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Doctoral thesis

Circular and sustainable fish nutrition

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

Nutrients and nutrition knowledge applications in circular aqua-food systems

1.1. Present global context and our food system

The world's population has grown tremendously from 2.5 billion people in 1950 to 7.7 billion people in 2019. The global population is expected to reach 8.5 billion in 2030, 9.7 billion in 2050, and 10.9 billion in 2100 (DESA, 2019). Our planet also has a carrying capacity, which is characterized by planetary health boundaries. The planetary boundaries framework defines the "safe operating space for humanity" and is represented by nine global processes that can destabilize the Earth system or have disastrous consequences if breached (Rockström et al., 2009). Therefore, human activities and resource consumption must stay within these limits for the planet to sustain itself in the future. On a continental scale, already in Europe, at least four out of nine planetary boundaries have been crossed because of human activity since the industrial era, *i.e.,* climate change, biodiversity loss, land-system change, and altered nitrogen and phosphorus cycles (Commission, 2019). For example, in Europe (EEA/FOEN, 2020), the limit for nitrogen (N) losses (footprint) has exceeded not only the European limits but also the global limit. The phosphorus (P) footprint and land cover anthropization have exceeded the European limits. However, Europe's freshwater use has not breached the planetary health boundary and lies much below the European limit for a safe operating space for its future generations (EEA/FOEN, 2020). It indicates the potential of a blue-based bioeconomy in the future (EUMOFA, 2018; Kuempel et al., 2021) while reducing the pressure on land and mitigating footprints as much as possible. Nonetheless, an urgent change is needed in how we produce food, the quality and quantity of products we eat. Besides, the inedible losses also need to be minimized and re-valorized.

Additionally, the latest climate change report is being dubbed as 'code red for humanity' (IPCC, 2021). The IPCC (IPCC, 2021) reports with 'very high' or 'high' confidence that in 2019, atmospheric CO_2 concentrations were higher than at any time in at least 2 million years, and concentrations of other more potent GHGs (CH_4 and N_2O) were higher than at any time in at least 800,000 years. The global surface temperature has increased faster since 1970 than in any other 50-year period over at least the last 2000 years. In the first two decades of the 21st century (2001-2020), the rise in global surface temperature was +0.99°C (confidence interval: 0.84–1.10 °C). While just within one decade (2011-2020), the current global surface temperature has risen to +1.09°C (0.95-1.20°C). As a result, in 2011–2020, the annual average Arctic sea ice area reached its lowest level since at least 1850 (IPCC, 2021).

Today, nearly 800 million people suffer from hunger, while 2 billion overweight people are at health risk. However, one-third of our food is wasted (Commission, 2019). In terms of our food systems, the challenges of rising populations and the need to feed 9 billion people will require a 60% increase in food production. Besides, there is an increasingly unsustainable global demand for meat and animal products. Presently, 25 to 30% of greenhouse gas (GHG) contributions come from farm to fork food production, half of this from meat production alone (Commission, 2020). On the other hand, if food loss and waste were a country, it would be the third most significant source of greenhouse gas (GHG) emissions (UNEP, 2021). A recent report estimates that around 931 million tons of food waste were generated in 2019; 61% of the wastage came from households (fork), 39% occurred from farm to fork (food processing, retail) (UNEP, 2021).

The future of our food system faces some challenges too. For example, 70% of global freshwater and 30% of global energy production are consumed in making our food, in the middle of a growing scarcity of natural resources. The challenge is to improve the diets of the 2 billion people who remain overweight or obese. There is also a big challenge of reducing food waste which presently accounts for 33% of our total food production, while 800 million people go hungry every day (Commission, 2020). Moreover, our food system's weak resilience and insecurity to respond to global shocks, such as the global COVID-19 pandemic, recently came to the forefront (Commission, 2020; Duguma et al., 2021; Selin, 2021). The COVID-19 pandemic drew all the necessary attention to rebuilding a more resilient, homogeneous, and highly connected global food system (Selin, 2021). Even the zoonotic transfer of COVID-19 contagion and various other contagions before (e.g., H1N1, Swine flu, Ebola and the Nipah virus) to humans is another grim reality of our food system (e.g., wild animal retail, livestock and poultry farming) (Aiyar and Pingali, 2020; Rohr et al., 2019). It is a reality we are going through at the moment. Following the official first anniversary of the global COVID-19 pandemic (WHO, 2019) and because of the environmental impacts (mentioned above), it has become even more critical to ponder whether we are producing (farming) and consuming (eating) within the limits of our planet (?). As of today, the answer is perhaps "no." Food production is among the most significant cause of global environmental change (Van Zanten et al., 2018; Willett et al., 2019).

1.2. Intensification of aquaculture and fish as a food

After the second world-war, increased production in food systems like agriculture has come at a price. They include pollution, pushing of planetary health boundaries, greenhouse gas (GHG) emissions, pests and diseases, poorer animal welfare, and perhaps the erosion of societal emotions surrounding agriculture. Many feel that modern agriculture equals large-scale, heavy chemicals and genetically engineered sectors (de Boer and van Ittersum, 2018). The focus on increasing production at any cost, with little consideration of the environment and planet's finite resources, has brought numerous problems (de Boer and van Ittersum, 2018).

In this aspect, aquaculture may not be far behind. Although situations with intensive aquaculture waste management, captive feed efficiency, fish-in fish-out ratio, and meeting the nutritional requirements of a diverse array of fed aquaculture species have improved, persistent or emerging pitfalls in the present aquaculture landscape exist (Naylor et al., 2021). For example, a recent documentary entitled 'Seaspiracy' has divided the global community on sustainability concerns surrounding aquatic food. On the other hand, the fisheries and aquaculture community have been dealing with some misinformation surrounding aquaculture (GSA, 2019). Even though fish is expected to be a part of our future healthy and sustainable diet (Bogard et al., 2019), the expectations should not be at the cost of the abovementioned problems. Fish must be produced sustainably to be part of a proposed futuristic 'planetary healthy diet' (Willett et al., 2019).

In general, fish intake has been associated with reduced risk of cardiovascular diseases, better cognitive functions, reproductive health, and therefore considered healthy. In general, fish has a high content of omega-3 fatty acids, which have many essential roles, including being precursors of eicosanoids, a large component of the central nervous system, a structural element of every cell of the body, and a regulator of cardiac rhythm (Bogard et al., 2019; Willett et al., 2019). Presently, fish (irrespective of capture fisheries or aquaculture) provide 3.1 billion people with about 20% of their daily animal protein intake. It is crucial for the world's poorest, for whom fish that are eaten whole constitute essential micronutrient security (Castine et al., 2017; Willett et al., 2019). With ~90% of global wild fish stocks being overfished or fished at capacity, future 'fish as healthy food' should come from 'aquaculture,' one of the fastest-growing food production sectors globally (FAO, 2020; Willett et al., 2019).

As per the latest FAO statistics (FAO, 2020), global fish production reached about 179 million tonnes in 2018, with a total first sale value estimated at USD 401 billion. The present estimated annual supply of fish for the global population is about 20.5 kg per capita (FAO, 2020). Aquaculture accounted for 46 percent of the total fish production and 52 percent of fish for human consumption. From the current global aquaculture production of 82.4 million tons (in 2018), global aquaculture production is projected to surpass 100 million tons by 2030 (FAO, 2020). In the last few decades, total fish production has seen important increases in all the continents, except Europe. Europe saw a gradual decrease from the late 1980s but recovered slightly in the last few years. Europe presently contributes only ~10% of total global fish production, capture fisheries, and aquaculture combined (FAO, 2020).

Fed aquaculture (57 million tonnes) has also outpaced non-fed aquaculture. The latter accounted for only 30.5 percent of total aquaculture production in 2018 (FAO, 2020). If the aquaculture sector sustains its current average annual growth rate of 5.70% (fish) and 9.91% (crustaceans), then the external provision of nutrient and feed inputs will have to grow at a similar rate. From 2000 to 2017, the commercial aquaculture feed sector has grown over three-fold, from 13.8 to 51.2 million tons. This increase represents an average percentage rate of 8.0% per year since 2000 and is expected to reach ~73 million tons by 2025, respectively (Boyd et al., 2020; FAO, 2020). Aquaculture's resource use needs to be re-scrutinized through overarching lenses, *i.e.,* from the farm to the fork, from animal/ farm level to industry level, and under a bird-view of food systems (Commission, 2020).

The critical resource constraints for the future of aquaculture include competition for feed resources and available land for freshwater farming. Research in sustainable aquaculture feeds also developing rapidly (Colombo and Turchini, 2021; Willett et al., 2019). The future environmental footprint of fish production depends on the aquatic species farmed, what they eat (or fed), and where aquaculture occurs (Willett et al., 2019).

1.3. Connection of fed aquaculture and its environmental impact

Aquaculture intensification also increases waste generation. The impact of waste products from aquaculture has increased public concern and threatens the sustainability of aquaculture practices. Therefore, aquaculture waste management has been one of the major problems, having the greatest impact on the environment (Cao et al., 2007; Dauda et al., 2019). The feed has been reported as the primary source of waste in aquaculture systems (Dauda et al., 2019; Martins et al., 2010). Waste production from feed depends on many factors like palatability, water stability, nutrient composition, method of production (extruded vs. pelleted), digestibility, the ratio of feed size to fish size, the quantity of feed per unit time, feeding method, and storage time (Dauda et al., 2019; Prabhu et al., 2019). The waste produced from aquaculture units can be categorized into soluble (e.g., ammonia, orthophosphate, and mineral ions) and solid waste (e.g., egested feces, uneaten feeds) (Prabhu et al., 2019). Other indirect nutrient emissions from intensively fed aquaculture to the environment may include diffusive losses of greenhouse gases (GHGs like nitrous oxide, methane) due to decomposition of aquaculture effluents (sludge from uneaten feed, feces) (Hu et al., 2012; Williams and Crutzen, 2010; Yuan et al., 2019). These GHGs are, in fact, several times more potent than the common carbon dioxide in causing global warming (Hu et al., 2012; Yuan et al., 2019).

Conclusions from previous life cycle assessments (LCAs) highlight the feed and feeding efficiency as fundamental to the environmental impact of most aquaculture production systems (Aubin et al., 2009; Biermann and Geist, 2019; Henriksson et al., 2015; Mungkung et al., 2013; Papatryphon et al., 2004). Long before it was predicted that a major future challenge for aquaculture would be influencing the composition and ratios of nutrients in aquaculture effluents and facilitating further water purification processes through proper diet formulation or nutrient provisioning to fish (Verdegem, 2013). Diet-related strategies to reduce aquaculture waste production have predominantly focused on improving feed efficiencies and digestibility (Gatlin III et al., 2007; Prabhu et al., 2019). Compared to improvement in digestibility of nutrients in feedstuffs (a priority strategy of aquafeed industry) (Glencross, 2020; Turchini et al., 2019), strategies focusing on improving metabolizability of feeds have become stagnant, *i.e.,* suppressing of reactive, non-fecal losses of N, P. Partly because metabolic losses are more challenging to manage than fecal losses (Bureau, 2004; Bureau et al., 2003; Hardy and Gatlin III, 2002). However, at times, these non-fecal losses (invisible) can seriously outweigh (e.g., N) or nearly match (e.g., P) the 'visible' fecal losses (Bureau, 2004; Hardy and Gatlin III, 2002; Kaushik, 1995; Roy et al., 2020; Sugiura et al., 2000). Therefore, addressing the issue of nutrient loading from feed to the environment (via fish) is a complex task, which, if addressed correctly, would take care of the most fundamental environmental impact of aquaculture production (Boissy et al., 2011).

Even the alternative feedstuffs of plant origin that are presently being dubbed as 'sustainable' (but may not be so; Colombo and Turchini, 2021) are known to contain anti-nutritional factors, nutritional imbalances, non-bioavailable form(s) of specific nutrients, which may increase excretory nutrient loading from fish to the environment (Kokou and Fountoulaki, 2018; Prabhu et al., 2019). There is another possibility that conventional plant-based feedstuffs or diets (which are presently being advocated in aquaculture) may fail to improve the environmental profile of fish farming (Boissy et al., 2011) and be counter-productive in a future circular bioeconomy framework. The primary reasons being high eutrophication potential (directly linked with digestibility of fed aquatic animals, fertilizers use in land-based cultivation of plant feedstuffs), high land occupation potential (linked with land-based cultivation), and high ecotoxicity potential (herbicides, pesticides use in land-based cultivation) surrounding decisions to switch entirely to 'presumably sustainable' plant-based choices in aquafeed (Boissy et al., 2011). Indeed, the rapidly expanding aquaculture sector can negatively affect coastal habitats, freshwater, and terrestrial systems (related to the area directly used for aquaculture and feed production) (Willett et al., 2019).

In this context, plant-, algal-, microbial- and insect- feedstuffs raised on wastes, integrated with the existing farming systems (as a bio-based waste recycling system component, end-of-pipe treatments) and producing biomass that do not go for direct human consumption (avoiding food-feed conflict) would be the face of future, circular origin, and sustainable fish nutrition sources in aquaculture. It should be one of the core mandates of evolving to 'Aquafeed 3.0' (circular aquaculture nutrition) from the present 'Aquafeed 2.0' (replacement of fish derivatives in aquafeed); which the scientific community is lacking clarity now (Colombo and Turchini, 2021). This paradigm shift of feed resources origin to produce the food (fish) would be much needed to neutralize environmental impact and increase aquatic food systems' growth within planetary health boundaries (Commission, 2020; EEA/FOEN, 2020).

1.4. Visions of circular bioeconomy in food systems and aquaculture

EAT-Lancet Commission proposes 'fish' as the first (in terms of the possible range of intake) or second (in terms of average intake) most important component among animal-sourced protein and lipid sources; in the future, reference and healthy planetary diet for humans (Willett et al., 2019). Hereinafter, referred to as 'healthy planetary diet.' For example, in Europe, to achieve such a healthy planetary diet, the consumption of red meat among animal protein sources needs to be significantly cut down, while the share of fish in the diet needs to be increased. This transition alone would offset many GHG emissions, N and P footprint from the territorial food system (Willett et al., 2019). Consumption of white meat (poultry and fish) is not associated with increased mortality while consuming red meat is associated with increased risk of stroke and type 2 diabetes (Willett et al., 2019). However, aquaculture production needs to be expanded sustainably, given its effect on and linkage with land and aquatic ecosystems (both). Aquaculture can help steer the production of animal source proteins in future planetary healthy human diet towards reduced environmental effects and enhanced health benefits (Willett et al., 2019). But even EAT-Lancet commission assessment (Willett et al., 2019) perhaps underestimate the role of blue (aquatic) food in the context of human and environmental health. Blue (aquatic) foods would be particularly indispensable for human nutrition and environmental health for the healthy planetary future we aim for (Ahern et al., 2021; Gephart et al., 2021; Golden et al., 2021).

In Europe, one in four of every fish product consumed comes from aquaculture. However, the majority of their origin is covered by imports from outside the European Union (EU). Presently around 60% of the total fish supply in the EU is covered by imports. Only 10% of EU fish consumption is of EU aquaculture origin (Commission, 2021). It shows a sizeable margin and future growth potential. Despite these commercial prospects, EU aquaculture production has only increased by +6% (since 2007) and reached 1.2 million tonnes in sales volume and 4.1 billion € in turnover in 2018. The EU's aquaculture contribution to world aquaculture production is less than 2% as of 2018 (Commission, 2021; FAO, 2020). To further boost EU fish production, the European Commission has adopted some strategic guidelines for more sustainable and competitive aquaculture over 2021–2030 (Commission, 2021). It emphasizes explicitly: (a) reducing pollution; (b) preserving ecosystems and biodiversity; (c) more circular management of resources (European Commission 2021; COM/2021/236 final).

To ensure aquaculture's booming growth (FAO, 2020) stays within the planetary health boundaries and societal emotions surrounding it remain intact, we need to revisit some *status quo* approaches and habits. Aquaculture's resource use needs to be re-scrutinized through overarching lenses, *i.e.,* from the farm to the fork, from animal/ farm level to industry level, and under a bird-view of food systems (Commission, 2020). Presently the European Commission, as part of its green deal and a global leader of a new concept in its territorial food systems (Food 2030 pathways) (Commission, 2020), vows on promoting a future that would embrace a 'circular bioeconomy framework'; combining agriculture, aquaculture, forestry, and other potential non-food industries (Commission, 2019, 2020). Adoption of this approach is expected to address multiple issues at once: food security, managing natural resources sustainably, reducing dependencies on non-renewable resources, mitigating climate change, and creating jobs (Commission, 2019, 2020). Many might be unfamiliar with such a concept.

In terms of definition, circularity means recycling and reusing wastes from one system as input in another system. In principle, there is no waste in the totalitarianism of circularity. The waste generated from one system serve as an input or resource in another system. In a circular bioeconomy, the circular part aims to maintain the value of land, products, materials, and resources for as long as possible. The bio-economy part targets renewable biological resources to produce food, materials, and energy (Commission, 2019; de Boer and van Ittersum, 2018). But what is new, or how does it differ from sustainability (?). Circularity demands a paradigm shift in thinking, changing focus from increasing productivity (presently) to increased resource use efficiency (future), from animal or farm level to the industrial scale (e.g., food system) (Commission, 2020). Circularity does not discriminate between agriculture, forestry (forest products), aquaculture, or capture fishery; instead, it links the same circle. Circular bio-economy aims to improve resource use efficiency (RUE) greatly, minimize environmental footprint, and avoid inedible human losses by design, reuse, recycle, remanufacture, and integrating of resources as much as possible (Colombo and Turchini, 2021; de Boer and van Ittersum, 2018; Roy et al., 2021). Circular and sustainable bio-economy initiatives also advocate using bio-based solutions to emerging problems in food systems, biodiversity preservation, and ecosystem services maintenance (Commission, 2019, 2020). Resource optimization but not productivity alone, of land, animals, and energy, is the desired outcome of a future circular bioeconomy (de Boer and van Ittersum, 2018). To achieve such a desired outcome, our policy, science, food sector, consumer habits, and farmers' practices should point to the same paradigm in a coordinated way (Regueiro et al., 2021; Van Zanten et al., 2018).

Presently, our food system is somewhat linear, production-oriented, but not resource-oriented, demanding large inputs and leaving a large environmental footprint (Fig. 1) (Global, 2021).

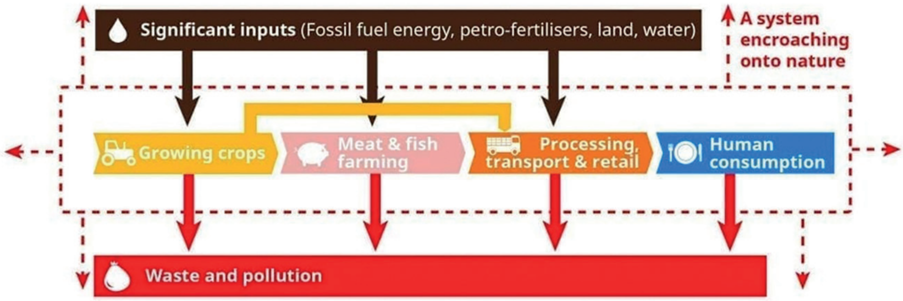

Figure 1. *Schematics of our present, linear local food systems. Adopted from Feedback global (2021) (Global, 2021).*

The basic principle of a circular food system is simple, *i.e.*, high RUE. However, its implementation is complex and challenging. Fig. 2 gives a simple overview of how circular bioeconomy in 'local' food systems may look like (Global, 2021). Fig. 3 shows how complex the implementation of the circular bioeconomy concept and its integration with other political goals might be on a 'territorial scale' (e.g., Food 2030 pathways) (Commission, 2020). If looked at closely, Fig. 3 is more like a circular puzzle than a circle filled with bubbles (Fig. 2); nonetheless, both are better than the present linear food system (Fig. 1). At the same time, the definition of circular food systems is still evolving (Dagevos and Lauwere, 2021; Muscat et al., 2021; **Roy et al., 2021**). From where the boundaries of circularity shall begin and where it must end remains to be seen. The concept is expected to evolve in this decade (Regueiro et al., 2021).

