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ORIGINAL PAPER

Utility of dried and fermented meal from the duckweed species *Spirodela polyrhiza* as fishmeal-protein replacer in diets for common carp fry

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ABSTRACT. The family of cyprinids comprises some of the most important fish species in global aquaculture. With increasing global fish production also increasing amounts of suitable and sustainably produced high-quality feeds other than fishmeal are needed. Duckweed (*Spirodela polyrhiza*) is a fast growing aquatic plant with the potential of high biomass and protein production in nutrient recycling systems. This study reports the effects on growth, feed conversion and proximate body composition of common carp (*Cyprinus carpio* L.) fry fed graded levels of differently processed duckweed (meals dried and fermented) each replacing 150, 300 or 450 g/kg of dietary fishmeal protein. Comparisons were made to a duckweed-free and fishmeal-based control, containing 400 g/kg of fishmeal, equivalent to 261.9 g/kg of fishmeal protein. Carp fed the highest inclusion rate of fermented duckweed showed a significantly reduced performance (growth and feed conversion rate) compared to all other groups. No differences regarding growth and feed conversion rate were found among all other groups. Whole body crude lipid content was generally lower and crude ash content generally higher in carp fed dried compared to those fed fermented duckweed. Whole body crude protein content was not influenced by treatments. Based on the results of this study, dried *S. polyrhiza* meal could be used to replace up to 450 g/kg of fishmeal protein, while fermented *S. polyrhiza* meal should only replace up to 300 g/kg fishmeal protein in diets for carp fry.

Introduction

The family of Cyprinids (carp-like fishes) are the most important cultured fishes. Their global production reached over 31 mln t equivalent to 52.2% of the 59.4 mln t of finfish totally produced in 2021 (FAO, 2023). The main countries producing carps are China, India and Bangladesh. Cyprinids are usually omnivorous (trophic level ± 3) or herbivorous (trophic level ± 2) and are well suited for polyculture production systems which can be operated as low or zero input systems (Woynarovitsch et al., 2010). Although low trophic level species are considered to be economically and ecologically more

sustainable compared to high trophic level species (Neori and Nobre, 2012), more recently a change towards increased intensification, also in carp production, was observed in China (Cao et al., 2015). The percentage of fishmeal in carp grow out feeds is usually low, while it is common practice that starter feeds for fish fry, including carp fry, contain very high shares of fishmeal (up to 50% or even higher) and are only gradually reduced in fingerling and even further in grow out diets. However, over the last decades fishmeal and fish oil production have stagnated. Furthermore, fishmeal production itself is unsustainable, putting additional pressure on marine ecosystems already heavily pressured by climate change, eutrophication, ocean acidification, oxygen depletion, plastic pollution and increased occurrence of harmful algae blooms (Cashion et al., 2017). In addition, the use of potentially edible fish as feed for production of other fish is questionable.

In 2012 herbivorous and omnivorous carp species were estimated to be the largest consumers of global aquaculture feeds with 11.0 mln t equivalent to 27.8% (Tacon and Metian, 2015). To compensate for fishmeal and to supply the steadily growing fed aquaculture sector, terrestrial plant-based feed ingredients, such as soybeans, rapeseed/canola, maize or wheat have been increasingly utilized and are now the most important aquafeed ingredients by volume (Tacon and Metian, 2015). Still, feeding such crops implies feed-food competition, land-use pressure and emissions from agriculture (Schader et al., 2015); thus, alternatives, which do not compete human food and which are based on circular economies are looked for.

