of duckweed (*Lemna minor*) harvested from IRAS as a partial replacement for fishmeal proteins in barramundi (*Lates calcarifer*) diets," *AACL Bioflux*, vol. 15, pp. 1663–1674, 2022.

[50] M. Sorensen, "A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods," *Aquaculture Nutrition*, vol. 18, no. 3, pp. 233–248, 2012.

[51] M. Sorensen, T. Storebakken, and K. D. Shearer, "Digestibility, growth and nutrient retention in rainbow trout (*Oncorhynchus mykiss*) fed diets extruded at two different temperatures," *Aquaculture Nutrition*, vol. 11, no. 4, pp. 251–256, 2005.