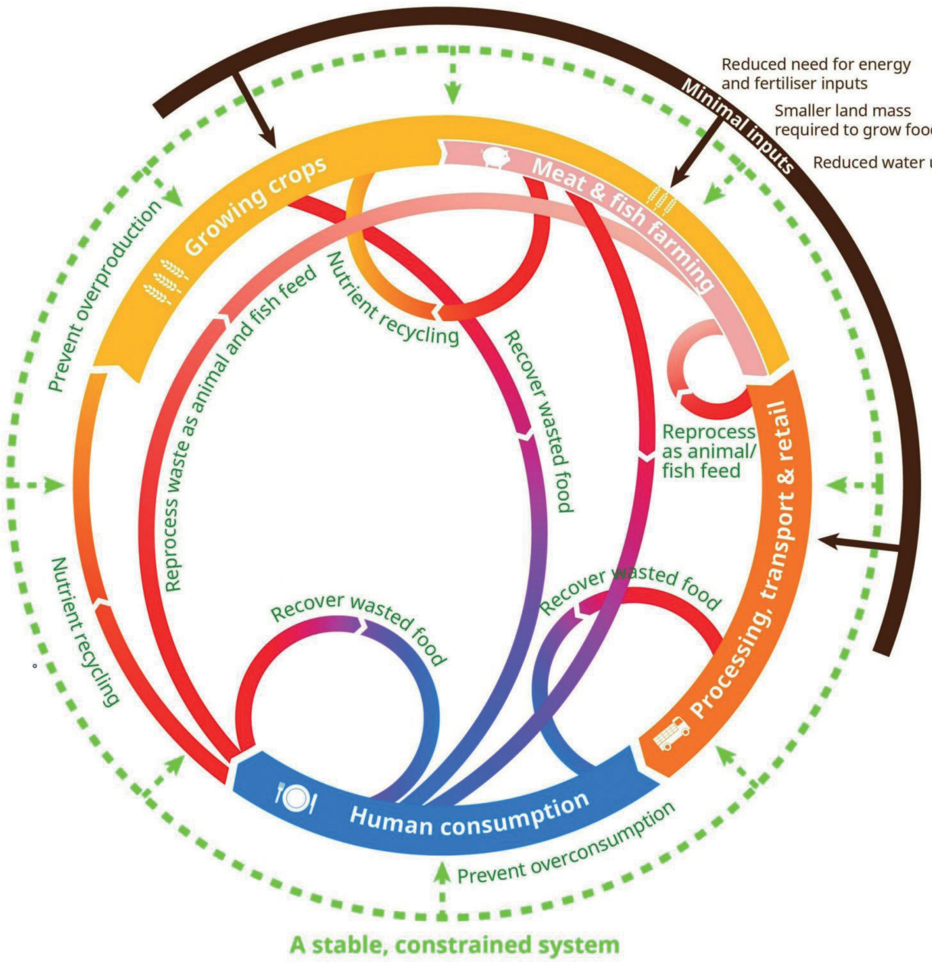

Figure 2. *Schematics of a future circular bioeconomy in local food systems. Adopted from (Global, 2021).*

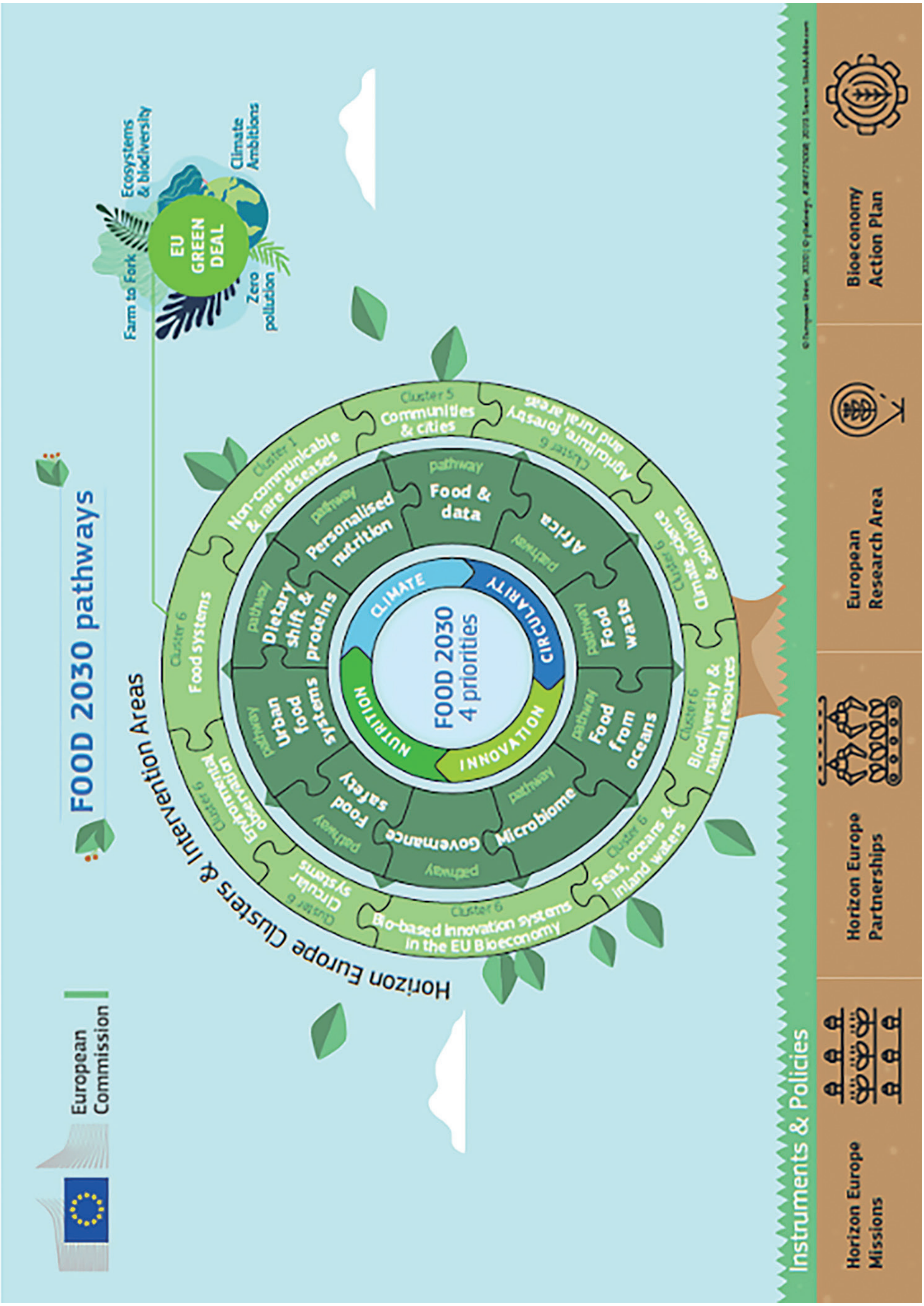

Figure 3. *Schematics of the future territorial circular bioeconomy in European union's food systems. Adopted from the "Food 2030" vision by the European Commission (Commission, 2020).*

In the abovementioned context of evolving definitions and boundaries, the author and colleagues (Roy et al., 2021) closely followed a previous blueprint (de Boer and van Ittersum, 2018). They laid out five contemporary puzzles that are needed "to be solved" for triggering circularity in aquaculture (illustrated in Fig. 4). First, plants being the engine of the carbon cycle by photosynthesis, are the basis of circularity in nature, and they should play the same role in aquaculture as well. The reviews (Oliveira et al., 2021; Reverter et al., 2021) point in this direction. Even if plants may not be directly used for aquaculture, they can still contribute to circularity – the review (Prazukin et al., 2020) points in this direction. Even the plants in aquaculture need to be drawn from the circular origin, from waste or side streams – the review (Ragaza et al., 2020) points in this direction. Besides, plants themselves can be raised on aquaculture effluents (e.g., aquaponics, flocoponics, use as fertilizers, or vermicomposting manures for plants).

Second, prevent human inedible by-products from piling up in fisheries and aquaculture. Most of it should be re-used for human food as much as possible, first, and then for other activities. We can make the most efficient use of animals to unlock biomass inedible for humans into valuable food, manure, and ecosystem services. Only when such options are exhausted should they be recycled to enrich the soil and fertilize plants. The recent reviews (Agboola et al., 2021; Siddik et al., 2021) touch on these parts of the story. Especially from the farm to the fork, a lot of inedible losses occur. For example, almost half of the fish (head, fins, viscera, carcasses with bones) are rendered inedible or given secondary importance during the filleting. They may still be converted to human edible food through low-cost value-added products, soups, spreads, and sausages, or can go for pet food production. In contrast, the carcasses or viscera itself can be a source of bioactive molecules (e.g., enzymes, fatty acids like arachidonic acid, minerals like organic or skeletal tissue bound selenium), which can be re-used as functional ingredients in fish feed (Jan Mraz personal communication, Mraz and Roy unpublished).

Third, reduce resource consumption and emissions to the environment by closing the loop of materials flow within aquaculture systems. Under this paradigm, losses should be prevented at all costs by recycling, reusing, or remanufacturing. The reviews (Khanjani and Sharifinia, 2020; Robles-Porchas et al., 2020) point in this direction. Even when a closed loop is achieved, optimizing it must not stop. Newer materials from non-food side streams may still be tried and tested – the review (Abakari et al., 2021) highlights this aspect. Few other real-life examples could be aquaponics (Baganz et al., 2021; Folorunso et al., 2021), flocoponics (Pinho et al., 2021), vermicomposting of aquaculture sludge (Kouba et al., 2018), and inclusion of earthworm in the fish feed while manure applied to horticulture, use of sludge from RAS to fertilize ponds. Besides, use of pond sludge to adjacent land farms and human-inedible plant by-products for inclusion in aquafeed or pond green manuring (AquaBridges consortium, Horizon 2020 consortium, personal communications).

Fourth, losses may be inevitable in aquaculture, and we need to understand why it is happening and think of all the possible side streams to put those lost materials into some use – the review (Schumann and Brinker, 2020) helps develop such understanding. For example, better technological advancements in aquaculture systems should be explored. Such as the advancement of filtration technologies, sludge digestion technologies (Martins et al., 2010), and raising of genotypes or phenotypes to reduce inedible human yield (Prchal et al., 2018; Prchal et al., 2021).

Fifth, while pursuing circularity, an important concern is the risk of disease transmission and resultant food safety. The reviews (Knipe et al., 2021; Melo-Bolívar et al., 2021) highlight some concerns that might be relevant in a circular aquaculture setup. Integrating terrestrial and aquatic ecosystem components in a closed-loop is a hallmark of circular food production (e.g., aquaponics). Addressing diseases and safety issues in the food generated from such systems is much complicated – the review (Folorunso et al., 2021) highlights such aspects. Besides, the use of animal origin wastes to feed other animals directly, re-manufacturing inedible carcass losses to human edible items, re-using human excreta, terrestrial livestock excreta in aquaculture would need epidemiological and microbiological safety of highest standards (Roy and Mraz unpublished); future research should be increasingly focused on this direction too.

Nevertheless, the abovementioned puzzles might not be all. Other socio-economic and food system dimensions need attention as well. For example, minimizing losses along the farm to fork, mitigating environmental footprint of farming methods per unit of consumable product, creating job opportunities or human resources development, addressing food-feed conflict in fed aquaculture, preservation of biodiversity and ecosystem services with aquaculture (Jan Mraz personal communication; Mraz and Roy unpublished).

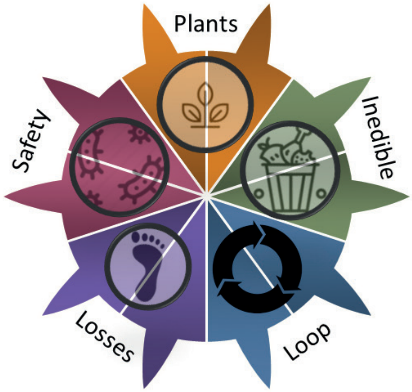

Figure 4. *Five contemporary puzzles "to solve" for triggering circularity in aquaculture (Roy et al., 2021). Photo source: Koushik Roy.*

The aquaculture research community feels responsible for promoting a future that would embrace circular aquaculture, adopt an aquaculture-centric bioeconomy, and find bio-based solutions in aquaculture or with aquaculture (author observations; AquaBridges consortium for HORIZON-CL6-FARM2FORK). Embarking on this challenge, we need to reset our thinking and focus more about the resources efficacy and how to utilize most material considered as waste. Of course, the change may not be possible immediately, rather it is a long-term coordinated effort (Roy et al., 2021). For this purpose, inter-disciplinary and complementary knowledge exchanges, resource use among agriculture, forestry, environmental solutions, or non-food industries must be triggered surrounding aquaculture (Roy et al., 2021). Here, such an application of 'fish nutrition knowledge' relevant to a futuristic, regional, aquaculture-centric circular bioeconomy (hereinafter referred to as 'circular blue bio-economy) is being demonstrated. The complexity and flow of nutrients in a circular blue bioeconomy in providing the future generations with a healthy planetary diet is graphically represented in Fig. 5.

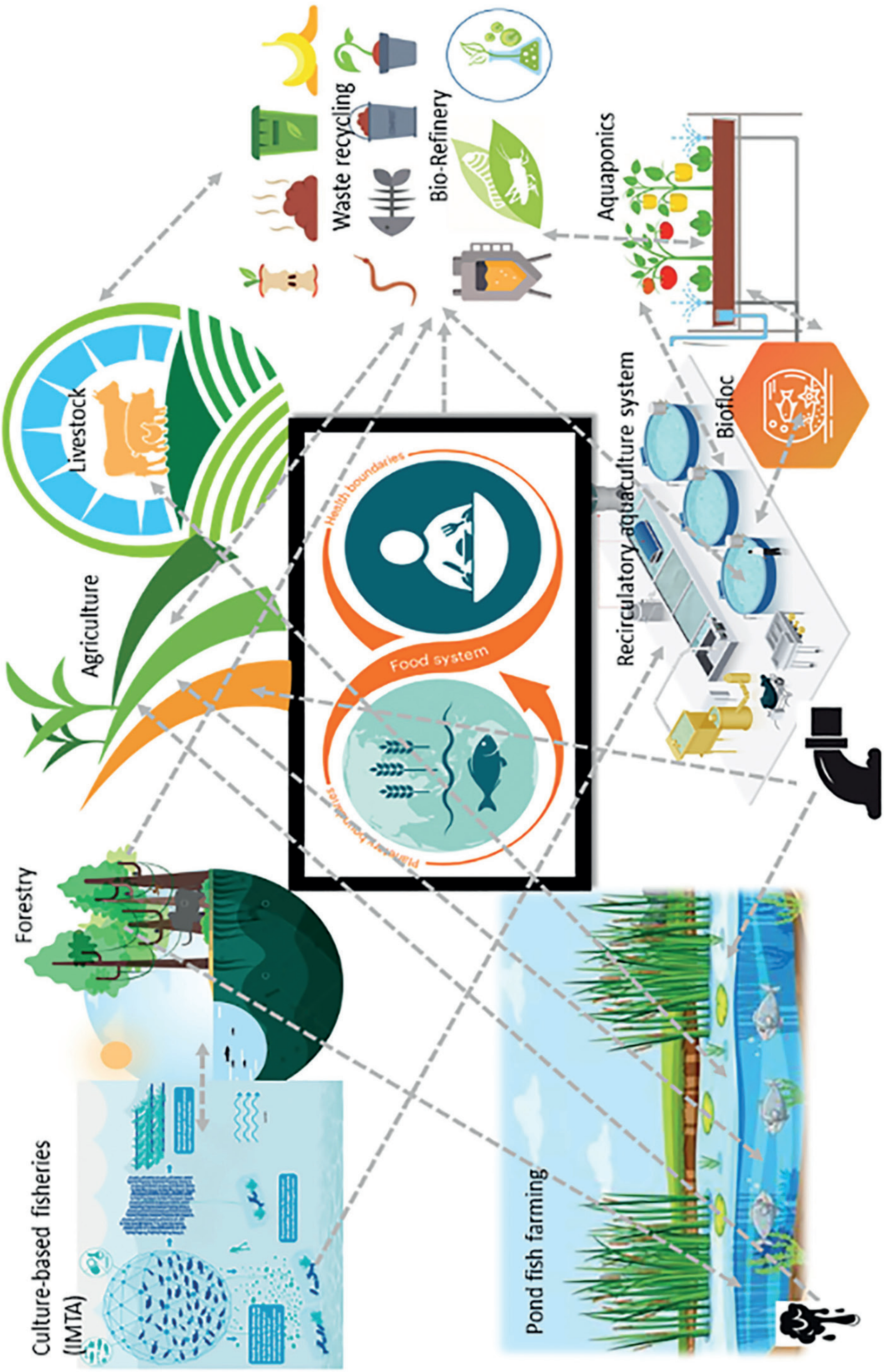

Figure 5. *The "complexities" and flows of nutrients in a future, circular blue bioeconomy for producing a healthy planetary diet in regional food systems. Photo source: Koushik Roy.*

1.5. Justification of fish nutrition as a tool and novelty of the work

The question of why nutrition was chosen as a tool lies in the presumption that perhaps nutrition (and resultant excretion) is the most dynamic and regular process in living organisms (besides respiration), which involves an exchange of nutrients to and from the environment. It is also one of the fundamental biological processes that connect *in-vivo* and *in-situ* in tandem. In order to address circularity and sustainability from the animal to farm level, such processes may be increasingly targeted in the future for assessments and bio-manipulation.

The present dissertation builds on the advances in fish nutrition that have been achieved after decades of research. Indeed, the subject of fish nutrition has come a long way. Some examples of conventionally known knowledgebase (among the many) are listed as follows: (a) feeding and nutritional requirements (NRC, 2011); (b) nutritional bioenergetics (Bureau et al., 2003); (c) amino acids metabolism (Li et al., 2009); (d) intermediary (carbohydrate) metabolism (Polakof et al., 2012); (e) *de-novo* fatty acids synthesis or metabolism (Xu et al., 2020); (f) feedstuff evaluation and anti-nutritional factors (ANFs) (Glencross et al., 2007; Hardy and Barrows, 2003; Kokou and Fountoulaki, 2018); (g) digestible and metabolic losses (Halver and Hardy, 2003, NRC, 2011) (h) effects of nutrient insufficiency (Guillaume et al., 2001; NRC, 2011) (i) growth trajectory (Dumas et al., 2007).