Duckweed species such as *Lemna gibba, Lemna minor, Lemna polyrhiza*, *Lemna perpusilla* and *Spirodela polyrhiza* of the Lemnaceae family are small aquatic plants. They might have a not yet fully quantified potential to reduce or mitigate several of the above-mentioned impacts, namely to reduce the amount of fishmeal or human-edible crops in feedstuff and potentially recycle nitrogen and phosphorus from animal manure (Stadtlander et al., 2019; 2023a). Growing duckweed on animal manure has been shown to be possible, thus combining efficient nitrogen and phosphorous recovery with the generation of feed-grade protein-rich biomass (Xu and Shen, 2011; Stadtlander et al., 2022; 2023a). Up to now, different duckweed species have been tested as feed or feed ingredient for pigs (Rojas et al., 2014), poultry (Haustein et al., 1990) and several fish species such carnivorous fish such as rainbow trout (Stadtlander et al., 2019; 2023b) or Eurasian perch (Stadtlander et al.. 2023b) and grass carp (Cui et al., 1992). However, investigations on effects of graded duckweed levels on growth performance, nutrient utilization and intestinal health of common carp fry are missing. Similarly, described positive effects of fermentation of duckweed (Bairagi et al., 2002) meal have not been studied in common.

In the present study, we hypothesised that substantial amounts of fishmeal protein could be replaced by *S. polyrhiza* protein in common carp (*Cyprinus carpio*). For that purpose we investigated graded inclusion levels of two differently produced meals from *S. polyrhiza* (one from dried and one from fermented *S. polyrhiza*, as described in Stadtlander et al., 2023b). Effects on the growth performance, feed and nutrient utilization and intestinal health were registered.

Material and methods

Duckweed production and fermentation

Production and processing, including fermentation, of the duckweed species chosen, *S. polyrhiza*, was basically conducted as described in Stadtlander et al. (2023b). In short, *S. polyrhiza* (collection number 9346) was provided by the Landolt Duckweed Collection (Zurich, Switzerland). The duckweed was propagated on a round-shaped tank of 1.7 m^3 volume, followed by mass production in two greenhouse-based pools of 20 m^2 each. The initial ammonium-nitrogen (NH_4-N) concentration was 20 mg/l and fresh fertilizer (modified Hoagland medium; for details see Stadtlander et al., 2023b) was added when concentrations declined to 0.1 mg/l to increase NH_4-N concentration back to around 20 mg/l. Duckweed was harvested and divided into two fractions. One was air-dried on a shaded bench inside a greenhouse, before complete drying in a drying chamber. The other one was fermented in a water bath at a stable temperature of 31–32 °C for two weeks using EM-1 (a mixture of lactic acid bacteria, phototrophic bacteria and yeasts; purchased from EM Schweiz AG, Arni, Switzerland; transformed to EM-A according to the manufacturer's specification) and *Pediococcus pentosaceus* (PP100-25,BIOAGRO S.r.l., Italy) as starter cultures. After fermentation, the duckweed was dried by the same way as the freshly harvested duckweed. Afterwards, all dried duckweed was ground to receive a fine powder and refrigerated until use.

Experimental diets

Seven different diets were formulated to be iso-nitrogenous (470 g crude protein (CP; defined as N × 6.25)/kg dry matter (DM)) and iso-lipidic

Table 1. Feed formulation and feed proximate composition (g/kg dry matter)

Treatments[1]	Control	Duckweed, dried			Duckweed, fermented		
		DWD 150	DWD 300	DWD 450	DWF150	DWF300	DWF450
Ingredients							
fishmeal[2]	400	340	280	220	340	280	220
duckweed, dried	–	115.7	231.4	347.0	–	–	–
duckweed, fermented	–	–	–	–	131.5	262.9	394.4
poultry by-product meal[3]	252.6	252.6	252.6	252.6	252.6	252.6	252.6
potato starch	185	140	95	50	130	70	15
α-cellulose	82.9	71.2	59.5	47.8	66.0	54.1	37.2
sunflower oil	59.5	60.5	61.6	62.6	60.0	60.4	60.8
vitamin and mineral premix[4]	20	20	20	20	20	20	20
Proximate composition							
dry matter	934	942	951	955	946	958	970
crude ash	130	136	140	151	131	129	129
crude protein	480	477	467	480	472	464	461
crude lipids	121	121	121	126	126	130	141
crude fibre	65	56	102	127	67	87	108
N-free extract[5]	204	210	170	115	204	190	160
gross energy (MJ kg^{-1} dry matter)	20.4	20.4	20.2	20.1	20.5	20.7	20.8

[1] duckweed (*Spirodela polyrhiza*) in dried (DWD) and fermented (DWF) forms replacing either 150, 300 or 450 g/kg equivalent to 39.3, 78.6 or 117.8 g/kg of fishmeal protein; [2] herring fishmeal (Bioceval, Cuxhaven, Germany, containing 654.7 g/kg dry matter of crude protein); [3] AquaTrac sol SD, GePro, Diepholz, Germany; [4] vitamin-mineral premix equal to that used by Stadtlander et al. (2019); [5] calculated as: 1000 − crude ash − crude protein − crude lipids − crude fibre

(125 g/kg crude lipids (CL) in DM). In detail, fishmeal was replaced by duckweed meal with its lower CP content on a calculated protein basis, and the replacement of a mixture of potato starch and α-cellulose was compensating for the additional gross energy provided by the duckweed meal. Still in the analysed diets there was some variability in CP and in CL content (Table 1). The CL content tended to be higher in the treatments with higher duckweed inclusion. The control diet was formulated based on fishmeal and a poultry by-product meal as main protein sources. Proportions of 150, 300 and 450 g/kg of fishmeal protein each were replaced by the protein from duckweed meal either being only dried (DWD) or fermented and dried (DWF). In order to work with high inclusion levels of duckweed meals and to come close to commercial starter diets, we chose to formulate a high fishmeal diet containing 400 g/kg fishmeal in the control. The formulation resulted in one control diet (C) and the experimental diets DWD150, DWD300, DWD450, DWF150, DWF300 and DWF450. In one batch for each diet, all dry dietary ingredients were mixed several minutes before adding the sunflower oil and mixing thoroughly again, until no lipid globules were visible anymore. The mixtures were extruded without additional water in a lab-scale single-screw extruder (Do-Corder C3, Brabender GmbH, Germany) at the Laboratory of Food and Materials of ETH Zurich (Zurich, Switzerland). After extrusion, the first 5 cm of the diets leaving the extruder were discarded, the rest was collected and dried. Subsequently, diets were crumbled and sieved to retain the pellet fractions of 0.8–1.0 mm and 1.0–1.6 mm. Afterwards diets were stored at −20 °C until use.

Experimental fish and setup

The experiment was approved by the veterinary authorities of the canton Aargau, Switzerland (approval number AG-75722).

A total of 700 common carp (*Cyprinus carpio* L.; R8R3 strain; Irnazarow, 1995) fry (0.69 ± 0.04 g, mean ± standard deviation (SD)) were obtained from Wageningen Aquatic Research Facility (University of Wageningen, Wageningen (The Netherlands). After arrival and one week of adaptation in 55 l aquaria, they were stocked into 28 aquaria with 10 l volume each, connected to a recirculation system at 20 fish per aquarium. The remaining 140 fish were divided into two groups of 70 fish each, euthanized by MS-222 (tricaine-methanesulfonate, 200 mg/l buffered with 400 mg/l sodium bi-carbonate) and frozen for later analysis. During a one-week adaptation to the new aquaculture system, the fish were fed the same feed as that used in Wageningen (F−1.0 MP Pro Aqua Brut, Skretting, Norway; 570 g CP and 150 g CL/ kg^{-1}, as fed) at 8% of their body mass per day to acclimatize them. At the start of the experimental feeding, the average body mass of the fish was 0.79 g ± 0.05. Each diet was fed to four replicated