Some of the grey or evolving areas in fish nutrition were also touched on in this dissertation (discussed in the chapters). In order to cite a few examples, there is a recent paradigm shift of focus to non-essential amino acids (NEAAs) along with essential amino acids (EAAs) in defining protein quality, ideal protein concepts for fed animals (He et al., 2021; Hou et al., 2016; Kim et al., 2019). Till now, non-fecal mineral losses (urinary, branchial losses) causing a spike in reactive forms of nutrients in aquatic systems (thus triggering eutrophication) was thought to be of less importance (except C, N); but it might not be so due to imbalances dietary (digestible, retained) nutrient imbalances (Roy et al., 2002; Sugiura et al., 2000; Vielma and Lall, 1998) or even bioconversions of amino acids at the renal axis (Hou et al., 2016, Tomlinson et al., 2011). There was limited knowledge how zooplankton derived enzymes boost the difficult-to-digest fiber, mineral fractions (like P) from plant-based food items in ponds (Avila et al., 2011; Wynne and Gophen, 1981); with particular reference to the relationship between fiber digestibility and mineral bioavailability (Goff, 2018). After digestible intake, how interactions happen between dietary amino acids (AAs), fatty acids (FAs), non-protein energy, and final impact happen on protein accretion (growth), *de-novo* lipogenesis (fattiness), reduced retention efficiency (Huang et al., 2020; Kersten, 2001; Li et al., 1996; Polakof et al., 2012; Zhang et al., 2017). Potentially new focus on digestible phosphorus to protein ratios (PPR) in diet taking hints from less explored evidence (D'Alessandro et al., 2015; Noori et al., 2010) to formulate environmentally responsible aquafeed besides conventional focus on digestibility (to improve bioavailability) or balancing energy to protein ratio (to trigger protein-sparing) (Bureau et al., 2003; NRC, 2011). Unification of ecosystem RUE concepts with optimum retention ratios of nutrients *in-vivo* to minimize losses (Hodapp et al., 2019). There was also limited knowledge of evolving proportions or ratios of reactive to suspended losses in fish excreta influenced by nutritional profiles of the diet (Chumchal and Drenner, 2004; Lamarra Jr, 1975; Vanni, 2002). Digestibility of minerals in fish primarily focus on N, P, lipid, and carbohydrates (mainly C), but a minor effort is given to explore the intake and excretion of other minerals that are important for plants if circular resource (waste) use is to be planned (*i.e.,* from fish to plants to fish).

The dissertation has few novelties hidden within its chapters. The work unified several concepts like *in-vivo* nutrient partitioning in fish, *in-vivo* and *in-situ* nutrient flow with ecosystem or system-level RUE, cycling of nutrients from environment to fish, and fish to the environment in different forms (suspended, reactive, organic-bound). Many multidisciplinary applications were made using fish nutrition and excretion, ecosystem, and aquaculture system knowledge (detailed in each chapter). They include: (a) metadata synthesis on nutrition (digestibility, retention, dependencies of growth, food quality); (b) autochthonous nutrient loading and eutrophication potential of fishes and farming methods (N, P and their environmental costs); (c) waste management and manipulation from feed to fish to plants in RAS and aquaponics; (d) alternative feedstuffs for aquaculture nutrition; (e) pond fish nutrition and eco-intensification of pond fish farming in line ecological principles (plankton ecology group model; ecosystem services; eutrophication); (f) vulnerability assessment in the valorization of wastes and invasive species from a perspective of nutrients and nutrition.

Another intangible novelty is knowledge use efficiency (KUE). We think that knowledge is an intangible resource too. If a greatly improved RUE is the need of the hour, then it applies to KUE as well. One of the core strategies of this dissertation was that the author and the colleagues applied the knowledge of fish nutrition and excretion in multidisciplinary, circular, and sustainable contexts (elaborated below). Additionally, if circular and sustainable bioeconomy advocates bio-based solutions (as mentioned above), the present dissertation shows that the knowledge of fish nutrition and/or excretion could be applied to develop some bio-based solutions. To the best of our knowledge, fish nutrition is often seen as an *in-vivo* topic for optimizing the growth or reproduction of aquatic animals for the sake of commerce, conservation, or nutritious food (fish). Besides, fish nutrition is seen as an isolated topic from fish excretion. The present dissertation is a humble attempt to enlarge these boundaries of perception.

1.6. Aim and objectives

This dissertation aims to build a collective awareness in improving KUE and encouraging bio-based solutions using fish nutrition and excretion knowledge.

The aim will be achieved through the following overarching objectives:

1. To study the fishes' digestibility, retention, digestible losses, and metabolic losses of nutrients driving environmental nutrient loading and scopes for minimizing losses.
2. To study the nutritional problems and prospects of valorizing wastes, valorizing societally discarded aquatic species in aquaculture, and exploring alternatives to finite resources in sustainable and circular aquaculture.
3. To apply fish nutrition knowledge in developing 'bio-based solutions' for improved resource use efficiency and improved valorization of wastes.

The works under these objectives is presented chronologically, as they developed over the course of doctoral program (2018–2022), arranged through chapters 2 to 7.

Chapters 3, 5 and 6 are related to the first objective. Chapters 2, 4 and 8 are related to the second objective. Chapters 6 and 7 are related to the third objective.

Chapters' relevance within circularity and sustainability goals

A mind-map of the dissertation chapters fitting into the circular and sustainable bioeconomy goals of the food system (mentioned above) is provided in Fig. 6.

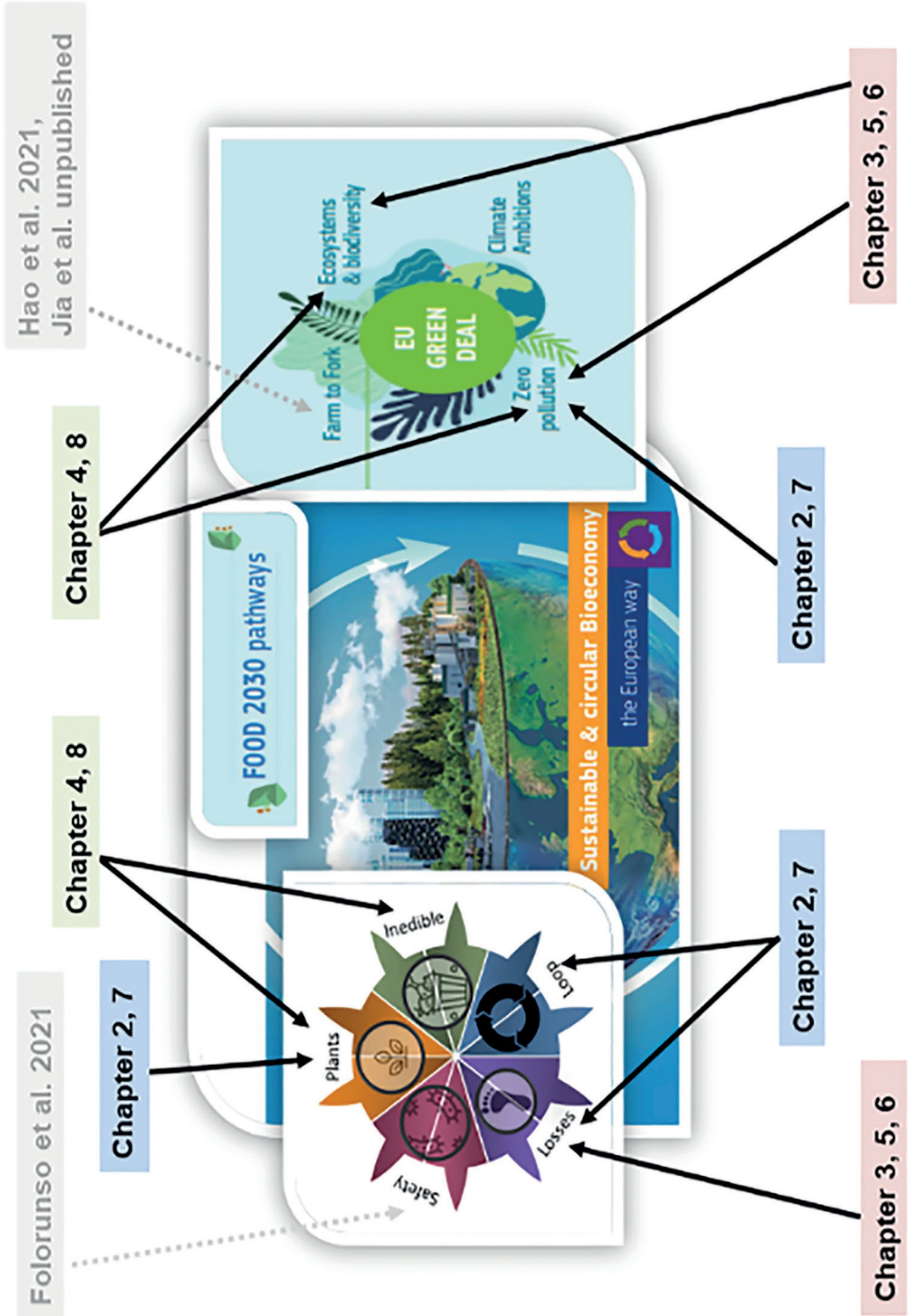

Figure 6. *Mind map of the chapters included in the dissertation and how they fit the sustainable and circular bioeconomy goals of the future food systems. Only first-authored or joint-first authored outputs are included and emphasized in the dissertation. Co-authored outputs that did not involve fish nutrition per se (not included in the dissertation) but fit the context are mentioned in the background (faded color) (Folorunso et al., 2021; Hao et al., 2021). Photo source: Koushik Roy.*

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CHAPTER 2

WASTE IS NOT WASTE, BUT RESOURCE: CLOSING THE LOOP BY NUTRITION AND EXCRETION

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Research article

Understanding nutrient throughput of operational RAS farm effluents to support semi-commercial aquaponics: Easy upgrade possible beyond controversies

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ABSTRACT

The present research attempted to address a key industry-level question amidst Recirculating Aquaculture System (RAS) waste throughput and aquaponics limitations controversies. Nutrient throughput of three operational RAS farms with progressive size proportions (16, 130, 1400 m^3), aquaculture intensity (24, 62, 86 kg stock m^{-3}) were studied. Results suggest - daily total efflux and potency of nutrients in effluents should not be generalized, extreme variability exists. Consistencies of nutrients in wastewater (except N, Ca and Na) are higher than in sludge. Asynchrony between patterns of nutrient loading and effluent nutrient concentrations exist for secondary macronutrients and micronutrients (S, Mg, Fe, Cu, Zn, B, Mo). Macronutrient output generally increases with increasing farm size and culture intensity but same cannot be said for micronutrients. Deficiency in wastewater can be completely masked using raw or mineralized sludge, usually containing 3–17 times higher nutrient concentrations. RAS effluents (wastewater and sludge combined) contain adequate N, P, Mg, Ca, S, Fe, Zn, Cu, Ni to meet most aquaponic crop needs. K is generally deficient requiring a full-fledged fertilization. Micronutrients B, Mo are partly sufficient and can be easily ameliorated by increasing sludge release. The presumption surrounding 'definite' phyto-toxic Na levels in RAS effluents should be reconsidered – practical solutions available too. No threat of heavy metal accumulation or discharge was observed. Most of the 'well-known' operational influences failed to show any significant predictable power in deciding nutrient throughput from RAS systems. Calibration of nutrient output from operational RAS farms may be primarily focused around six predictors we identified. Despite inherent complexity of effluents, the conversion of RAS farms to semi-commercial aquaponics should not be deterred by nutrient insufficiency or nutrient safety arguments. Incentivizing RAS farm wastes through semi-commercial aquaponics should be encouraged - sufficient and safe nutrients are available.

1. Introduction

The lack of space for expansion and new sites (resource competition from other users), limited fresh water availability, and concerns over pollution are considered as key obstacles for further expansion of commercial intensive aquaculture systems (e.g. cage-based and flow-through aquaculture systems). Therefore, most European countries have promoted Recirculating Aquaculture Systems (RAS) as one of the possible solutions and opportunities to further develop aquaculture (Badiola et al., 2012). In European countries, the development of RAS has been positive (Badiola et al., 2012; Eurostat, 2018; Martins et al., 2010). Aquaculture production data from freshwater RAS at the whole-EU scale is only accessible till 2010 - estimated at 20,658 tons. Denmark followed by Netherlands are the most prolific RAS producers within EU, together comprising around 90% of the total aquaculture produce from RAS. The example of the Czech Republic, a landlocked central European country, is one of its kinds. It clearly demonstrates the progressive expansion of RAS with production increasing from mere 36 tons in 2009 to 237.7 tons during 2016 *i.e.* nearly a 7-fold increase in 8 years, most intensely during 2013–2016 (Eurostat, 2018). However, there might be both good and bad sides to this prolific growth as discussed by several authors over the years (e.g. reviewed in, Badiola et al., 2012). A detailed account on the history, status and research development of RAS industry in Europe can be found in Martins et al. (2010); hence skipped from further introduction.

From the industrial point of view - fish waste management has been one of the problems having the greatest impact on the environment. Negative effects of waste from aquaculture to aquatic environment are increasingly recognized, although they are negligible to land-based pollutants (Cao et al., 2007). The varieties of wastes produced in RAS and waste recycling or disposal methods available have been well discussed in scientific literature (Badiola et al., 2012; Ebeling and Timmons, 2012; Martins et al., 2010; Rijn, 2013; Schneider et al., 2005). The overall waste treatment efficiency employing various microbial degradation techniques (the most common one in RAS) is still too low and leads to a mismatch in surface areas between fish production and microbial reactors (Schneider et al., 2002; Martins et al., 2010). Same mismatch often occurs between the mechanical filter surface area and culture water volume (Murray et al., 2014). The slow adoption of RAS technology is in part due to the high initial capital investments required by RAS (Martins et al., 2010). The average

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pay-back period under normal circumstances has been estimated at 8 years which is quite long (Badiola et al., 2012). This often compels RAS managers to employ high stocking densities in pursuit of higher system productivity to be able to cover the investment costs. This also results in an increase in both quantity and potency of 'in-system' and 'off-system' wastes. Consequently, waste management concerns concurrently arise (Martins et al., 2005, 2010). RAS investors rarely present properly researched plans and investment for farm waste utilization which quickly becomes a 'headache' as production expands (Badiola et al., 2012; Murray et al., 2014). Another ground reality being - substantial track record of RAS company failures exists in Europe and worldwide. There may be many RAS who may have ceased to exist, or production levels are quite insignificant (< 100 tons per annum) (described in Murray et al., 2014). Here the subject of integrating hydroponics (resulting into aquaponics) comes under discussion and often attains a *'prima-facie'* status among the producers. Introduction of such new 'commercially reap-able' compartments such as 'aquaponics production' is viewed as a 'by-pass' to overcome environmental or economical constraints of commercial RAS ventures. The aquaponics offer a variety of solutions- (a) decrease final environmental output, (b) valorize nutrients taking advantage of produced byproducts and, (c) generate products to supplement economical input on a regular basis (Badiola et al., 2012; Palm et al., 2018; Rijn, 2013).

Technologically speaking - RAS systems were developed for intensive fish farming, mainly where land and/or water availability is restricted: they enable up to 90–99% of the water to be recycled that too within a limited land-area. These systems allow the operator a greater control over the culture-climate, biosecurity and water quality parameters, reduced food miles (*i.e.* producing in urban set-up close to the markets) and improved product security (Badiola et al., 2012; Murray et al., 2014). Technicalities of RAS have been discussed in detail in Ebeling and Timmons (2012). Conventional RAS farms ensure > 90% water recirculation (< 10% replacement per day) or recirculation @0.1–1 $m^3\,kg^{-1}$ feed (Martins et al., 2010; Murray et al., 2014). In this process they generate limited but concentrated (nutrient rich) volumes of wastewater and sludge on daily basis; providing an opportunity for improved waste management and nutrient recycling (Martins et al., 2010). Irrespective of whether a RAS farm is marine or freshwater, the wastes generated have real economic values (if re-utilized) and a wide range of recycling options is available (Badiola et al., 2012; Murray et al., 2014; Rijn, 2013). Many environmental groups support RAS over open-production systems for the same reasons (Murray et al., 2014). In recent years, the EU environmental policy directives have become more stringent bringing serious implications for aquaculture sector. These include clumping down of aquaculture input use, farm waste effluent penalties and lowered ceilings in waste nutrient concentrations (Hlavač et al., 2016; Hoevenaars et al., 2018). RASs have been modified to respond to such increasing environmental regulations in countries with limited access to land and water (Martins et al., 2010).

Aquaponics combines two technologies: recirculation aquaculture systems (RAS) and hydroponics (soil less plant production) in a closed-loop system where either complete or majority (> 50%) of nutrients sustaining the optimal plant growth is derived from RAS effluents (Forchino et al., 2017; Palm et al., 2018). Aquaponic systems range from traditional RAS and hydroponic units combined in a single loop that deems fish feed as the only plant fertilizer source (called '1-loop' or coupled aquaponics) to separated aquaculture and hydroponic units (called '2-loop' or decoupled aquaponics) with higher investment, significant nutrient addition and water control (Monsees et al., 2017a, b). Aquaponic units have also been classified as 'extensive' (with integrated RAS sludge usage) and 'intensive' (with sludge separation) (Junge et al., 2017). Aquaponics are effective at nutrient removal when sized correctly (plant surface area: fish culture volume) to balance nutrient production by fish culture and nutrient uptake by plants. It introduces vegetable crops as biofilter (phytoremediation) that reduces nutrient load from the effluents and/or improves quality of 'returning' water. The plants (vegetable crops) represent an additional 'saleable' commodity for the fish farmer; an interim income source between the periodic fish harvests that also acts as 'leverage' to accidental fish losses (Blidariu and Grozea, 2011; Buzby and Lin, 2014). Research in the field of aquaponics has been 'trending' over the last decade (Junge et al., 2017; Palm et al., 2018). Ample literature exists in terms of its history and classification (Palm et al., 2018), system variants and technicalities (Junge et al., 2017; Rakocy et al., 2006), nutrient dynamics and requirements (Bittsanszky et al., 2016; Maucieri et al., 2018), sustainability assessment (Forchino et al., 2017; Konig et al., 2016), challenges (Goddek et al., 2015, Yavuzcan Yildiz et al., 2017) and policy needs (Hoevenaars et al., 2018; Joly et al., 2015). FAO (2018) has deemed aquaponics (RAS + Hydroponics) as a major player in coping with the increased demand of a growing world population. However substantial doubts exist in this regard as many key questions about the overall feasibility of aquaponic production remain unanswered (Goddek et al., 2015; Monsees et al., 2017a, b; Short et al., 2017).

Unlike in the case of RAS, there is no dedicated database on aquaponics to probe their adoption and production successes. This leaves only few and published surveys conducted so far as the only means to gain insights on ground-level realities (e.g. Love et al., 2014, 2015; Mchunu et al., 2018; Short et al., 2017). Most of those surveys pointed out promising nature of aquaponics and tagged it as an emerging practice worldwide. However, the stigma of its scaling issues remains at large - still being a niche or 'backyard activity' performed at hobby or subsistence scale (Mchunu et al., 2018; Love et al., 2014). Owing to the scaling issues and lack of farmers' knowledge in addressing plant nutrition at larger scales, these systems have not proved commercially lucrative (Bostock et al., 2010). Nonetheless, aquaponics is indeed highly scalable to commercial systems if the basic principles and ratios of fish stocking density, feeding rates, crop growing area are maintained and coupling-decoupling needs are realized (Buzby and Lin, 2014; Monsees et al., 2017a, b; Rakocy et al., 2006). The present research addresses a key industry-level question in the middle of such contradictions: whether and, if yes, how easily European (more precisely, Czech) 'operational RAS farms' can afford to upgrade to 'semi-commercial (non-backyard) aquaponics' taking into consideration the quantity and nutrient potency of their daily discharged effluents (wastewaters, sludge) (?). By the term 'upgrade' – we imply to the primary intent of the farms in managing their waste in a eco-friendlier (vis-à-vis policy abiding) and 'commercially reap-able' way. In order to address the question, we attempted to quantify and characterize – (a) nutrient concentration in RAS effluents, (b) average system influx and effluxes of total nutrients, (c) potency of nutrient concentrations in effluents in relation to release (discharge) percentages, (d) relationships between system management protocols and nutrient discharge, (e) some empirical budgeting models based on identified relationships, and, (f) capacity of the farms to meet the nutritional needs of some common aquaponic crops.

2. Materials and methods

2.1. System selection

Two commercial RAS farms (Anapartners s.r.o., Prague http://www.ftn-aquaart.com/en/home-englisch/and Fish farm Bohemia s.r.o., Rokytno, https://www.fishfarmbohemia.cz/) and one experimental RAS facility (FROV, University of South Bohemia in Ceske Budejovice, http://www.frov.jcu.cz/en/institute-aquaculture-protection-waters/lab-nutrition) were studied during 2015–2017. Hereinafter, the farms are termed as 'FROV' (Farm A), 'ANAPARTNERS' (Farm B) and 'ROKYTNO' (Farm C); selected based on their progressive size proportions 1: 8: 80 (A: B: C). A detailed account of their operational and technical specifications (supplementary) can be found in Table 1 and Table S1, respectively. All the systems have been 'operational' for at least 5 years or more prior to the initiation of the present study; justifying our purpose of studying established systems with well laid SOPs (standard operational procedures). Furthermore, the systems were characterized by increasing intensity of aquaculture operations (e.g. no. Of species cultured, stocking density, feed rations, production) from A (lowest) to C (highest).

2.2. Sampling program

Sampling for RAS effluents were conducted intermittently at intervals of 4–5 months. By the term 'effluents', we imply 'wastewater' and 'sludge'. Sampling program were repeated 3 times for farm A (FROV), 4 times for farm B (ANAPARTNERS) and 5 times for farm C (ROKYTNO) depending on their increasing size proportions; back-stopping measure to minimize sample variability due to unknown size (scaling) influences, if any. Further details on sampling is included in supplementary text S1.

2.3. Sample analyses

Wastewaters and sludge were analyzed separately in a certified third-party laboratory (AGRO-LA, spol. s.r.o., Jindřichův Hradec) employing 'Czech standard' analytical methods (ISO verified and certified protocols in Czech Republic). Some selected 'plant-essential' elements were quantified. It includes – *primary macronutrients* (N, P, K), *secondary macronutrients* (Mg, S, Ca) and *micronutrients* (Na, Fe, Zn, Cu, B, Mo, Ni) (Resh, 2016). Additionally, some environmentally hazardous heavy metals (As, Cd, Hg, Pb, Ni, Cr) were measured from the sludge. In general, the lowest detectable limits on dry matter basis were 0.01 $mg\,kg^{-1}$, 0.01% and in wet matter 0.001 $mg\,L^{-1}$. Some elements, especially heavy metals, had element specific lower detection thresholds. All analyses were done in triplicate.

2.4. Database compilation, parameterization and descriptive statistics

Data were coded farm wise and then compiled to generate both farm-specific and pooled information. The categories of information were: (a) influx of various aquaculture inputs (b) efflux of various nutrients from the system, (c) total efflux of some 'inevitable' RAS nutrients at hypothetical exchange rates, and, (d) comparing the nutrient status in effluents with standard hydroponic solution concentrations for some common aquaponic crops. Keeping the space limitations into consideration - the parameters, their derivations (formulas) and assumptions-conditions have been provided in Table S2, category-wise.

Descriptive statistics were generated through SPSS 16.0. Mean values were

Table 1
Operational specifications of the studied RAS farms (*arranged in ascending order of size*).

Parameters	FROV (Farm A)	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED*
Volume (m^3)	16	130	1400	16-1400 (630.67 ± 680.61)
Fish species cultured (no.)	2	2	4	2-4
Water exchange (% day^{-1})	5.81	1.65	0.89	0.89-5.81 (2.37 ± 2.1)
Stock density (no. m^{-3})	30	70	75	30-75 (62.08 ± 19.48)
Stock mass (kg m^{-3})	24.38	62.46	85.71	24.38-85.71 (62.63 ± 25.32)
Feeding rate (% biomass day^{-1})	2	2.5	3	2-3 (2.58 ± 0.42)
Feed input (g m^{-3} day^{-1})	490	1560	2570	490-2570 (1713.33 ± 866.19)
Feed crude protein (%)	44.2	52	32	32-52 (41.72 ± 9.11)
Feed-N Input (mg L^{-1} day^{-1})	30	130	130	30-130 (105 ± 45.23)
Feed-P (%)	1.42	1.2	1	1-1.42 (1.17 ± 0.17)
Feed-P input (mg L^{-1} day^{-1})	10	20	30	10-30 (21.67 ± 8.35)
Feed micronutrient (%)[a]	9.18	6.32	9	6.32-9.18 (8.15 ± 1.35)
Feed micronutrient input (mg L^{-1} day^{-1})	40	100	230	40-230 (139.17 ± 83.61)
Food Conversion Ratio (FCR)	1.4	1.2	1.2	1.2-1.4 (1.25 ± 0.09)
pH buffer input (mg L^{-1} day^{-1})[b]	35	20	32	20-35 (28.75 ± 6.58)
Temperature (°C)	24.7	23.1	22.4	18.5-25.5 (23.2 ± 2.3)
pH (units)	6.74	7.53	7.64	6.47-7.95 (7.38 ± 0.43)
Total Suspended Solids (mg L^{-1})	12.19	39.04	64.29	12.19-64.29 (42.85 ± 21.69)
Electrical conductivity (µS m^{-1})	1.7	2	2.3	1.7-2.3 (2.05 ± 0.25)
Dissolved oxygen (mg L^{-1})	7.38	10	7.61	5.85-10.53 (8.35 ± 1.55)
Wastewater volume (m^3 day^{-1})	0.75	1.3	5	0.75-5 (2.7 ± 2.04)
Sludge volume (m^3 day^{-1})	0.1	0.2	0.5	0.1-0.5 (0.3 ± 0.18)
Sludge Dry Matter (%)	2.7	4.9	5.84	0.5-9.3 (4.74 ± 2.63)
Wastewater: RAS-Volume ratio (%)	4.7	1	0.4	0.004-0.047 (0.017 ± 0.018)
Sludge: RAS-Volume ratio (%)	0.63	0.15	0.04	0.0004-0.0063 (0.0022 ± 0.0025)
Sludge: Wastewater volume ratio (%)	13	15	10	0.10-0.15 (0.12 ± .0.023)
Parameters significantly differing [c] ($p < 0.05$)	volume, feed input, stocking density, stocking biomass, temperature, total suspended solids, crude protein of feed, sludge dry matter content, sludge: RAS-volume ratio, sludge dry matter: RAS-volume ratio, sludge: wastewater-volume ratio			
Parameters non-significantly differing [c] ($p > 0.05$)	fish species cultured, water exchange, pH buffer input, feeding rate, dissolved oxygen, pH, electrical conductivity, sludge volume, wastewater volume, FCR, feed phosphorus, feed micronutrient, wastewater: RAS volume ratio			

* Pooled values contain range and mean ± SD (in parentheses)
[a] Total ash content of the feed (excluding P)
[b] $Ca(OH)_2$ and KOH used @1:1 in Farm A; $NaHCO_3$ used in Farms B and C.
[c] Results from Kruskal-Wallis H Test.

checked for their fitness of representation by estimating their coefficient of variation (CV = standard deviation/mean). Parameters with CV > 1 were flagged as 'extremely variable' and were considered as unfit for generalization (pooling) and comparison (Snedecor and Cochran, 1989). In view of high variability, 95% confidence intervals (C.I.) were calculated for nutrient concentrations in effluents to obtain best fitted representative data.

2.5. Mapping of inter-system operational variability and effluent nutrient consistency

Data was coded farm-wise and subjected to Kruskal-Wallis One Way-ANOVA based on Ranks (Kruskal-Wallis H Test) (McDonald, 2014). Details of the test is included in supplementary text S1.

2.6. Modeling of operational influences on nutrient output through effluents

Attempts were also made to identify the most important operational influences that play a key role in influencing nutrient generation. The data was analyzed in multiple steps, employing various statistical tools (stepwise multiple regression, log-10 transformation and non-linear LOESS smoothing). The details are included in supplementary text S1.

3. Results

3.1. System characteristics, operational variability and effluent nutrient consistency

A descriptive account of system characteristics is presented in Table 1 and Supplementary Table S1. Keeping the motto of this section in mind, we skipped presenting the trends of individual system parameters from tables to the text. Nevertheless, a generally increasing trend in system parameters from Farm A to C is easily perceptible; function of increasing size and aquaculture intensity (A < B < C). *Three farms were significantly different ($p < 0.05$) from each other in the following aspects*: volume (m^{-3}), feed input (g m^{-3} day^{-1}), stocking density (no. m^{-3} day^{-1}), stocking biomass (kg m^{-3} day^{-1}), temperature (°C), total suspended solids (mg L^{-1}), crude protein of chosen feed (%), sludge dry matter content (%), sludge release ratio (sludge volume: RAS volume), sludge dry matter release ratio (sludge dry matter: RAS volume), sludge: wastewater volume ratio (%) – hinting these as probable 'set of factors' responsible for significantly differing effluent nutrients if the management regimes in Czech RAS farms are normalized. *The farms did not varied significantly ($p > 0.05$) in terms of* fish species cultured (nos.), water exchange (%), pH buffer input (mg L^{-1} day^{-1}), feeding rate (% biomass day^{-1}), dissolved oxygen (mg L^{-1}), pH (units), electrical conductivity (µS m^{-1}), sludge volume (m^{-3} day^{-1}), wastewater volume (m^{-3} day^{-1}), FCR of the chosen feeds (units), phosphorus and micronutrient contents of chosen feed (%), wastewater release ratio (wastewater volume: RAS volume) - probably acting as the 'set of factors' behind maintaining coherence in effluent nutrients (if any) in spite of diverse management regimes in RAS farms (Table 1).

Digging deep into the daily input and loading (by fish, see Table S2 for derivations) of certain nutrients into the systems, we found out that – feed-N, P and micronutrients input (mg L^{-1} day^{-1}) varied significantly ($p < 0.05$) among the farms in conjunction with significantly different daily feed input. In terms of nutrients loading by fish, estimated N and micronutrient loadings varied significantly ($p < 0.05$) while P-loading (mg L^{-1} day^{-1}) was similar ($p > 0.05$). In terms of nutrient consistencies in wastewaters (concentrations, mg L^{-1}) among the farms, 9 out of 12 nutrients *viz.* Total-P, K, S, Mg, Fe, Cu, Zn, B, Mo were found to be consistent (non-significant differences, $p > 0.05$) irrespective of farm-specific variations. Total-N, Ca and Na were found to be significantly differing among the farms; probably due to significant differences in feed crude protein alongside fish stocking biomass (vis-à-vis nitrogen) and choice of pH buffering agents ($Ca(OH)_2$ and KOH in farm A; $NaHCO_3$ in farms B and C). If the above rationale applies true, the absence of an 'equally anticipated' K from the list despite being used in farm-A (as KOH) is questionable; although K was present in sludge at much higher concentrations (Table 4). Interestingly, a closer look in our dataset revealed that the cluster of micronutrients (Fe, Cu, Zn, B, Mo) which appeared consistent across farms might be attributed to their 'trace concentrations' (≤0.01 mg L^{-1}) in the wastewater (Table 3); concentrations in sludge being much higher (Table 4). *Synchrony between the patterns of nutrient loading and nutrient concentrations in wastewater was observed for N, P and Na.* In other words, N-concentration in wastewater differed significantly across farms as did the N-loading by fish. Similarly, P-concentration in wastewater followed the same pattern as P-loading *i.e.* not differing significantly among farms. Presence of Na in the 'non-consistent nutrient list' was excluded from interpretation since complete data on Na input was unavailable; only 2 out of 3 farms had measurable Na-input (using $NaHCO_3$) (Table S. *Asynchrony between the patterns of nutrient loading and nutrient concentrations were observed for the micronutrients* (represented by S, Mg, Fe, Cu, Zn, B, Mo). Despite significantly different micronutrient loadings among the farms, their concentrations in wastewater did not reflect such trend. This also hints us a ***significant partitioning of micronutrients probably from wastewater to sludge compartment of the effluents*** (further elaborated below) (Table 3).

On the other hand, the significant differences observed in the sludge dry matter (%) content among farms was double-checked with another proxy parameter *i.e.* sludge-ash content (%). We found significant differences ($p < 0.05$) in sludge ash content too. After such dual confirmation, we infer that the sludge matrix is highly inconsistent and unpredictable among the farms – making any of its comparison impractical. We restrained from analyzing nutrient consistencies in sludge to avoid unknown, random interferences in our results due to variable sludge matrix consistency (also clarified under methodology section).

3.2. Nutrient output (concentration, total efflux and potency)

Keeping the space limitations into consideration, only the highlights of results have been presented in this sub-section. Detailed presentation can be found in supplementary text S2.

3.2.1. Primary macronutrients (N, P, K)

Wastewater: For total-N, nitrate was the most dominant fraction overall i.e. about 85% of the total-N concentration in wastewaters. K-concentration in wastewaters can be manipulated by using KOH as pH buffer in RAS even to the extents that it surpasses farm size influences on deciding the concentration (Farm B's K concentration < Farm A's, despite larger size). Overall in terms of primary macronutrients in wastewater – (a) the primary macronutrient efflux and potency were extremely variable in nature making it difficult to present any representative (pooled) scenario, (b) the nutrient output progressively increases with increased farm size (culture water volume) and aquaculture intensity, (c) there is a order in primary macronutrient output through wastewaters (N > K > P) and, (d) concentration of K can be manipulated beyond pre-existing 'farm size influences' by the use of .KOH as pH buffer in RAS systems (Tables 3 and S3, Fig. S1).

Sludge: All nutrient outputs through sludge are given on 'wet sludge' basis *i.e.* sludge with dry matter content of 0.5–9.3% (pooled mean 4.74 ± 2.63%). Sludge total-N concentration was over 2 times (210%) higher than in wastewater; Ammonia fractions dominating over nitrates. In the absence of nitrites and organic bound-N data we could not conclude that ammonia is the most dominant fraction. There might be a possibility that organic bound-N dominates the overall nitrogen fraction in sludge – scope for mineralization. Sludge had extremely higher concentration of total-P as compared to wastewater - 37 times higher (37873%). Sludge had almost 3 times (260%) higher K content than in wastewaters. Like in the case of wastewaters, K output through sludge can also be manipulated using KOH as a pH buffer in RAS even beyond influences of size and aquaculture intensity (Farm A's sludge K content was higher than both Farms B and C). Overall in sludge – (a) daily efflux of primary macronutrients are extremely variable making it difficult to present a generalized (pooled) picture, (b) the concentration of primary macronutrients in sludge does not necessarily increase with farm size and aquaculture intensity, (c) primary macronutrient concentrations in sludge are 2–3 times higher than in wastewaters (extremely high for P, beyond comparison with N and K), (d) the order of primary macronutrient output is N > P > K, and, (e) K output through sludge can be improved significantly by the use of KOH as pH buffer in RAS (Tables 4 and S3, Fig. S2).

Wastewater and sludge combined: All the results presented in this sub-section is estimated from a simulated release scenario where wastewater release is to the tune of 1% of total RAS volume and sludge release at 0.1% (see Table S2 for further details). Only efflux (g day^{-1} 1.1% $release^{-1}$) and potency data (mg L^{-1} day^{-1} 0.1% $release^{-1}$) were calculated. Overall in wastewater and sludge combined- (a) the order of primary macronutrient output was found to be N > K > P – matching the trend as in wastewater, (b) the macronutrient effluxes and potencies have generally extreme variability making them difficult to generalize or compare as such, (c) size and culture intensity matters, i.e. more the size and intensity, more is the nutrient output (Table S7).

3.2.2. Secondary macronutrients (Ca, S, Mg)

Wastewater: Interestingly, a peculiarity was noticed in Ca concentration among the farms. Despite not using $Ca(OH)_2$ as a pH buffer by farms B and C (as reported), they had comparable (farm B) or even higher (farm C) Ca concentration in wastewaters than farm A (used $Ca(OH)_2$ as pH buffer). We suspect an 'unreported' use of $Ca(OH)_2$ by the farms (especially farm B) as an emergency contingency measure to tackle greater drop of system pH; beyond rapid remedial capacity of the commonly used $NaHCO_3$. Especially for the revamped 'soviet-era' farm C, we suspect calcium leaching from some old calcified/cement tanks or water channels in the farm. There was some unexpected farm-level extreme variability in Mg output by farm A; unexplained. Overall in wastewater – (a) the concentration of secondary macronutrients did not generally increased as expected with increase in farm size and aquaculture intensity, (b) extreme variability exists in pooled efflux and potency of secondary macronutrients and hence cannot be generalized, (c) Ca concentration can be influenced by even emergency use of $Ca(OH)_2$ as pH buffer or leaching from old calcified structures, and, (d) the order of secondary macronutrient output is: Ca > S > Mg (Table 3, Fig. S3).

Sludge: All nutrient outputs through sludge are given on 'wet sludge' basis (also mentioned above). Due to methodological error sulfur (S) could not be measured in the sludge; although there may be significant amount locked. As presented in the case of wastewater, peculiarity in sludge Ca concentration was also observed. In fact, the lower concentration of Ca in farm A (using $Ca(OH)_2$) than both farms B and C was far from our anticipation. Moreover, higher Ca concentration in farm B than farm C reinforced our suspicion of an unreported $Ca(OH)_2$ use in farm B, probably to ameliorate high pH fluctuations (*clarified above*). The concentration Ca and Mg in sludge were almost 10 times (997%) and 4 times (388%) higher than in wastewater. Overall in sludge – (a) secondary macronutrient output unanimously increased with increasing farm size and aquaculture intensity, (b) the efflux of secondary macronutrients was extremely variable and hence cannot be generalized, (c) secondary macronutrient concentrations are over 4 times higher than in wastewater, and, (d) the order of secondary macronutrient output is: Ca > Mg, ignoring the Sulfur. Extrapolating our results from the other two secondary macronutrients, we assume that there might be approximately 3–9 times higher sludge S concentration than in wastewater (Table 4, Fig. S4).

Wastewater and sludge combined: All the results presented in this sub-section is estimated from a simulated release scenario; wastewater release (1%) and sludge release (0.1%) (clarified above). Data on sulfur could not be presented because it was not measured in sludge (mentioned above). Overall in wastewater and sludge combined – (a) secondary macronutrient output increased with increasing farm size and culture intensity, (b) extreme variability in efflux and potency exists making them difficult to generalize, and, (c) Ca is the most dominant secondary macronutrient (Table S7).

3.2.3. Micronutrients (Na, Fe, Zn, Cu, B, Mo, Ni)

Wastewater: Ni was not detected; probably absent. Unlike in other class of nutrients, the concentration of micronutrients did not show any prominent increasing trend from farm A to C. Interestingly the concentration of Na did not increased from farm B to C as anticipated due to increase in total $NaHCO_3$ input (Table 2). Cross matching this data with sludge Na concentration revealed a 'balanced' partitioning of Na from wastewater to sludge; masking the anticipated effect of increased Na concentration in wastewater with $NaHCO_3$ use (presented under sludge sub-section). Overall in wastewater – (a) the concentration of micronutrients did not exhibit any prominent increase with increasing farm size and culture intensity, (b) the output of most micronutrients except Fe are extremely variable and unfit for generalization, (c) concentration of Na did not increased with increasing $NaHCO_3$ input (pH buffer) in farms, (d) the order of micronutrient output is: Na > Fe > Zn > B > Cu > Mo, and, (e) the output of Mo was extremely low to comment upon and Ni was absent (Table 3, Fig. S5).