aquaria. The overall experiment, including the one-week adaptation as described above, lasted for 6 weeks. Due to an initially insufficiently working biofilter, the feed allowance was reduced for the first two experimental weeks to 5% of body mass per day and increased for another three weeks to 6% of body mass per day. Feed was distributed 2–3 times per day. Once a week, all fish were group-weighed per aquarium and the amount of feed offered was adapted to the newly determined body mass. Each aquarium was aerated by air stones connected to a membrane air-pump (Mistral 4000, Aqua Medic, Bissendorf, Germany). During the experiment, the average water flow rate for each aquarium was 14.4 l/h. Water quality (oxygen, pH, nitrite and ammonia) was controlled twice per week. Oxygen, pH and temperature were measured with a WTW 3410 hand-held oxygen meter and the respective probes for oxygen and pH (Xylem Analytics Germany Sales GmbH & Co KG, Weilheim, Germany). Nitrite and ammonium concentrations were measured by photometer using test kits from Merck (ammonia: 1.14752.0001, nitrite: 1.14776.0002; Merck KGaA, Darmstadt, Germany). Water temperature throughout the experiment was 19.4 ± 1.08 °C (mean ± SD). The oxygen concentration ranged between 7.68 and 8.55 mg/l (average 8.07 mg/l), the oxygen saturation between 86.5 and 95% (average 91.9%), the pH between 7.86 and 8.23 (average 8.03), nitrite between 0.04 and 0.39 mg/l (average 0.18 mg/l) and ammonia between 0.09 and 0.30 mg/l (average 0.43 mg/l).

Sampling and analysis

At the end of the experiment, all fish were euthanized aquarium-wise in the same way as the fish that had been euthanized initially. Group weight for each aquarium was determined to the nearest 0.1 g. In the three largest and smallest fish of each aquarium total length was measured to the nearest 0.1 cm and their individual weight to the nearest 0.01 g. The three largest fish of each aquarium were dissected and the visceral weight was measured to the nearest 0.01 g. Of two fish per aquarium a 0.4 cm long section of the hindgut was dissected and fixed in 10% buffered formalin for histological analysis, embedded in paraffin, and routinely processed for histological examination. Sections of 3 µm were cut, stained by haematoxylin-eosin and examined microscopically for the amount of immune cells (macrophages/monocytes and neutrophils) infiltrating the lamina propria and the amount of basophilic granular cells in the mucosa. All fish of each aquarium, including the dissected ones, were frozen, cut into small pieces and autoclaved at 121 °C for 15 min as one batch. Afterwards, samples were homogenized with an Ultra-Turrax T18 (IKA-Labortechnik, Staufen, Germany), frozen again and lyophilized in a Beta 1−16 (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Following lyophilisation, the samples were finely ground with a M23 mill (IKA-Labortechnik, Staufen, Germany) and subjected to further analysis. DM and crude ash (CA) were analysed in a TGA-500 (Leco, St. Joseph, USA), total nitrogen was measured by the Dumas method with the Analysator CN-2000 (Leco, St. Joseph, USA), and CP was calculated as N × 6.25. Crude lipids were measured by the Soxhlet method using an Extraktionssystem B-811 (Büchi Labortechnik GmbH, Flawil, Switzerland) and petroleum ether as solvent. Crude fibre was measured with a Fibertherm FT 12 (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and gross energy (GE) in a C7000 bomb calorimeter (IKA-Labortechnik, Staufen, Germany).

Calculations and statistics

The following calculations were conducted in order to describe growth of and utilisation by the fish of the different treatments:

- relative weight gain (%): [final body weight (g) – initial body weight (g)] / initial body weight (g) × 100;
- specific growth rate (%/day): [ln final body weight (g) – ln initial body weight (g)] / days of experiment × 100;
- feed conversion ratio (g/g): total dry feed intake (g) / [final body weight (g) – initial body weight (g)];
- protein productive value (%): [final fish protein content (g) – initial fish protein content (g)] / total protein intake (g) × 100
- lipid productive value (%): [final fish lipid content (g) – initial fish lipid content (g)] / total lipid intake (g);
- condition factor: final body weight (g) / total body length $(cm)^3$ × 100;
- viscerosomatic index (%): viscera weight (g) / final body weight (g) × 100.