Sludge: Mo and B were below detection limits; could not be presented. Due to methodological error, Fe could not be measured for farms B and C. The concentrations of Cu, Zn were 15 times (1561%) and 17 times (1774%) higher than in wastewater, respectively. Interestingly, concentration of Na was 63.6% lower than in wastewater – the only nutrient showing such opposite trend. Ni was only detected in sludge and could not be compared with wastewater. Data on the concentration of Fe is only present for farm A. Comparing with Farm A's wastewater Fe concentration, we estimated a 562% (5 times) higher Fe concentration in sludge. Unlike in wastewater, almost all micronutrients in sludge showed an increasing concentration with increasing farm size and culture intensity. The increase in sludge Na concentration (farm A vs. farms B and C; Farm B to Farm C) corresponded with the increasing $NaHCO_3$ use at farm level (Table 2). Cross-matching this data with wastewater Na concentration hints a 'somewhat balanced' partitioning of Na between wastewater and sludge that on one hand masks the anticipated increasing of Na in wastewater with increased $NaHCO_3$ use and retains maximum Na in wastewater on the other hand. Overall in sludge – (a) the output of micronutrients have extreme variability, like other classes of nutrients, making them difficult to generalize or compare, (b) the concentration of micronutrients increases with increasing farm size and culture intensity (unlike in wastewater), (c) the concentration of micronutrients are usually 5–17 times higher than in wastewater, (d) Na concentration is almost 60% lower than in wastewater in spite of increasing with $NaHCO_3$ use in farms – a balanced partitioning with wastewater is apparent, (e) the order of micronutrient output is: Na > Fe (extrapolated) ≥ Zn > Cu > Ni, and, (f) Mo and B were below detectable limits (Table 4, Fig. S6).

Wastewater and sludge combined: Results on B, Mo and Ni were purposively excluded due to unavailability of concentration data in either wastewater or sludge (*explained above*). Overall in wastewater and sludge combined – (a) micronutrient output increased with increasing farm size and culture intensity, (b) extreme variability in efflux and potency exists making them difficult to generalize, (c) the order of micronutrient output (excluding B, Mo and Ni) is: Na > Fe (extrapolated) ≥ Zn > Cu (Table S7).

Table 2
Influx of various aquaculture inputs in the studied RAS farms.

Parameters*	FROV (Farm A)	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED#
N-loading (mg L^{-1} day^{-1})[a]	15.38	31.96	32.38	15.38-32.38 (27.99 ± 7.61)
P-loading (mg L^{-1} day^{-1})[a]	2.77	3.75	5.14	2.77-5.14 (4.08 ± 1.01)
Micronutrients loading (mg L^{-1} day^{-1})[a]	17.9	19.74	46.29	17.9-46.3 (30.34 ± 14.1)
Ca input (mg L^{-1} day^{-1})[b]	9.47	-	-	0-9.47 (2.37 ± 4.28)
K input (mg L^{-1} day^{-1})[b]	12.2	-	-	0-12.2 (3.05 ± 5.52)
Na input (mg L^{-1} day^{-1})[b]	-	5.47	8.76	0-8.76 (5.47 ± 3.62)
TSS (mg L^{-1})[a]	12.19	39.04	64.29	12.19-64.29 (42.85 ± 21.7)

[a] From selected feed
[b] From selected pH buffer
* See *Table S2* for clarification regarding calculations.
# Pooled values contain range and mean ± SD (in parentheses)

3.3. Heavy metal discharge (As, Cd, Hg, Pb, Ni, Cr)

The output of some environmentally hazardous heavy metals through sludge is given in Table S4. *The surveyed RAS farms were completely 'safe' in terms of their heavy metal discharge potential.* The concentration of all the heavy metals tested were 'far below' their respective pollution thresholds (Czech EPA limits, Table S4). Further details can be found in supplementary text S2.

3.4. Suitability of effluents in meeting nutrient requirements of common aquaponics crops

Based on our results of nutrient outputs through farm effluents, a self explanatory 'capacitogram' was generated in respect to the standard nutritional requirements of some commonly raised aquaponics crops (plants) (Table 5). Overall, considering both the capacities of wastewater and sludge, the macronutrient K is generally deficient requiring a full-fledged fertilization intervention (K fertilizers). Micronutrients like B, Mo are partly sufficient that can be easily ameliorated employing a variety of management decisions – (a) supplemental fertilization (not full-fledged), (b) by increasing wastewater exchange, or, (c) manipulating more sludge release. Nutrients like N, P, Mg, Ca, S, Fe, Zn, Cu are 'sufficiently meet-able' to plant needs using either wastewater or sludge or both 'as-it-is'. It should be noted that - *even if some nutrients are deficient in wastewater* (P, K, Ca, Mg, Ca, S, Fe, Cu) *to meet the plant needs, it can be completely masked by the use of raw or mineralized sludge* which contains almost an estimated 3–17 times (or even more, e.g. phosphorus 37 times) higher concentration of those nutrients than in wastewater. Cases on individual crops have not been elaborated here and can be easily interpreted from the capacitogram (Table 5).

The 'capacitogram' has four color blocks (green, light green, yellow, red and black) that have been defined in the legends. Counting the number of individual color blocks for each plant (Red + Black blocks; Yellow blocks; Green + Light green blocks as 'Green') and comparing the counts among plants, we prioritized the crops in terms of their 'nutritional management interventions'. By the term 'management interventions', we imply a combination of decisions on complete fertilization, supplementary fertilization or increase in wastewater exchange, sludge release manipulations. It is arranged in the descending order of 'nutritional management interventions' required: Chilli (Red + Black 10 + 2/Yellow 1/Green 9) > Cucumber (8 + 2/2/10) ≥ Tomato (7 + 2/4/9) > Lettuce and herbs (7 + 2/3/10). This order of priority should not be viewed as 'difficulty level' of culturing from plant nutrition perspective, as majority of the nutrients can be easily delivered from the effluents.

3.5. Modeling of operational influences on nutrient output

Keeping the space limitations into consideration, only the highlights of results have been presented in this sub-section. Detailed presentation can be found in supplementary text S3.

3.5.1. Wastewater

The results suggest that the concentrations of P and Mg cannot be predicted by any predictor (operational factors or variables) hinting some degree of unidentifiable, random influence on them. N, K, Ca, S and the whole cluster of micronutrients (Na, Fe, Zn, Cu, B) had some identifiable key driver influencing their concentration in wastewater. The notable factors that had key manifestation(s) on wastewater nutrient concentrations (in parentheses) were: fish species (K, Ca, S, Fe, Zn, Cu) > wastewater volume (N) > FCR (Na) > micronutrients loading (B). From practical point of view, the appearance of 'number of fish species cultured' as a key driver in determining most of nutrient concentrations in wastewater seems somewhat unrealistic. We infer it as a statistically abstract output since the data on 'fish species' had a very narrow variability (2–4 species; 2 species being the most common combination – farm A and B). Nonetheless, *it remains an interesting area to explore for future research whether increased cultured fish diversity in RAS farms generate more nutrient rich effluents (wastewater)* i.e. more the combination of fish species cultured, better the nutrient quality of wastewater (?). Appearance of FCR as a driver for Na was also partly unrealistic. Although fish feeds are known to contain 'some' amount of common salt (NaCl) in their composition, but that is far negligible in comparison to the input of Na into RAS systems through $NaHCO_3$ (as pH buffer). FCR also differed too little – by degrees of 1/10th of decimals (± 0.1) perhaps making the parameter very sensitive to predict nutrient (Na) concentrations (Table S5).

The empirical budgeting models suggest that per unit increase of wastewater volume (m^3) may lead to a corresponding change of +602.59 mg L^{-1} (standard error, SE ± 254.81) in N content of wastewater (R = 0.599). Likewise, a unit increase in micronutrients loading (mg L^{-1} day^{-1}, see Table S2 for derivation) may result in a change of +0.014 mg L^{-1} (SE ± 0.001) B in wastewaters (R = 0.955). A unit increase in FCR (units) corresponds to a change of −1760.28 mg L^{-1} (SE ± 295.51) Na (R = 0.883). Such large change in Na concentration should be carefully interpreted keeping in mind that the changes in feed FCR usually occur at the scale of 1/10th (e.g. changes by ± 0.1 units); therefore, concentration of Na in wastewater changes by −176.03 mg L^{-1} (SE ± 29.55) per 0.1 unit increase in FCR (R = 0.883). *All the above empirical estimates may presumably be considered as 'good-fit' within a range of aquaculture intensity but not universally*; *i.e.* the range of aquaculture intensity within which the models were generated (culture volume 16–1400 m^3, fish species 2–4, water exchange 0.89–5.81%, Stock mass 24.38–85.71 kg m^{-3}, Feeding rate 2–3% biomass day^{-1}, FCR 1.2–1.4, pH buffer input 20–35 mg L^{-1} day^{-1}). LOESS models between Total-N efflux and potency in respect to wastewater volume showed a slow but steady increase, slightly hinting a tendency of leveling-off at higher wastewater discharge (Fig. S7). The pattern of B efflux and potency in relation to increasing micronutrient loading showed an initial 'burst' followed by a 'gradual increase' at higher loading scenarios, also having an ultimate tendency to level-off like total-N (Fig. S8). LOESS models for Na could not be generated because changes in FCR were too small to generate any model.

In terms of multicollinearity between wastewater and sludge nutrient concentrations - we observed a mildly positive but non-significant partial correlation (r = 0.4, p > 0.5) between wastewater and sludge K concentrations. A mildly negative but insignificant partial correlation was observed in the case of Na (r = −0.317, p > 0.05). No partial correlation was observed for Total-N, Total-P, Ca, Mg, Zn and Cu for concentrations between wastewater and sludge.

3.5.2. Sludge

The results suggest – concentration of macronutrients in sludge (i.e. total-N, total-P, K, Ca, Mg) cannot be predicted by any predictor (operational factors or variables) hinting some degree of unidentifiable, random influence on them. However, the concentrations of micronutrients (Na, Cu, Zn, Ni) were influenced by some key drives and can be predicted. The notable factors that had key manifestation(s) on sludge micronutrient concentrations (in parentheses) were: sludge-RAS volume ratio (Na) > feeding rate (Cu) > stock mass (Zn) > fish species (Ni). The model of Ni with 'fish species' was excluded from presentation (clarified under wastewater) (Table S6).

As per the empirical models generated – (a) per unit increase in sludge release % (sludge: RAS volume ratio) may result in a decline of sludge Na concentration by 362.23 ± 74.65 mg L^{-1} (R = 0.838); (b) per unit increase in feeding rate (%) may increase sludge Cu concentration by 4.25 ± 1.21 mg L^{-1} (R = 0.744); (c) per unit increase in stock mass (kg m^{-3}) may increase Zn by 0.75 ± 0.22 mg L^{-1} (R = 0.726). It should be noted that, in practical situations, changes in sludge release % and feeding rate % usually occur at the scale of 1/100th (i.e. ± 0.01%) and 1/10th (± 0.1%) respectively. Therefore, interpretation from the models should be made carefully. For example - sludge Na concentration will decrease by 3.62 ± 0.75 mg L^{-1} per 0.01% increase in sludge release. Likewise, Cu concentration may only increase by

Table 3
Efflux of some selected plant-essential nutrients through released wastewaters from RAS.

Parameters	FROV (Farm A)	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED
		Primary macronutrients		
Total N ($mg\ L^{-1}$)[a]	350±308.36	673.18±427.02	2907±2689.51	41.64-7272.51 (1523.1±2050.82)
N efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	56±49.34	875.14±555.12	40698±37653.15	6.66-102000 (17263±30719.22)
N potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	74.67±65.79	673.18±427.02	8139.5±7530.63	8.88-20363.04 (3634.5±6045.05)
Total P ($mg\ L^{-1}$)[a]	2.29±0.84	1.79±0.39	2.94±1	1.31-4.24 (2.39±0.9)
P efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	0.37±0.14	2.33±0.51	41.16±14.02	0.2-59.4 (18.02±22.13)
P potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	0.49±0.18	1.79±0.39	8.23±2.81	0.31-11.87 (4.15±4.02)
K ($mg\ L^{-1}$)[a]	43.28±35.32	18.4±5.39	109.1±32.08	7.96-155 (62.42±49.03)
K efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	6.92±5.65	23.92±7.01	1527.4±449.17	1.27-2170 (646.12±823.78)
K potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	9.23±7.54	18.4±5.39	305.48±89.83	1.7-434 (135.73±159.44)
		Secondary macronutrients		
Ca ($mg\ L^{-1}$)[a]	88.53±26.45	84.3±3.35	234.76±134.84	62.1-463 (148.05±112.26)
Ca efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	14.17±4.23	109.59±4.35	3286.6±1887.73	9-94-6482 (1409.5±2010.71)
Ca potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	18.89±5.64	84.3±3.35	657.33±377.55	13.25-1296.4 (306.71±385.1)
S ($mg\ L^{-1}$)[a]	18.3±3	19.56±8.12	90.26±31.48	9.6-141 (48.7±41.5)
S efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	2.93±0.9	25.42±10.55	1263.7±440.66	2.93-1974 (535.75±695.44)
S potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	3.9±1.2	19.56±8.12	254.74±88.13	3.9-394.8 (112.8±134.68)
Mg ($mg\ L^{-1}$)[a]	30.82±40.12	8.92±0.81	41.17±16.27	4.57-77 (27.83±24.55)
Mg efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	4.95±6.45	11.59±1.05	576.44±227.82	0.73-819 (245.28±323.02)
Mg potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	6.6±8.6	8.92±0.81	115.29±45.56	0.97-163.8 (52.66±61.85)
		Micronutrients		
Na ($mg\ L^{-1}$)[a]	41.75±26.65	407±54.71	383.25±129.37	15.1-549 (305.79±180.27)
Na efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	6.68±4.26	529.1±71.12	5365.5±1811.13	2.42-7686 (2413.7±2833.08)
Na potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	8.99±6.66	407±54.71	1073.1±362.23	3.22-1537.2 (585.02±508.78)
Fe ($mg\ L^{-1}$)[a]	0.96±0.91	0.33±0.2	14.32±13.56	0.05-37.62 (6.32±10.82)
Fe efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	0.15±0.14	0.43±0.25	200.41±189.85	0.01-526.7 (83.69±154.02)
Fe potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	0.21±0.2	0.33±0.2	40.08±37.97	0.01-105.3 (16.86±30.73)
Zn ($mg\ L^{-1}$)[a]	0.1±0.07	0.12±0.05	4±3.9	0.03-10.66 (1.73±3.09)
Zn efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	0.02±0.01	0.15±0.07	56.03±54.66	0.01-149.24 (23.41±43.77)
Zn potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	0.02±0.01	0.12±0.05	11.21±10.93	0.01-29.85 (4.71±8.74)
Cu ($mg\ L^{-1}$)[a]	0.02±0.01	0.01±0.001	0.41±0.37	0.01-1.04 (0.18±0.31)
Cu efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	0.003±0.001	0.02±0.001	5.80±5.21	0.01-14.55 (2.42±4.33)
Cu potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	0.001±0.001	0.01±0.001	1.16±1.04	0.1-3 (0.49±0.87)
B ($mg\ L^{-1}$)[a]	0.04±0.02	0.05±0.001	0.42±0.1	0.02-0.58 (0.21±0.2)
B efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	0.01±0.002	0.06±0.001	5.94±1.41	0.004-8.18 (2.5±3.16)
B potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	0.01±0.01	0.05±0.001	1.19±0.28	0.01-2 (0.51±0.62)
Mo ($mg\ L^{-1}$)[a]	0.01±0.001	0.01±0.001	0.01±0.004	0.01-0.02 (0.01±0.001)
Mo efflux ($g\ day^{-1}$ % $release^{-1}$)[b]	0.001±0.001	0.01±0.001	0.12±0.06	0.001-0.22 (0.05±0.07)
Mo potency ($mg\ L^{-1}$ % $release^{-1}$)[c]	0.001±0.001	0.01±0.001	0.02±0.01	0.001-0.01 (0.01±0.01)
Consistent nutrients ($p<0.05$)[d]	Total-P, K, S, Mg, Fe, Cu, Zn, B, Mo			
Inconsistent nutrients ($p>0.05$)[d]	Total-N, Ca and Na			

[a] Observed concentration.
[b] Estimated at system scale (based on average daily wastewater output).
[c] Estimated at solution scale (based on resultant concentration of wastewater per percent culture water *i.e.* release ratio (percentage) – wastewater: RAS volume).
[d] Results from Kruskal-Wallis H Test.
Grey highlighted cells: Indicate extreme variability (CV>1) in values; rendering them unfit for generalization and comparison.

0.43 ± 0.12 per 0.1% increase in feeding rate. These models may be considered as 'good-fit' only within a range of aquaculture intensity but not universally (clarified above). LOESS models on effluxes and potencies of Na, Cu and Zn with respect to their key predictor(s) revealed some general trends. With increasing sludge release there is a steady but continuous decline in Na efflux and potency (Fig. S9). Efflux and potency of Cu and Zn seem to increase initially but gradually stagnates with increasing feeding rate and stocking biomass decisions, respectively. The effect is more pronounced in efflux rather than in potency (Figs. S10–S11). Multicollinearity results between sludge and wastewater nutrients have been presented under 'wastewater'.

4. Discussions

4.1. System characteristics, operational variability and effluent nutrient consistency

The natural feeding habit of fish species cultured, fish stocking density, total fish biomass, selection of feed, feed input rate, water quality and water management regimes are known to have decisive impact on the assimilation of nutrients in RAS and ultimate wastewater production. The main source of nutrients being – uneaten feed, fish feces, soluble excreta, pH buffer input and in-system solids or bioflocs (Ebeling and Timmons, 2012; Goddek et al., 2015). Most of the aquaponics viability studies till now have focused on the fact that waste generation by fish is directly related to the quantity and quality of feed being applied; that too predominantly from N and P perspectives (Buzby and Lin, 2014; Fornshell and Hinshaw, 2008; Schneider et al., 2005). Factors like - manipulations in wastewater-sludge release to amend nutrient concentrations, utilization of sludge as a major player in proving plant nutrition, seeing pH buffer input as a 'fertilization opportunity' have been always perceived as secondary thoughts. Under the current practices in RAS, solid wastes are only partially solubilized as they are mechanically filtered out daily (Goddek et al., 2015); soluble nutrients in RAS wastewater being the primary focus to plan aquaponics. Nonetheless, fish feed is the main nutrient input and defines, to a large extent, the sustainability of the aquaponics operation (Junge et al., 2017). We beg to differ a bit regarding the sustainability of operation by inserting 'wastewater-sludge release manipulations' and 'sludge recycling' as equally important co-factors besides the feed input. *The present study showcased that operational RAS farms are already capable of sustaining aquaponic operations with their present rate of feed input, given that they slightly increase their effluent discharge intensity.* For example - +2–3% for wastewater (by longer draining) and +0.1% for sludge (by adding more mechanical filter surface area); further discussed under nutrient output section.

Hu et al. (2015) suggested that aquaponics, with concomitant nutrient recovery, will probably become one of the widely used methods of sustainable food production soon. The contributions of such globally prevailing speculations are although 'positive vibes' for RAS farm managers or consultants to rely upon, but they are often insufficient to rationalize a decision. Especially the multitude of studies reasoning against the nutrient production from RAS being inferior for sustaining plant growth in hydroponic component – negative vibes (reviewed in Bittsanszky et al., 2016). There are already some 'established combinations' of fish and plant species that are perceived as gold-standards for venturing into aquaponics; presumably due to lower chances of failure adopting such combinations. The most common fish species are Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Onchorynchus mykiss*), common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*) which can be integrated with leafy vegetables, such as lettuce (*Lactuca sativa*), basil (*Ocimum basilicum*), spinach (*Spinacia oleracea*) (Forchino et al., 2017). Entrepreneurs often plunge into 'aquaponic ventures'

Table 4
Efflux of some selected plant-essential nutrients through discharged sludge from RAS.

Parameters	FROV (Farm A)*	ANAPARTNERS (Farm B)	ROKYTNO (Farm C)	POOLED
Primary macronutrients				
Total N (mg L^{-1})[a]	2000	3850±2816.91	3400±1272.79	400-7300 (3200±1821.46)
N efflux (g day^{-1} 0.1% $release^{-1}$)[b]	32	500.5±366.2	4760±1781.91	32-7280 (2158.17±2549.58)
N potency (mg L^{-1} 0.1% $release^{-1}$)[c]	320	2502.5±1831	9520±3563.82	260-14560 (4880.83±4800.57)
Total P (mg L^{-1})[a]	354	1200±898.15	1000±353.55	100-2300 (905.17±619.68)
P efflux (g day^{-1} 0.1% $release^{-1}$)[b]	5.66	156±116.76	1400±494.97	5.66-2100 (636.74±741.8)
P potency (mg L^{-1} 0.1% $release^{-1}$)[c]	56.64	780±583.8	2800±989.95	56.64-4200 (1440.8±1403.69)
K (mg L^{-1})[a]	200	125±77.57	170±42.43	30-230 (162.5±56.71)
K efflux (g day^{-1} 0.1% $release^{-1}$)[b]	3.2	16.25±10.1	238±59.4	3.2-322 (105.38±122.64)
K potency (mg L^{-1} 0.1% $release^{-1}$)[c]	32	81.25±50.42	476±118.79	20-644 (233.42±228.16)
Secondary macronutrients				
Ca (mg L^{-1})[a]	520	2165±1596.25	1500±431.34	210-4120 (1476.67±1088.44)
Ca efflux (g day^{-1} 0.1% $release^{-1}$)[b]	8.32	281.45±207.51	2100±603.87	8.32-2954 (970.9±1072.92)
Ca potency (mg L^{-1} 0.1% $release^{-1}$)[c]	83.2	1407±1037.56	4200±1207.74	83.2-5908 (2240±2022.63)
Mg (mg L^{-1})[a]	60	110±73.49	135±67.18	20-320 (107.92±63.83)
Mg efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.96	14.3±9.55	1890±94.05	0.96-322 (83.76±109.09)
Mg potency (mg L^{-1} 0.1% $release^{-1}$)[c]	9.6	71.5±47.77	378±188.09	9.6-644 (183.73±208.55)
Micronutrients				
Total Ash (%)[a]	6.42	16.4±2.45	14.7±1.48	6.42-19.4 (13.2±4.44)
Ash-efflux (g day^{-1} 0.1% $release^{-1}$)[b]	6420	32800±4898.98	73500±7424.62	6420-84000 (43200±29191.97)
Ash potency (mg L^{-1} 0.1% $release^{-1}$)[c]	10.27	106.6±15.92	411.6±41.58	10.27-470.4 (209.6±184.22)
Na (mg L^{-1})[a]	50	215±61.24	265±81.32	50-380 (194.58±107.4)
Na efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.8	27.95±7.96	371±113.34	0.8-532 (164.1±195.45)
Na potency (mg L^{-1} 0.1% $release^{-1}$)[c]	80	139.75±39.8	742±227.69	8-1064 (357.75±370.19)
Cu (mg L^{-1})[a]	0.34	2.45±1.84	4.59±2.11	0.2-7.57 (2.81±2.34)
Cu efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.005	0.32±0.24	6.43±2.95	0.01-10.6 (2.79±3.68)
Cu potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.05	1.59±1.19	12.85±5.9	0.05-21.2 (5.9±7.15)
Fe (mg L^{-1})[a]	5.4	-	-	-
Fe efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.09	-	-	-
Fe potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.9	-	-	-
Zn (mg L^{-1})[a]	2.72	29.3±22.29	48.6±22.63	2-80.6 (30.7±26.1)
Zn efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.04	3.81±2.9	68.04±31.68	0.04-112.84 (29.63±38.97)
Zn potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.44	19.05±14.49	136.08±63.36	0.44-225.68 (63.16±75.59)
Ni (mg L^{-1})[a]	0.06	0.06±0.01	2.77±1.79	0.03-5.29 (1.22±1.74)
Ni efflux (g day^{-1} 0.1% $release^{-1}$)[b]	0.001	0.001±0.001	2.5±0.65	0.001-7.41 (1.62±2.49)
Ni potency (mg L^{-1} 0.1% $release^{-1}$)[c]	0.01	0.001±0.001	5±0.65	0.01-14.81 (3.26±4.97)

[*] FROV samples were pooled and sent as one sample
[a] Observed concentration.
[b] Estimated at system scale (based on average daily sludge output).
[c] Estimated at solution scale (based on resultant concentration of 'wet sludge' per percent culture water *i.e.* release ratio (percentage) – sludge: RAS volume).
Grey highlighted cells: Indicate extreme variability (CV>1) in values; rendering them unfit for generalization and comparison.

compelled by the responsibility to dispose their increasingly problematic RAS wastes to avoid legal penalties by environment regulation agencies or simply to diversify their income. Very often they are faced by lack of quantified reports or clear-cut recommendations that advocates the suitability (or unsuitability) of RAS farm effluents in upgrading to aquaponics. In such lack of confidence, some RAS farm managers take a 'leap-of-faith' while some deter their decision to upgrade to aquaponics (Anon, 2017). The present study besides commenting on the nutrient outputs by RAS farms also commented on the set of operational parameters that significantly differ or does not differ among the RAS farms (see results, Table 1). The set of operational parameters that do significantly differ among the RAS farms are the ones most likely to contribute to the success (degree of success) of the upgraded aquaponics venture, if focused upon and calibrated properly. On the other hand, the set of operational parameters which does not generally differ (significantly) among the farms can be overlooked from further calibration. This is the first kind of study which generated such type of information that too from operational RAS farms which are not yet converted to aquaponics.

In RAS systems, minerals have different solubilization rates and do not accumulate equally, which influences their concentrations in the water (Goddek et al., 2015). This was also reflected in the asynchronies we observed for some nutrients between their input and output *(see results)*. It is a well accepted notion that characteristics of RAS effluents are highly erratic and complex in nature (Goddek et al., 2015; Rijn, 2013; Seawright et al., 1998). Knowledge gap exists on identifying nutrients in RAS effluents that significantly differ or does not differ with varying scale and culture intensity of the farms. The present study gave a firsthand look on those nutrients – classified as consistent or inconsistent (see results). Future research should focus on investigating consistencies in nutrient stoichiometry and mass balance equations of effluents with varying farm conditions.

4.2. Nutrient output and meeting plant requirements

Contradictory views exist on the suitability and safety of RAS effluents to sustain plant growth under aquaponics condition. In a recent review, Bittsanszky et al. (2016) presented the diplomatic side of nutrient sustainability issues for aquaponics. Although the nutrient concentrations in fish process water (RAS) are significantly lower for most nutrients compared to hydroponic systems, plants do thrive in such sub-standard hydroponic solutions (Bittsanszky et al., 2016). They further attributed it to recent developments in the field of plant nutrition. Recently, the nearly two-century-old "Liebig's law" (briefly, plant growth is controlled by the scarcest resource) has been superseded by complex algorithms that take interactions between the individual nutrients into account (Parent et al., 2013; Baxter, 2015). These methods do not allow a simple evaluation of the effects of changes in nutrient concentrations in a hydroponic or aquaponic system (Bittsanszky et al., 2016). Generally speaking - nitrogen, mainly nitrate, is the predominant macronutrient recycled from the RAS (Bittsanszky et al., 2016*); also supported by the present study. P and K are often scarce in RAS water and need to be supplemented (Bittsanszky et al., 2016; Monsees et al., 2017a, b); agreeing only with K in the present study as sludge had adequate P. Rakocy et al. (2006) opined otherwise – K, Ca, Mg are usually deficient to support plant growth; present observations contradict this view as sludge may completely mask deficiencies observed in RAS wastewater. Additional K, Ca and Mg supply can be improved by modifying the choice of pH buffers used (e.g. $Ca(OH)_2$, KOH, $CaMg(CO_3)_2$ used alternatively in combination) (Rakocy et al., 2006). Data from Bittsanszky et al. (2016) clearly show that most plant nutrients except Cu, S and Ca were at significantly lower concentrations in fish water; complying to our observations in water phase (wastewater). In terms of micronutrients - Fe, Mn, B, Mo do not accumulate significantly in RAS waters with respect to cumulative feed input (Rakocy et al., 2006); partly agreeing to our observations on B and Mo. Fe is the most commonly supplemented micronutrient supplementation in aquaponics (Rakocy et al., 2006); although we suspect Fe to be present in sufficiently high amount in sludge. Yavuzcan Yildiz et al. (2017) adds Cu and Zn to the aforementioned list of deficient micronutrients; not deficient as per our estimate if sludge taken into consideration. Promising studies have shown higher plant productivity in aquaponics comparable to hydroponics despite lower concentrations of macronutrients; attributed to 'plant beneficial micro-organisms' present in RAS effluents that can be taken up for future studies (Palm et al., 2018). Thus, a high level of

Table 5
Capacitogram of RAS farms in meeting some (prioritized) plant-essential nutrient thresholds for common aquaponics crops.

Crop*	N		P		K		Mg		Ca		S		Fe		Zn		B		Cu		Mo	
	WW	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*	*WW*	*SLG*
Cucumber	Adequate	Adequate	Deficient	Adequate	Deficient	Deficient	Deficient	Adequate	Deficient	Adequate	Deficient	Sufficient	Deficient	Sufficient	Adequate	Adequate	Partly sufficient	Not detected	Deficient	Adequate	Partly sufficient	Not detected
Chilli	Adequate	Adequate	Deficient	Adequate	Deficient	Deficient	Deficient	Adequate	Deficient	Adequate	Deficient	Sufficient	Deficient	Sufficient	Partly sufficient	Adequate	Deficient	Not detected	Deficient	Adequate	Deficient	Not detected
Lettuce & Herbs	Adequate	Adequate	Deficient	Adequate	Deficient	Partly sufficient	Deficient	Adequate	Deficient	Adequate	Deficient	Sufficient	Deficient	Sufficient	Adequate	Adequate	Partly sufficient	Not detected	Deficient	Adequate	Partly sufficient	Not detected
Tomato	Adequate	Adequate	Deficient	Adequate	Deficient	Partly sufficient	Deficient	Adequate	Deficient	Adequate	Deficient	Sufficient	Partly sufficient	Sufficient	Deficient	Adequate	Partly sufficient	Not detected	Deficient	Adequate	Partly sufficient	Not detected

*In reference to standard hydroponic nutrient solution concentrations (Resh 2012, Resh and Anguilla 2011). *Abbreviations used:* WW= Wastewater, SLG= Sludge

Legends

- **Adequate**= above recommended concentration
  (apply wastewater or sludge 'as-it-is')
- **Sufficient=** extrapolated based on observations (refer text)
  (apply sludge 'as-it-is')
- **Partly sufficient**= above Q3 of recommended concentration and below recommended concentration.
  (supplementary fertilizer to be used or increased water exchange/sludge release necessary)
- **Deficient**= below Q3 of recommended concentration
  (Complete fertilization necessary. Beyond capacity of water exchange or sludge release manipulation to reach desired concentration)
- **Not detected =** suspected absence.
  (Complete fertilization necessary. Beyond capacity of water exchange or sludge release manipulation to reach desired concentration)

disparity in information on nutrient status of RAS effluents to sustain plant growth is evident from these examples. The present study attempted to 'clear the air' regarding these discrepancies under practical conditions (commercial RAS farm effluents) and beyond experimental systems.

The present study strongly advocates re-use of sludge as-it-is or in mineralized form. Information on available sludge digestion technologies can be found in Goddek et al. (2015), Martins et al. (2010), Palm et al. (2018), Yavuzcan Yildiz et al. (2017). According to Lennard (2015), at least 80% by weight (and often more) of the nutrients required for optimal plant growth are derived from fish waste alone. We infer this is not possible without taking sludge into consideration. Rakocy et al. (2006) estimated that in closed RAS with water exchange as low as 2%, dissolved nutrients accumulate in concentrations like those in hydroponic nutrient solutions. Nevertheless, most nutrients can be recycled from the fish sludge, to sustain an aquaponics operation without significant external fertilizer input (Monsees et al., 2017a); strongly supported by our data. Brod et al. (2017) applied dried fish sludge from RAS on 'agricultural' land and achieved a relative agronomic efficiency compared with mineral fertilizer of 50–80%. A crucial item in aquaponic systems is pH stabilization. Maximum nutrient absorption by plants occurs in mildly acidic conditions (pH 5.5–6.5 units) while pH in RAS waters are purposively kept neutral to alkaline (7–8 units) (Yavuzcan Yildiz et al., 2017). Allowing sludge digesta or raw sludge itself may likely overcome the pH conflict by dampening the pH values of resultant solution to be more skewed towards plant requirements; sludge has acidic reaction (Rijn, 2013). On the other hand, re-using sludge or its digesta to mask nutrient deficiencies in RAS wastewaters may make the process water returning to fish culture units progressively turbid; undesirable for RAS especially biofilters (Junge et al., 2017; Badiola et al., 2012). If the situation demands, decoupling of fish rearing and plant culture unit is a safer option to manipulate acidic pH conditions for plants and clearer water for fish – to address welfare and aesthetic issues in culture systems (Monsees et al., 2017a, b, Yavuzcan Yildiz et al., 2017).

Addition of sodium bicarbonate ($NaHCO_3$) to aquaponic systems for pH control is not advised; high Na^+ in the presence of Cl is phytotoxic and retards uptake of other nutrients. Rakocy et al. (2006) recommended an upper ceiling of Na^+ concentration of $50\ mg\ L^{-1}$; which was clearly breached in our findings. Contradictions occur on this aspect as well. Reviewed in Resh (2016) - the use of saline water for hydroponic growing of crops have been investigated by several workers; possibilities exist within upper ceiling as high as $1180\ mg\ L^{-1}$ Na (molar mass basis from $3000\ mg\ L^{-1}$ NaCl) given a few 'simple' considerations (see, Resh, 2016). Our data indicates - Na concentration (in either wastewater or sludge) seldom crossed $350\ mg\ L^{-1}$ (i.e. around Q1 of the critical limit). In this light, *the prejudice of a 'definite' Na toxicity for plants should be re-visited, preferably less prioritized.* Nonetheless, with readily available RO (reverse osmosis) equipments and more complicated desalinization units these days, it is easy to remove the salts from RAS effluents (Goddek and Keesman 2018; Resh, 2016); not suggested as it may also reduce other nutrients (salts, e.g. S) in the solution. This situation can be easily avoided if the RAS farms use a combination of $Ca(OH)_2$, KOH and $CaMg(CO_3)_2$, discontinuing $NaHCO_3$ (Rakocy et al., 2006); strongly advised and backed by our data from farm A. Contrary to concerns raised from time to time regarding heavy metal accumulation and/or discharge by RAS farms (Cao et al., 2007; Martins et al., 2010), we found no such threats since the concentration of heavy metals in effluents were 'absolutely safe' (concentrations far below Q1 of pollution thresholds); also highlighted by Ebeling and Timmons (2012).

4.3. Modeling of operational influences on nutrient output

Limited information is available on modeling operational influences on nutrient output through RAS effluents. Based on our personal experience and literature search, this can be attributed to two reasons: (a) due to inherent complex nature of RAS systems itself (Monsees et al., 2017a, b), and, (b) most of the modeling attempts going un-reported due to non-realization of 'convincing' models. Limited modeling efforts, till now, have mostly concentrated on optimizing 'fish feed input (fish culture volume): plant culture area ratio' (*reviewed in*, Buzby and Lin, 2014) and recently on 'desalinization needs of aquaponics' (Goddek and Keesman 2018). Some thumb-rule models have also been listed in Ebeling and Timmons (2012) that are instrumental in planning RAS systems for emerging entrepreneurs. Interestingly, most of well-understood operational influences in RAS having implications on nutrient outputs (e.g. Ebeling and Timmons, 2012; Martins et al., 2010; Rakocy et al., 2006; Rijn, 2013) failed to make direct 'statistical appearances' as predictors in our modeling attempt. Apart from six predictors identified in the present study (viz. wastewater volume, sludge: RAS-volume ratio, feeding rate, feed micronutrient loading, FCR and stocking biomass) most of the 'well-known' operational influences failed to show any significant predictable power in deciding nutrient throughput from RAS systems. Moreover, not all the nutrients can be directly predicted or have clear cut dependencies between wastewater and sludge concentrations. Concentrations of some nutrients increase with increasing farm size and culture intensity, while in others no such tendency is apparent (see results). The limitations of our modeling approach have been clarified above. Despite that - *calibration of nutrient output from operational RAS farms may be primarily focused around the abovementioned (six) predictors.* By 'calibration' - we suggest adjusting these predictors *aka* six identified operational parameters for optimizing overall nutrient throughput from RAS farms; not merely viewing them as nutrient-specific calibration (as the models appear). The present modeling attempt generated some baseline information, with intentions to draw-in contemplations from the global community on whether and how the predictors of nutrition output can be further precised. Nonetheless, some degree of predictability exists in RAS nutrient throughputs using limited but few available means.

5. Conclusion

Contradictory views exist on the suitability and safety of RAS effluents to sustain plant growth under aquaponics condition. The present study attempted to 'clear the air' regarding these discrepancies under practical conditions (commercial RAS farm effluents) and beyond experimental systems. Diplomatic advisories and lack of clear-cut scientific conclusion tend to retard adoption of any emerging technology. The purpose of the present study was concluded by generating applied information that can aid in future conversions, rather 'upgrades', of operational RAS farms to semi-commercial Aquaponic ventures. We emphasize - *despite inherent complexity of RAS effluents,* the *conversion of RAS farms to semi-commercial aquaponics should not be deterred by nutrient insufficiency or nutrient safety arguments.* Incentivizing RAS farm wastes (nutrients) through semi-commercial aquaponics should be encouraged - sufficient and safe nutrients are available.

Declaration

The study was a part of a national project of the Czech Republic. All necessary data have been provided in the manuscript and through supplementary files.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.05.130.

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CHAPTER 3

AQUACULTURE-ENVIRONMENT INTERACTIONS: NUTRITION FOR FISH OR NUTRIENTS FROM FISH

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Feed-based common carp farming and eutrophication: is there a reason for concern?

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Abstract

Metadata from 70 research articles on *Cyprinus carpio* digestibility published between 1973 and 2017, covering 71 feed ingredients, were analysed. Interquartile range (IR) of nitrogen (N) and phosphorus (P) content in feedstuffs was 5–8% and 0.7–1.2% of dry matter, respectively, with digestible N:P 7.2:1–44.1:1. IR of N digestibility (79–99%) was high, whereas IR of P digestibility (27–47%) was rather poor. Dietary energy digestibility (gross energy and non-protein energy) was >76%. Higher P in feedstuffs caused significant negative interferences for N digestibility. IR of nutrient content in carp faeces was estimated at 0.5–1.7% N and 0.4–0.9% P. Considering the metabolic losses, the carp excreta have an 'eutrophic' N:P ratio (2.1:1–5.8:1). Eutrophication potential from feeding seems linked to P digestibility followed by bad protein profile of diets. While brewery wastes, microbial protein and natural prey offer high P digestibility (75–90%), large knowledge gaps still exist in P digestibility of various ingredients. Thermal processing does not always improve P digestibility; acidic pre-incubation with phytases (optimum: 1500–2000 IU kg^{-1} feed) is worth exploring. Under semi-intensive system, digestible 'supplementary' nutrients (N: 3.3–4.9%, P: 0.2–0.5%; even lower) can support at least 0.6–1.2 thermal growth coefficient (reasonable growth) and be ecologically relevant. We further considered validity of data within experimental conditions; effects on N/P utilization; non-faecal losses (IRs 17–59% of N intake; 9–18% of P intake); and controversies over eutrophication. Recent eutrophication of carp fishponds might have been rather 'management-driven' than carp's biological limitations. Ameliorative measures are outlined.