The statistical analysis was performed using SPSS version 24 (IBM corporation, Armon, USA). The normal distribution was analysed by a Shapiro-Wilks test and the homogeneity of variance was analysed by the Levene test. All data except histological data, was analysed by a one-way analysis of variance with treatment as fixed factor and aquarium as experimental unit. Subsequently, the Bonferroni post-hoc test was applied for multiple comparisons among means except for the proximate composition data, where a one-sided Dunnett post-hoc test was

additionally applied to compare the final composition of each treatment versus the initial composition. The histological data (immune cells counted in lamina propria and amount of basophilic granular cells) was compared by the non-parametric Kruskal-Wallis H test. In all statistical tests, $P < 0.05$ was applied as level of significance. Data were given in tables as means ± SD.

Results

Most of the diets were accepted immediately. Only the diets containing higher amounts of fermented duckweed (DWF300 and DWF450) took around 2 days to be well accepted and consumed equally fast as the other diets. Afterwards, all feed was consumed during the first 2 min after serving. In both duckweed forms it was visually observed that the water inside the respective aquaria started to change colour (towards green in DWD fed aquaria and towards brown in DWF fed aquaria), and the change was more intense with increasing duckweed content of the diet.

The carp groups grew to final group weights of between 27.8 g (DWF450) and 36.2 g (DWD 45) (Table 2). Fish fed DWF450 showed a significantly lower final group body weight compared to all other groups except fish fed DWF300. In fish fed DWF450 the growth was significantly lower in terms of absolute group weight gain (11.8 vs. 16.6–20.3 g in the other groups), relative weight gain (73.8 vs. 107–129%) and specific growth rate (1.13 vs. 1.49–1.69 % day^{-1}). No differences in growth performance were found between control-fed fish, all DWD fed fish and fish fed DWF150 and DWF300. The feed conversion ratio was highest for DFW450 fed fish (5.20) while that of the other treatments was not different from each other and ranged between 3.47 (DWD150) and 3.97 (DWF300). The same pattern was found for the protein productive value, where also fish fed DWF450 performed inferior compared to all other treatments. Different from that, fish fed DWF150 had a significantly higher lipid production value compared to fish fed DFW450, DWD150, DWD300 and DWD450.

No differences were found between the treatments regarding the condition factor and the viscerosomatic index (Table 2). Histological analysis revealed no differences for the amount of immune cells in the lamina propria (Figure 1). No eosinophilic granular cells were found in the intestinal tissue of any of the treatments.

Whole body CL and GE contents declined significantly between the carp analysed initially at the start of the experiment and all carps fed all treatments at the end of the experiment, whereas the CA content increased (Table 3). Only CP remained unchanged. At the end of the experiment, significant differences among treatments were found for CL, where all fish

Table 2. Growth and performance parameters for common carp fry fed diets with different duckweed proportions (n = 4; means ± standard deviation)

Treatments[1]	Control	Duckweed, dried			Duckweed, fermented			*P*-value
		DWD 150	DWD 300	DWD 450	DWF150	DWF300	DWF450	
Initial group body weight, g	15.4 ± 1.3	15.6 ± 0.8	15.9 ± 1.1	16.2 ± 1.3	15.6 ± 0.9	15.5 ± 0.6	16.0 ± 0.9	0.892
Final group body weight, g	35.0 ± 4.3^{a}	35.7 ± 2.2^{a}	36.1 ± 3.8^{a}	36.2 ± 3.9^{a}	35.4 ± 1.1^{a}	32.1^{ab} ± 1.0	27.8 ± 1.5^{b}	0.004
Group body weight gain, g	19.7 ± 3.2^{a}	20.1 ± 1.5^{a}	20.3 ± 2.9^{a}	20.0 ± 2.6^{a}	19.8 ± 1.2^{a}	16.6 ± 0.5^{a}	11.8 ± 1.2^{b}	0.004
Relative body weight gain, %	128 ± 12^{a}	129 ± 6^{a}	128 ± 12^{a}	123 ± 6^{a}	127 ± 13^{a}	107 ± 4^{a}	73.8 ± 8^{b}	<0.001
Specific growth rate, %/day	1.68 ± 0.10^{a}	1.69 ± 0.06^{a}	1.68 ± 0.11^{a}	1.63 ± 0.06^{a}	1.67 ± 0.12^{a}	1.49 ± 0.04^{a}	1.13 ± 0.10^{b}	<0.001
Feed conversion ratio, g/g	3.56 ± 0.24^{a}	3.47 ± 0.16^{a}	3.55 ± 0.24^{a}	3.67 ± 0.15^{a}	3.54 ± 0.25^{a}	3.97 ± 0.10^{a}	5.20 ± 0.44^{b}	<0.001
Protein productive value, %	8.23 ± 0.55^{a}	8.24 ± 0.35^{a}	8.49 ± 0.55^{a}	8.09 ± 0.32^{a}	8.31 ± 0.56^{a}	7.58 ± 0.18^{a}	5.91 ± 0.46^{b}	<0.001
Lipid productive value, %	7.67 ± 0.96^{ab}	6.99 ± 0.56^{b}	6.17 ± 0.96^{b}	6.14 ± 0.49^{b}	8.80 ± 1.03^{a}	7.25 ± 0.33^{ab}	4.20 ± 0.79^{c}	<0.001
Condition factor, g cm^{-3}	2.29 ± 0.10	2.26 ± 0.03	2.23 ± 0.12	2.20 ± 0.05	2.27 ± 0.13	2.27 ± 0.03	2.27 ± 0.09	0.821
Viscerosomatic index, %	10.8 ± 0.7	11.0 ± 0.8	10.7 ± 0.3	10.5 ± 0.4	10.2 ± 1.8	11.4 ± 0.5	11.3 ± 0.7	0.456

[1] duckweed (*Spirodela polyrhiza*) in dried (DWD) and fermented (DWF) forms replacing either 150, 300 or 450 g/kg equivalent to 39.3, 78.6 or 117.8 g/kg of fishmeal protein; [ab] means within the row with different superscripts are significantly different at $P < 0.05$ (one-way ANOVA, Tukey HSD post-hoc)

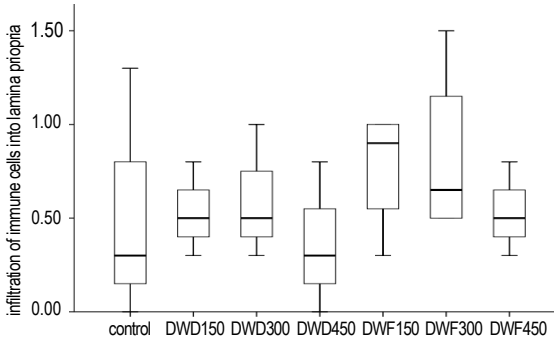

Figure 1. Box plots of immune cell infiltration into lamina propria in carp fry 0 = no infiltration, 3 = severe infiltration. DWD = duckweed dried; DWF = duckweed fermented; 150, 300 and 450 = 150, 300 or 450 g of fishmeal protein replaced by duckweed protein

Table 3. Initial and final whole body proximate composition (g/kg wet weight) of carp fry fed with diets with different duckweed proportions (n = 4; means ± standard deviation)

Treatments[1]	Initial[2]	Control	Duckweed, dried			Duckweed, fermented			*P*-value
			DWD 150	DWD 300	DWD 450	DWF150	DWF300	DWF450	
Moisture	756 ± 4*	773 ± 6	777 ± 1	775 ± 5	773 ± 4	772 ± 2	773 ± 2	772 ± 4	0.487
Crude ash	26.0 ± 1.7*	30.4 ± 0.5^{ab}	32.8 ± 1.7^{bc}	34.0 ± 2.1^{c}	34.6 ± 0.5^{c}	29.7 ± 1.3^{ab}	29.4 ± 0.7^{ab}	29.1 ± 2.4^{a}	<0.001
Crude protein	183 ± 3	187 ± 5	183 ± 3	185 ± 6	186 ± 4	185 ± 4	184 ± 3	183 ± 4	0.728
Crude lipids	112 ± 3*	73.5 ± 4.3^{abc}	70.1 ± 2.0^{ab}	68.0 ± 2.4^{a}	70.3 ± 3.7^{ab}	78.2 ± 3.2^{bc}	79.0 ± 4.4^{bc}	80.5 ± 6.4^{c}	0.001
Gross energy, MJ kg^{-1}	8.60 ± 0.10*	7.12 ± 0.27	6.80 ± 0.14	6.77 ± 0.26	6.83 ± 0.16	7.17 ± 0.11	7.15 ± 0.27	7.15 ± 0.25	0.047