Key words: common carp nutrition, digestibility data modelling, ecological trade-offs, global metadata analyses, nitrogen phosphorus footprints, responsible carp farming.

Introduction

Cyprinids contribute about 38% of all aquaculture (by weight) and also very much as an edible protein source coming from aquaculture. Carps, feeding lower on the food chain, use a relatively large amount of land per unit of protein produced (Waite *et al.* 2014). The common carp (*Cyprinus carpio*) is the oldest domesticated aquaculture species in the world and the most popular representative of cyprinids in aquaculture (Balon 1995, 2009). It contributed around 4.67 million tons (Mt) on a global scale during 2015–2016, roughly accounting for 7.4% of the total global inland fisheries production. In Europe, common carp contributed 1.8% (0.17 Mt) of the total inland fisheries production (9.42 Mt) during 2015–2016 (FAO FishStat 2017). It is a major farmed species in European freshwater aquaculture with production localized in central and eastern European countries. The Russian Federation (0.06 Mt) followed by Poland (0.02 Mt), Czech Republic (0.02 Mt), Hungary (0.01 Mt) and Ukraine (0.01 Mt) represents about 70% of carp production in Europe during 2016 (FAO FishStat 2017). In fact, the land-locked central European countries rely heavily on common carp aquaculture in fishponds. For example, in Czech Republic with 41 080 ha of fishponds (70% of which has 0.5–3 ha area), common carp has consistently comprised >85% of total aquaculture production (CZ-Ryby 2019). Average productivity of carp culture systems in central European countries ranges between 0.3 and 1 ton ha^{-1} (Sterniša *et al.* 2017). The European common carp production, in terms of volume, reached its peak (0.18 Mt) during 2009–2010 and has been declining since. In terms of value, the decline was realized late – peaked during 2011–2012 (0.45 million USD) and declining afterwards (0.38 million USD in 2016) (FAO FishStat 2017). This declining popularity of common carp aquaculture may be attributed to several factors: (i) diversification of alternative aquaculture species (FAO FishStat 2017), (ii) decreasing popularity of common carp among farmers and consumers (Hlaváč *et al.* 2015, 2016a), (iii) recent eutrophication concerns associated with carp farming (Pechar 2000; Weber & Brown 2009; Rahman 2015) and (iv) poor P digestibility and retention by carps (Nose & Arai 1976; Ogino *et al.* 1979; Watanabe *et al.* 1999; Ellestad *et al.* 2002). Like other aquaculture practices worldwide, the common carp aquaculture has pronouncedly intensified over the years. This has led to an increase in both stocking density and provision of supplementary feeding to enhance the yield (Potužák *et al.* 2007; Hlaváč *et al.* 2014). The perception of eutrophication risk resulting from such intensification has been perceived as a threat to aquatic ecosystem sustainability (Pechar 2000). This has led to a situation where the desire to increase carp culture productivity is often offset by the rising concerns of eutrophication of fishponds (see Hlaváč *et al.* 2016b). The freshwater eutrophication potential driven by aquaculture is presently estimated at 0.38 Mt P equivalent and forecasted to increase to 0.88 Mt P eq. by the year 2050, despite optimizing aquaculture management (Mungkung *et al.* 2014; Waite *et al.* 2014).

Carp farming is often criticized as an anthropogenic driver of eutrophication of inland freshwater bodies (Prikryl 1983; Pechar 2000; Potužák *et al.* 2007; Petrovici *et al.* 2010). Over the past three decades, common carp farming in Europe and elsewhere has undergone intensification (Pechar 2000; Potužák *et al.* 2007). Manuring and supplementary feeding have been the basis for improving natural productivity and production from semi-intensive carp fishponds (Kaushik 1995; Tacon 1996; Pechar 2000; Potužák *et al.* 2007). For example, in the Czech Republic, current legislation (Act No. 254/2001 Coll. – 'the Water Act') recognizes manuring in fishponds as an environmental threat and recommends avoiding it (Hlaváč *et al.* 2016b). Under these circumstances, supplementary feeding (also controlled by water authorities) is deemed as the only available tool for intensifying fish production (Hlaváč *et al.* 2016b). Other legislative regulations include (e.g. in Poland, Mazurkiewicz 2009) – limit imposed on the scale of carp production (<1.5 tonne ha^{-1}), nitrogen (N) and phosphorus (P) ceilings in post-production waters. Feed and feeding practices elicit major environmental impacts of aquaculture through waste discharged into the surrounding environment (Kaushik 1995; Searchinger *et al.* 2014; Waite *et al.* 2014). Globally, carp aquaculture was estimated to consume about 13.5 Mt of aquafeeds, that is 27% of the global aquafeed produced during 2015 (Tacon & Metian 2015). Extrapolating this figure with production data (FAO FishStat 2017), we estimated common carp alone consumed ~37.5% (~5.1 Mt) aquafeeds destined for carp aquaculture globally during 2015, among which ~0.2 Mt aquafeed used in Europe. The Czech Republic, which moved from compound feed to cereal grain-based supplementary feeding, used ~0.04–0.05 Mt cereals in fishponds to support its carp production (relative feeding coefficient considering contribution from natural food: ~2–2.5 kg cereals kg^{-1} total fish yield) (J. Mraz – unpublished results).

Over the years, feed-based aquaculture industry has reduced the share of fishmeal in fish feed. Compared to the scenario of 1990–2000s, the share of fish meal in carp feeds has come down (global average: 1–2%) in recent years (Searchinger *et al.* 2014; Waite *et al.* 2014; Tacon & Metian 2015). This has led to intensive research and use of alternative plant protein sources. Plant origin ingredients can contain several anti-nutritional factors such as anti-tryptic factors or phytate-bound phosphorus (P) affecting digestibility of N and P (Francis *et al.* 2001). The tendency of farmers to overuse feeds further aggravates N and P loading issues. There has been some debate between environmentalists and carp farmers concerning eutrophication of water bodies (Kestemont 1995; Knösche *et al.* 2000; Pechar 2000; Potužák *et al.* 2007, 2016; Hlaváč *et al.* 2014, 2016b). Recent commentary by Duras and Potužák (2016) expressing strong dissent on 'farmer lobbied' amendment (Act No. 275/2013 Coll. 39(12)) of a formerly 'environmentally strict' water act (Act No. 254/2001 Coll.) regulating supplementary feeding in Czech fishponds is a real example. Primary concern is how efficiently the carps are utilizing dietary N and P to retain (assimilate) or load nutrients from/into the aquatic environment (Hlaváč *et al.* 2014). On a related debate in Germany and Hungary, the federal state decided to substantiate predominantly experience-based arguments of carp culture supported with quantified data (Knösche *et al.* 2000). Prologue on a similar situation in Japan (Watanabe *et al.* 1999) and Korea (Kim *et al.* 1995a,b) can be referred to as well. More such cases might exist – unreported, clandestine or neglected. Here, we imply the nutrients excreted through fish faeces, gills and urine that end up in the aquatic environment, sometimes causing nutrient enrichment. The increasing nutrients coupled with the impact of climate change are blamed to push the 'closed' fishponds towards eutrophication (Pechar

2000; Potužák *et al.* 2007). In comparison with the global warming and associated concerns of algal bloom, the anthropogenic drivers of eutrophication are easier to control. The present European scenario of aquaculture development focuses more on 'ecological responsibility' rather than simply enhancing production. Upper permissible limits of PO_4 (0.4 mg L^{-1}) and NH_4 (1 mg L^{-1}) have been imposed for carp culture waters (EU Directive 2006/44/E Article 3 & 5, Annex I). Greater retention of dietary N and P by farmed fish is the key to balance aquaculture and environmental sustainability goals.

Being the oldest domesticated aquaculture species, research on common carp nutrition and feed utilization has been subject to many analyses. Studies have focused on different aspects of the common carp nutrition, spanning over the last six decades (1960–2018). Focused reviews on common carp (or carp *per se*) by Kaushik (1995), Takeuchi *et al.* (2002), Weber and Brown (2009), Hua and Bureau (2010) and Hlaváč *et al.* (2014) are particularly informative on aspects like nutrient utilization, optimum nutrition, effects on aquatic ecosystem, phosphorus digestibility and effects of supplementary feeding in carp fishponds, respectively. Unfortunately, a systematic analysis of literature data accrued over the years on nutrient input, availability and utilization in one place is lacking, especially from the perspectives of dietary N and P footprints and faecal eutrophication potential in common carp farming. Such a meta-analysis of data will be of use to feed formulators, farmers, nutrition researchers and policymakers in devising 'responsible and optimal' feeding of common carp or any species *per se*. In this instance, our goal is to compile and provide objective data on potential environmental impacts of carp farming as affected by different factors involving husbandry and feeding. Our aim is to undertake an updated comprehensive analysis addressing nutrition, aquaculture and environmental issues. The objectives of this metadata analyses were to (i) better understand the pre-conditions associated with any nutrient utilization data; (ii) be informed on the range of artificial feedstuffs considered in different diets; (iii) quantify the ranges of digestible (faecal) N and P losses under different feedstuffs; (iv) review metabolizable (non-faecal) N and P losses by common carp; (v) review and understand various potential interferences on nutrient utilization; (vi) review and model different dietary N and P levels with growth to find out optima and any environmentally responsible levels relevant for semi-intensive farming; (vii) assess potential of carp-excretion driven eutrophication and present studies corroborating or contradicting it; and (viii) highlight solutions that may be of importance to achieve cleaner production. All these objectives are sequentially addressed and presented in the review under appropriate headings.

Materials and methods

System of review

The collection and compilation of available published data were performed using Web of Science, Scopus, ScienceDirect and Google Scholar online databases. Keywords such as '*Cyprinus carpio*' and/or 'common carp' and 'digestibility' and/or 'nutrition' and/or 'feed digestibility' and/or 'ingredient digestibility' or 'common carp digestibility studies' or 'common carp growth trials' were used to get matches. Only peer-reviewed and published articles in English language or with an English abstract were retrieved – the 'primary' articles. Out of the 58 primary articles that fulfilled our search criteria, further papers (n = 12) were obtained by mining the relevant cross-references contained therein (Table S1).

Assumptions and interpretations

By dietary N and P utilization capacity, we imply digestibility data (apparent digestibility coefficients, ADCs) from trials on digestibility undertaken only with common carp. The term 'ingredients' or 'feed ingredients' or 'feedstuff' has been used inter-changeably. Nitrogen digestibility values in percentage correspond to protein digestibility. 'Common carp' and 'carp' were also used in the same sense. Those ingredients, whose digestibility studies have been encountered only once–twice or never encountered in our literature survey, have been flagged as 'poorly studied' or 'lacking data', respectively. By 'ingredient/category dominated diets', inclusion levels of >30% by weight of particular ingredient/category feedstuff (with exception for amino acid and P supplements) were considered.

Metadata analyses

Data (n = 220) from 70 'carp digestibility' research articles published between 1973 and 2017, spanning over 24 ingredient categories and covering 71 feedstuffs (or ingredients), were analysed. Protein content was converted to N-equivalent using standard conversion factor 0.16 (AOAC 2000); Jones factor or default factor (5.6) by Mariotti *et al.* (2008) was not used. Although re-calculation of N content with the latter factor(s) can be made in future, it will not affect the results surrounding digestibility data (expressed in percentage). For understanding the trends of digestibility experiments with common carp, a total of 58 research articles excluding some cross-references (n = 12) were screened (Table S1). All data are presented on dry matter (DM) basis.

Digestibility data were statistically tested with multiple linear regression under ANCOVA framework. Feedstuff category, ingredient, N content and P content were deemed as independent variables; N or P digestibility was the response variable. Analyses were performed in R (R Development Core Team 2015). Interquartile range (IR) was calculated using 'summary' function (25th or 1st quartile to 75th or 3rd quartile), and all ranges were expressed as IR. Graphical modelling (jitter boxplot, LOESS plot) was performed using 'ggplot2' package in R (Wickham 2016). To estimate IR of N or P content in carp faeces, percentage not digested by carp (100−IR of ADCs) was multiplied with IR of nutrient content. The N:P ratio in feedstuff and faeces was calculated (N divided by P) from their respective nutrient content IR.

Metadata (n = 170 for N, 113 for P) were also collected from some growth trials on common carp, testing multiple levels of dietary nutrient and/or feedstuff inclusion. Data on initial body weight (W_i in g), final weight (W_t, g), water temperature (T, °C), period of rearing (Δt, days) and dietary N or P levels (%) were compiled. Thermal growth coefficient (TGC) was calculated following the formula: $TGC = [(W_t^{1/3} - W_i^{1/3})/(T * \Delta t)] * 1000$ (Iwama & Tautz 1981; Cho 1992). Calculated TGC was inclusive of the size range (0.1–1650.7 g), dietary N (0.04–54.5%) and P (0.06–2.34%). Upper semi-interquartile range (upper semi-IR) of TGC, that is, median to 3rd quartile was identified. Jittered bubble plots (over-layered with multiple LOESS curves) were constructed feedstuff-wise using 'ggplot2' package (Cleveland *et al.* 1992; Wickham 2016). A generalized additive model (GAM), each for N and P, was simulated with TGC as response variable and dietary N or P levels as predictor variable (Hastie & Tibshirani 1986). We ascertained 'reasonable growth and ecologically relevant' dietary N or P levels by identifying cross-sections of (i) emergence of GAM function into upper semi-IR of TGC, (ii) exit of lower prediction belt (95% confidence interval) of GAM function from upper semi-IR of TGC and (iii) converted to digestible values by multiplying with IR of N or P digestibility. All analyses were done in R (R Development Core Team 2015).

Results and discussions

Trends in digestibility, nutrient utilization experiments with common carp

The experimental conditions for the studies undertaken so far on digestibility are summarized to serve as supplementary information along with data on digestibility coefficients. On the one hand, this information may be used for presenting data on digestibility in carp. On the other hand, the purpose is to enable future researchers to spot the limitations of experiments conducted so far and provide additional information. A detailed account can be found in the Appendix S1. In terms of the most common experimental conditions (>60% of surveyed literature), the information is listed in Table 1. The quantified digestibility metadata (presented in the subsequent sections) may be deemed valid within these ranges of experimental conditions. Based on these observations, it is felt that despite quite well laid out digestibility research with common carp, some areas are quite less explored. The recommendations are listed in Table 1. Filling up these gaps from N and P perspectives may yield better understanding of carp's ability to digest dietary N and P.

Table 1 Most common experimental conditions of digestibility/nutrient utilization trials with common carp

Parameter	Conditions
Strain	Scally carp, mirror carp
Trial type	Digestibility trials, growth trials
Duration (digestibility trials)	17–42 days
Experimental set-ups	Indoors (recirculatory aquaculture systems, tanks/aquaria)
Water exchange rate	0.2–0.8 L min^{-1}
Stocking density	<10 kg m^{-3}
Carps weighing	3–120 g
Water temperature	22–27°C
Dissolved oxygen	5–7 mg L^{-1}
pH	6.8–8 units
Ammonium and nitrite	<1 mg L^{-1}
Photoperiod	Natural to 15 h light: 9 h dark
Feed ration	Up to satiation, 1.4–2.5% of body wt. day^{-1}
Feeding frequency	manually 2–8 times day^{-1}, multiple splits within 1.5–6 h
Feeding time	Daytime
Acclimatization time with experimental feed	2–7 days before initiating faeces collection
Faeces collection period	Continued for 9–14 days
Faeces collection strategy	Passive collection (from sedimentation columns or by siphoning)
Dietary marker	Cr_2O_3 (0.5–1% DM basis)

Recommendations

(1) Digestibility assessment (referred as 'trials' below) and growth trials should be combined.
(2) More trials around lower (~15°C) and upper (~28–30°C) critical temperatures of feeding.
(3) Trials with respect to dissolved oxygen tensions.
(4) Effect of mildly acidic pH conditions on digestibility.
(5) Experiment with carps > 200 g size or mixed assortment of sizes.
(6) Trials with multiple markers in diet, in addition to conventional Cr_2O_3, to check susceptibility of ADC values due to marker-specific leaching.
(7) Comparative passive faeces collection with long- vs. short-duration faeces residence in water – to reflect fitness of previous estimates.

Ingredients tested and/or used in practical carp diets

A total of 71 feed ingredients have been used in feed formulation for common carp (Table S1). Cereals as whole or by-products thereof have been used most frequently (frequency, $f = 65$) in compound feed formulation followed by fish derivatives ($f = 54$), oilseeds as whole or meals thereof ($f = 39$), simple or complex carbohydrates ($f = 36$) and vegetable oils ($f = 28$). The prevalence of ingredients from different groups studied with carp is depicted in Figure S1.

Digestibility studies have been concentrated on some classes of feedstuff, for example oilseeds (number of digestibility studies, $n = 30$), cereals ($n = 21$), fish derivatives ($n = 17$), terrestrial animal protein ($n = 8$), starch ($n = 5$) and inorganic phosphorus supplements ($n = 5$). In contrast, live feed (zooplankton), vegetable oils, land animal fat, legumes-pulses, brewery wastes and microbial protein sources (yeast) have been subject to limited number of studies (Table S1, Fig. 1). The ADCs of macronutrients are available in good numbers for fish meal and its derivatives (number of digestibility data, $n = 12$) followed by soya bean meals and derivatives ($n = 11$), and corn and wheat ($n = 8$ each; Fig. 2). Digestibility of P from feedstuffs has been studied only to a limited extent in common carp. Further trends can be found in Appendix S1.

Dietary N and P utilization capacity of common carp

Overall

Digestibility data across 15 feedstuff categories comprising 55 ingredients were compiled for metadata analyses. The interquartile range (IR) of N content was 5–8% (Fig. S2). The IR of P content was 0.68–1.17% (Fig. S3). Carps seem to have apparent digestibility of dietary N in the IR of 79.1–99.2%. In terms of apparent digestibility of dietary P, the IR is 26.7–47.1%. This implies carp's poor capacity to utilize dietary P from feedstuffs. Breaking down the P digestibility across various fractions of P in feedstuffs (carp specific model; Hua & Bureau 2010), we have bone P ADC 0 ± 1%; phytate P ADC 0 ± 1% (also proved by Ellestad *et al.* 2002); organic-P ADC 72 ± 7%; inorganic monobasic P ADC 86 ± 3%; and inorganic dibasic P ADC 30 ± 6%. Average ADC (37.6%) of all these 'P fractions' from Hua and Bureau (2010) matches with the median (37.4%) of our estimated P digestibility IR. This also hints reliability of our present estimates despite using different set of research articles for metadata compilation. The IR of digestible N and P content in feedstuff was 3.96–7.94% and 0.18–0.55%, respectively. The IR of N and P content in carp faeces was estimated at 0.47–1.68% N and 0.36–0.86% P, with a N:P ratio of 0.55:1–4.67:1 (further discussed below). The information on P digestibility, however, is limited in comparison with that on N digestibility. There is indeed a large knowledge gap about the ADC of P for various ingredient/diet categories, either limited (1–2 attempts) or complete lack of data. Substantial information on P utilization from cereals and oilseeds is still needed to be generated. Distribution of carp N and P digestibility across various feedstuff categories and category dominated diets is depicted in Figures 3 and 4.

Animal proteins (terrestrial) in carp diets appear to have highly variable and comparatively poor N digestibility than the other feedstuff categories and might be due to cheap and poor quality of ingredient(s) used. In terms of P digestibility, brewery wastes, microbial protein (Brewer's yeast) and purified proteins are comparatively better than the others. Fish derivatives (fish meal) were the largest contributor of dietary P in carp diets, being richest in P content (Fig. S3). Inclusion range (15–50%) of various ingredient categories is depicted in Figure S4.