[1] duckweed (*Spirodela polyrhiza*) in dried (DWD) and fermented (DWF) forms replacing either 150, 300 or 450 g/kg equivalent to 39.3, 78.6 or 117.8 g/kg of fishmeal protein; [2] n = 2; ^{abc} means within the row with different superscripts are significantly different at $P < 0.05$ (one-way ANOVA, Tukey HSD post-hoc); * significant difference between initial fish and final fish ($P < 0.05$, one-way ANOVA, one-sided Dunnett post-hoc)

fed with DWF, independent of its concentration, were richer in CL compared to fish fed with DWD. Feeding DWF also resulted in significantly reduced CA contents compared to DWD300 and DWD450.

Discussion

With its fast growth and relatively high crude protein content of between 20–40% (Leng et al., 1995; Stadtlander et al., 2022), duckweed could be a potential protein source for animal feeds, with the possibility to produce it in circular systems, thus recycling nutrients from liquid animal manure (Stadtlander et al., 2019; 2023a).

The overall performance of the carp fry during our experiment appeared to be low with specific growth rates below 2%/day. Specific growth rates of 4–6%/day have been reported for carp fry at water temperatures of 23–26 °C (Kaushik, 1995). The water temperature of around 19 °C in our experiment probably contributed to the reduced overall performance. This may also be the reason for the unfavourably high feed conversion rations of between 3.5 and >5. Another potential reason for the comparatively low performance could be the low feeding frequency. However, Charles et al. (1984) reported that the optimum feeding frequency for *C. carpio* fry was three times per day and a similar performance was reported for other cyprinids, i.e. the Indian major carps catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) (Biswas et al., 2006). When carp fry was fed levels up to 200 g/kg of *L. minor* meal and kept over different temperatures, no differences compared to a control group were found for body weight gain, feed conversion ratio and protein utilization. However, the overall performance including growth, feed conversion and protein utilization was remarkably higher compared to the performance in our study. (Yilmaz et al., 2004).

Independent from the overall low performance, our results show that the protein of dried *S. polyrhiza* can be utilized to replace up to 450 g/kg of the fishmeal protein and that of fermented *S. polyrhiza* could replace up to 300 g/kg of fishmeal protein without significantly impairing performance. The high inclusion level of 400 g fishmeal/kg in the control diet of our study and the obtained results, especially for DWD, suggest that dried *S. polyrhiza* would be a valuable feed ingredient for common carp. Fermented *S. polyrhiza*, on the other side, did not show similar results in the present study. Although no significant difference was found in growth, feed and nutrient utilization up to a fishmeal protein replacement of 300 kg^{-1}, the numerical value showed already a decline, which became significant when the replacement level reached 450 kg^{-1}. This inferiority of DWF compared to DWD was unexpected because feeding rohu fingerlings of around 6.5 g with fermented *L. polyrhiza*, independent of its inclusion level, had been found to significantly improve performance (growth, feed conversion and protein utilization) compared to control fed fish (Bairagi et al., 2002). However, for fermentation, these researchers used a bacterial strain isolated from common carp intestines, while in the present study, *S. polyrhiza* was fermented using a mix of EM−1 and *Pediococcus pentosaceus*.

Feeding the experimental diets for 5 weeks in the present study resulted in significantly declining whole body lipid concentrations and, subsequently, gross energy content in all treatment groups. This could likely be attributed to the different composition of the standard feed used before the experiment being significantly higher in protein and lipids compared to the experimental diets. Across groups, the lipid content was significantly higher in carps fed DWF450 compared to all carps fed dried duckweed (DWD150, DWD300 and DWD450). This difference could have either resulted from the slightly

higher lipid content in diet DWF450 or from the lower growth rate at an unchanged lipid deposition in the body, as per unit of growth more energy was available from feed intake. The latter seems also likely as carps fed DWD300 had a significantly lower crude lipid content and no growth depression. The whole body crude protein content did not show any differences between initial and final fish or between any of the treatment groups. Only little and inconsistent differences in whole body composition were reported by Yilmaz et al. (2004) for common carp fry fed increasing proportions of *L. minor*. In rohu fingerlings, compared to control feeding dry and fermented *L. polyrhiza* resulted in increased carcass concentrations of of whole body crude protein at the higher inclusion levels of dry and all inclusion levels of fermented *L. polyrhiza* (Bairagi et al., 2002).

Physiological utilisation of feed as a whole and of the individual feed components depends mainly on digestibility and absorption of nutrients for metabolic processes. Carp species are lacking a true stomach, and stomach-less fish do not have an acidic digestion with optimum pH of 6.0 for intestinal microbiota reported for *C. carpio* (Kuz'mina et al., 2014). In addition, carp species are lacking pepsin, which is an important protease for protein digestion. Common carps showed reduced protein digestion and reduced apparent absorption efficiency of amino acids when fed soybean meal as sole protein source compared to carps fed fishmeal as sole protein source (Dabrowski, 1983). Still, it can reasonably be assumed that, due to the long relative intestinal length, the common carp can utilize duckweed more efficiently compared to carnivorous fish species such as rainbow trout (*Oncorhynchus mykiss*) or Atlantic salmon (*Salmo salar*) (Karachle and Stergiou, 2010). Accordingly, rainbow trout fry fed two low dietary duckweed inclusion levels (62.5 g/kg and 125 g/kg) performed slightly but significantly less well, although no difference was found between both duckweed groups (Stadtlander et al., 2019). Two carnivorous fish species fed two differently processed *S. polyrhiza* meals, one dried and one fermented, reacted totally different. Eurasian perch (*Perca fluviatilis*) fry was unable to even utilize the lowest amounts of either *S. polyrhiza* meal type, while rainbow trout fry were capable to utilize both *S. polyrhiza* meals rather well (Stadtlander et al., 2023b).

Feeding large quantities of plant-based diets over a prolonged time can induce enteritis as has been shown for example in Atlantic salmon (Baeverfjord and Krogdahl, 1996) and in common carp fed soybean meal (Urán et al., 2008). Intestinal inflammation in carp is associated with a shortening of the mucosal folds, subsequent loss of supranuclear vacuolization, a thickening of lamina propria and sub-epithelial mucosa, infiltration of inflammatory cells such as macrophages and eosinophilic granulocytes and an increased number of goblet cells in the epithelium (Urán et al., 2008). In our study, none of the described effects was observed in any of the treatment groups. In general, induction of enteritis by soybean meal is considered to be due to the presence of anti-nutritional factors, most importantly soya saponins as reported for Atlantic salmon (Knudsen et al., 2007). For *S. polyrhiza* the presence of cyanides, tannins and phytic acid has been reported (Fasakin, 1999), whereas oxalic acid and calcium oxalate was found in *L. gibba* and *L. minor* (Franceschi, 1987). These secondary compounds could play an important role in nutrient availability and utilization and more detailed studies on presence and concentrations of anti-nutritional factors are needed.

Conclusions

Our results show that the protein of dried meal of the duckweed species *Spirodela polyrhiza* could replace fishmeal protein by up to 450 g/kg in diets for slow-growing carp fry. Fermented *S. polyrhiza* was less suitable and could only replace fishmeal protein up to 300 g/kg without negative effects on growth and nutrient utilization. Utilizing duckweed grown on wastewaters could help closing nutrient cycles, especially for nitrogen and phosphorus. Duckweed production is, however, currently only a niche market and practiced mostly at small scale with few large scale producers being the exception. For large-scale application still some biosafety aspects have to be clarified.

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Conflict of interest

The Authors declare that there are no conflicts of interest.

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