Statistical patterns

Among four factors (feedstuff category, ingredient, N content and P content) considered, only 'ingredient' itself (F-value 2.88, $P < 0.05$) and 'P content' of feedstuff (F-value 8.16, $P < 0.05$) impart significant variability on carp's ability to utilize dietary N. Increasing P content has statistically significant negative impact on N digestibility (t-value −2.86, $P < 0.05$). Dietary/ingredient N content (ING.TN) showed no significant influence ($P > 0.05$) on N digestibility (ADI.CP), being stable across the range (Fig. 5). About P digestibility, only 'feedstuff category' (F-value 5.64, $P < 0.01$) imparts significant variability. However, P digestibility is not significantly dependent ($P > 0.05$) on N

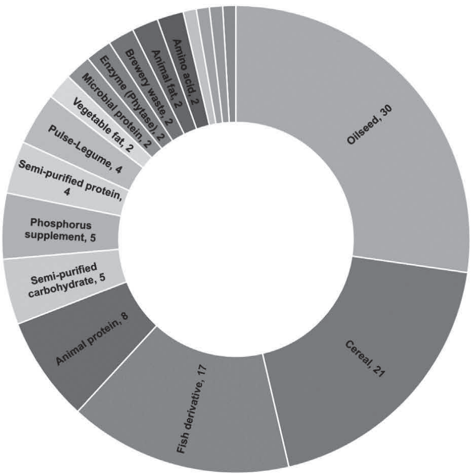

Figure 1 Research focus on digestibility estimation for different feedstuff groups during 1960–2018 (*numbers indicate count of studies*). Blank spaces indicate miscellaneous categories of feedstuffs. Fish derivatives classified separately from animal proteins (terrestrial) and animal fat (terrestrial).

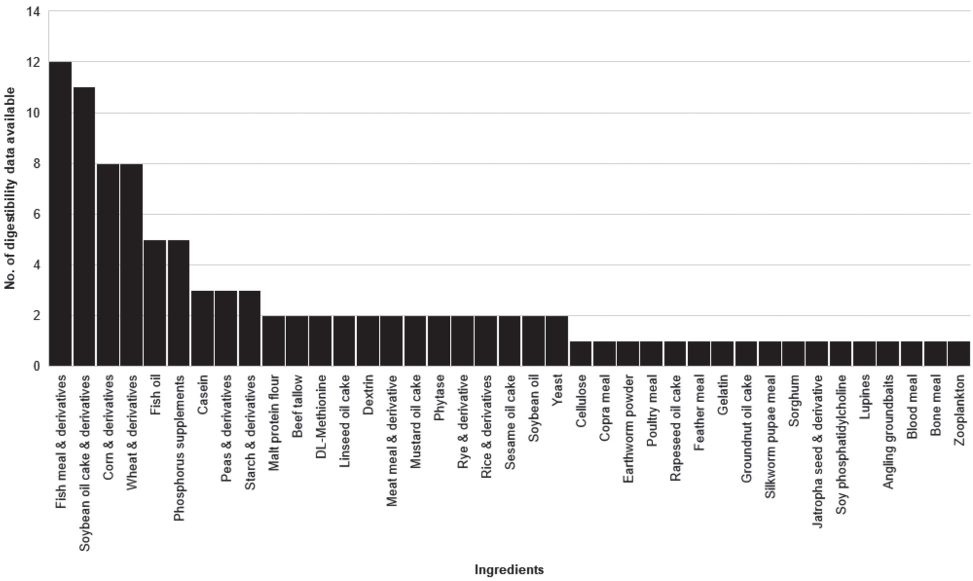

Figure 2 Frequency distribution of digestibility studies performed on common carp with different feed ingredients (1960–2018, references provided in Table S1). Ingredients with digestibility data less than 3 may be taken up for future investigations.

or P content of ingredients/diet. The dependency of P digestibility (ADI.TP) on P content of feedstuff (ING.TN) shows no definite pattern (Fig. 6).

Conventional feedstuffs

Cereals, oilseeds, fish derivative and animal (terrestrial) proteins were grouped as conventional feedstuffs in

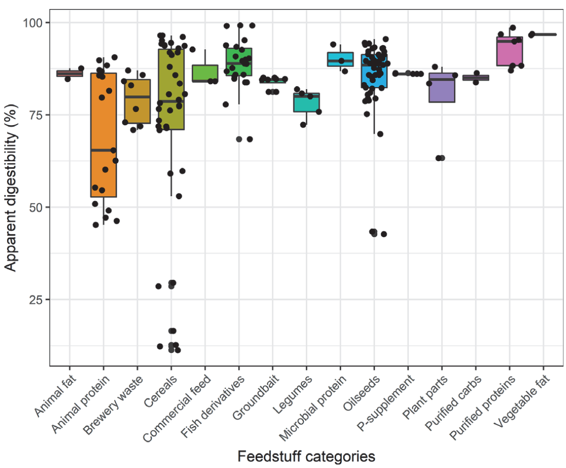

Figure 3 Data on Apparent digestibility of nitrogen from different feedstuff categories in common carp. Jitter boxplots – black dots are observed data points. Narrow boxes or heavy dashes are indicative of limited data availability.

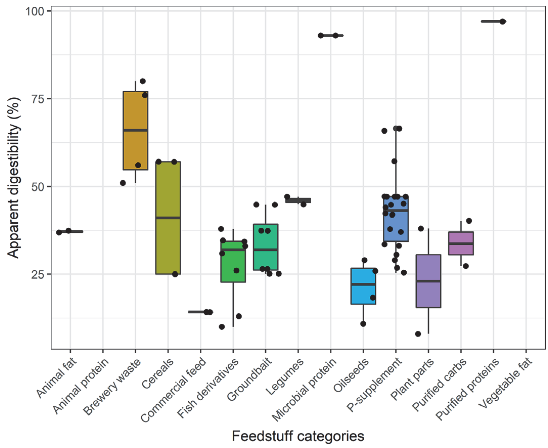

Figure 4 Data on phosphorus availability from different feedstuff categories in common carp. Jitter boxplots – black dots are observed data points. Narrow boxes or heavy dashes are indicative of limited data availability. Blank spaces indicate knowledge gap (e.g. animal protein, vegetable fat).

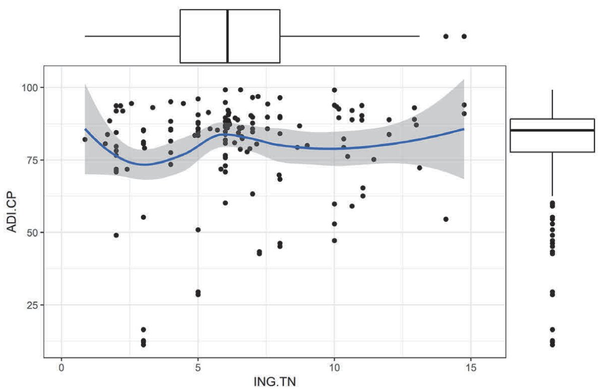

Figure 5 Relation between total N content of feedstuff (ING.TN, %) and apparent digestibility coefficient (ADC, in %) of nitrogen in common carp. LOESS smoothed regression with 95% CI band. No pattern observed.

practical carp diets. The IR of their N and P content and digestibility are summarized in Table 2. Ingredient-wise distribution of N and P digestibility can be found in supporting information figures (cereals: Figs S5,S6; oilseeds: Figs S7,S8; fish derivatives Figs S9,S10; terrestrial animal proteins Fig. S11). Too few data points or blank spaces in figures indicate 'less studied' or 'lack of data', respectively. Among cereals, rice bran and wheat germ meal offer comparatively inferior N digestibility than other cereals or their variants. Unfortunately, P digestibility data of only rice bran and wheat germ meal were encountered, wheat germ meal having a relatively high value for the ADC of P. Among oilseeds, there is a consistency in N digestibility across the oilseeds and their variants. Again, data on P digestibility data were available only for soya bean meal and soy protein concentrate. More data on P digestibility of commonly used cereals, oilseeds and their variants need to be generated. Soy protein concentrate seems to be the best choice among oilseed by-products, having comparatively good N and P digestibility. In case of fish derivatives, P digestibility is highly variable compared to N digestibility (consistent). Among animal proteins (terrestrial), blood

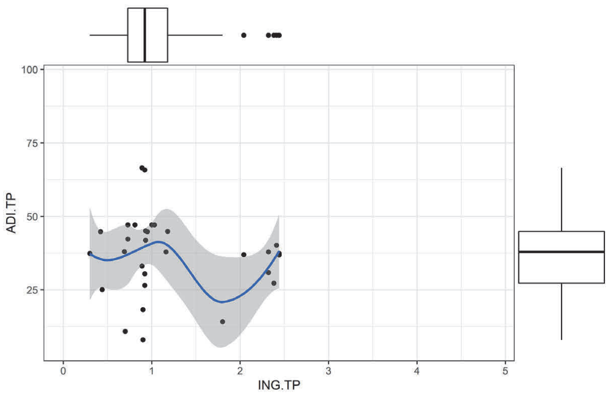

Figure 6 Relation between total P content of feedstuff (ING.TP, %) and apparent digestibility coefficient (ADC, in %) of phosphorus in common carp. LOESS smoothed regression with 95% CI band. No pattern observed. Lack of data support between 1 and 2% ING.TP.

meal, meat and bone meal, poultry meal and silkworm pupae have poor N digestibility. There is also a clear lack of data on P digestibility from animal protein sources.

Inorganic P supplements

P supplement used in practical carp diets included monocalcium phosphate (with guaranteed P content of approx. 22%), sodium dihydrogen orthophosphate and phytase enzyme. Phytase, which was purposively grouped under this category for easy interpretation, helps in digestion of phytic P that is unavailable to fish, not an inorganic P source *per se*. Our metadata revealed an inclusion range of phytase between 750 and 2250 IU phytase activity kg^{-1} feed. With the inclusion of P supplements in carp diets, IR of P digestibility stayed between 34.38% and 47.1% (Fig. 7). Contrary to the claim (Yang *et al.* 2005), monocalcium phosphate (MCP) inclusion did not exhibit greatly improved P digestibility; phytase performed comparatively better. P digestibility in phytase-supplemented diets was comparatively better (>45%) than MCP. However, it is not impressive. Sardar *et al.* (2007) found no significant difference in P digestibility of diets (0.2–0.3% phytate P) with

Table 2 Nitrogen and phosphorus content and apparent digestibility (interquartile ranges) of conventional feedstuffs in practical common carp diets

Category name	Content		Apparent digestibility	
	N (%)	P (%)	N (%)	P (%)
Cereals	2.08–5	0.26–0.69	70.9–93	25–57
Oilseeds	6.1–7.55	0.76–1.47	82.4–91.3	16.4–26.7
Fish derivatives	6.56–11	0.9–2.35	85.6–93	22.8–34.4
Animal proteins (terrestrial)	5–11.04	0.34–0.35	52.8–86.2	N.A.

microbial phytase inclusion (500 FTU kg^{-1} diet; P ADC 35.8–45.9%) or without it (P ADC 23.9–43.1%). This might be because phytases in general perform better in acidic pH environments with optimum pH between 3 and 6 units (Dersjant-Li *et al.* 2015). Gut pH of 'agastric' common carp has been reported above this range; minimum pH is ~6 units (Schaefer *et al.* 1995; Solovyev & Izvekova 2016). Therefore, pre-incubation of feedstuffs with phytases in acidic medium 'before' diet formation might improve P utilization by carps (Schaefer *et al.* 1995). Our metadata also revealed some optima of phytase activity in carp diets that warrants best dietary P utilization, that is 1500–2000 IU kg^{-1} feed (Fig. 8).

Other protein sources tested

Legumes (peas, lupines), brewery wastes (malt protein flour, corn DDGS), microbial protein (Brewer's yeast, petroleum yeast) and corn gluten meal have also been tested as feed ingredients in practical carp diets. The IR of digestibility values of these ingredients/alternative ingredients dominated diets varied between 73.7% and 85.38% for N and 47.1% and 80% for P (Figs 9, 10). We should recognize, however, that P digestibility of many of these ingredients is still missing for carps. From the limited available data, we could infer brewery wastes (malt protein flour, corn DDGS) and microbial proteins (yeast) are environmentally responsible choices for future carp diets. Corn gluten meal appears to be poorly digested by carps in terms of N (Heinitz *et al.* 2016) and P (diet PHYT4, Nwanna & Schwarz 2007).

Angling ground baits

Angling ground baits applied in natural water bodies during carp fishing include boilies (boiled paste of

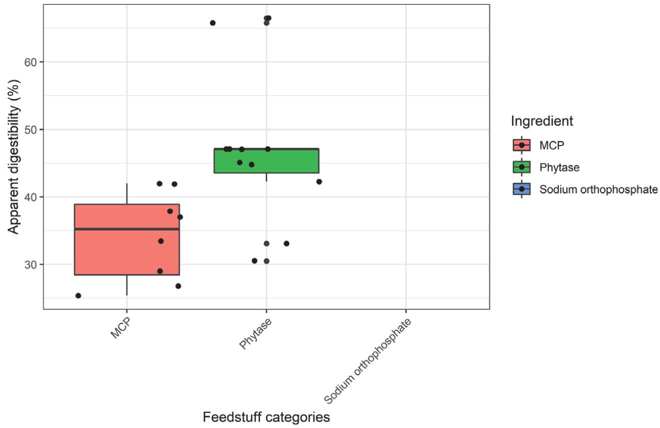

Figure 7 Effect of different phosphorus supplements on P digestibility of common carp diets. Jitter boxplots – black dots are observed data points. No available data on sodium orthophosphate supplementation.

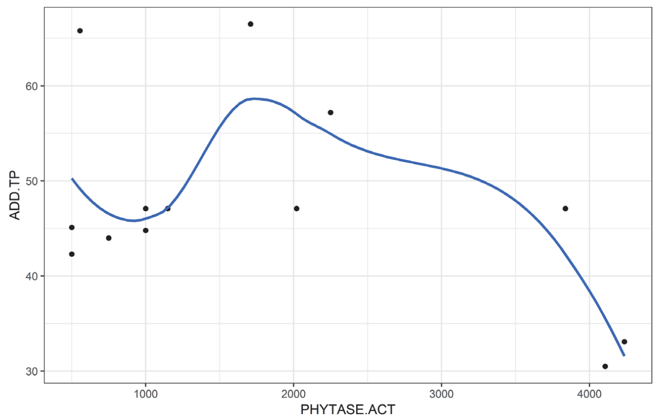

Figure 8 Inclusion level of phytase enzyme (PHYTASE.ACT in IU kg^{-1} feed) with respect to apparent diet digestibility of total phosphorus (ADD.TP %). Here, total P is inclusive of phytate P. Identified optima from LOESS smoothing: 1500–2000 IU phytase activity kg^{-1} feed.

ingredients), particles and feed mashes. The IR of N and P digestibility of angling ground baits was estimated to be 84.1–84.9% and 25.1–37.4%, respectively.

Lipid and carbohydrate digestibility

We also looked at the digestibility of lipid and carbohydrates by common carp that are in interest to the supply of non-protein energy and improved N utilization. The IR of content (lipid: 3.7–11.8%, carbohydrate: 20.95–58.45%) and digestibility (lipid: 80.31–92.9%, carbohydrate: 52.2–88.8%) of non-protein energy sources in feedstuffs is presented in Table 3, both overall and category-wise. These data are also graphically presented in Figures S12 and S13. Our data show a high variability in the digestibility of carbohydrate by carp. Lipids seem highly digestible by carps with less variability. We also estimated dietary energy digestibility by common carp: >77.2% (gross energy) and >76.3% (non-protein energy) (Table 3). Our estimate

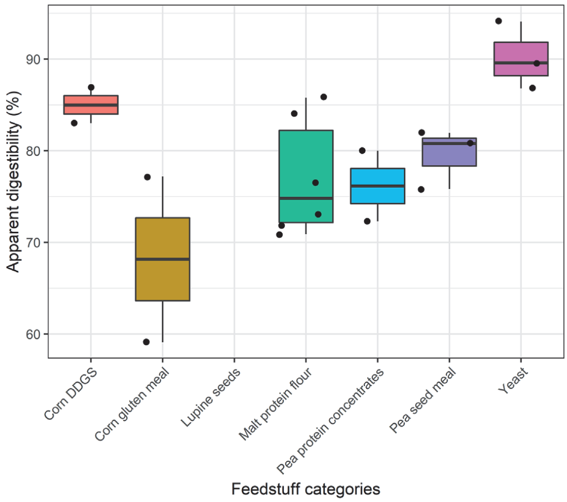

Figure 9 Data on apparent digestibility of nitrogen from some alternative feedstuffs (new age alternatives over conventional feedstuffs) in common carp. Jitter boxplots – black dots are available data points. No available data on lupine seeds.

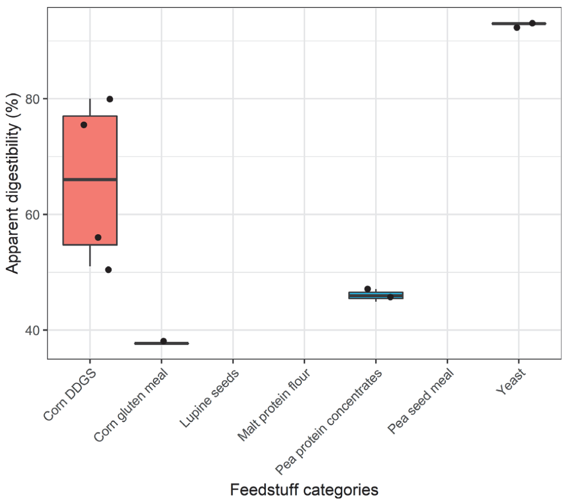

Figure 10 Data on phosphorus availability from some alternative feedstuffs (new age alternatives over conventional feedstuffs) in common carp. Jitter boxplots – black dots are available data points. Narrow boxes or heavy dashes are indicative of limited data availability. No available data on lupine seeds, malt protein flour and pea seed meal.

corresponds to the results (>76% digestible of gross energy) of Watanabe and Ohta (1995). Carp's capacity to utilize dietary energy from feedstuffs looks good. Moreover, the digestibility of gross energy and non-protein energy appears to be representative of each other.

Metabolic (non-faecal) losses

Nitrogen

In carps, as in all teleost, catabolism of ingested proteins releases NH_3 as the primary end-product excreted through gills and urine (Kaushik 1995). Soluble N losses (NH_4), predominantly branchial losses, are the most important losses in carps. Metabolic N losses account more than faecal losses (Kaushik 1980; Watanabe & Ohta 1995). From the handful of studies directly measuring metabolizable losses (Kaushik 1980; Kaushik & Dabrowski 1983b; Kaushik *et al.* 1983a; Watanabe & Ohta 1995), carps (size range 122–1356 mg) excreted 1.2–2.6 g NH_4–N kg body weight^{-1} day^{-1} or ~17–30.7% of dietary N intake and recalculated from original data into a common unit. This non-faecal part of N excretion changes depending on the diets or conditions like feed deprivation. For example, starving carps, despite no feed intake, would have an endogenous N excretion around 0.7 g NH_4–N kg body weight^{-1} day^{-1}. Carps fed on good quality animal protein (e.g. zooplankton, fish meal alone) would have ~3.1–3.7 times higher (*i.e.* 2.2–2.6 g NH_4–N kg body weight^{-1} day^{-1}) metabolic N excretion than in fasting conditions. In casein-based or readily absorbable amino acid mixture diets with perfectly balanced protein profile (amino acid composition) and 'extraordinary' apparent protein digestibility (>97%), the metabolic N losses are lower – up to 1.2 g NH_4–N kg body weight^{-1} day^{-1}. For carps solely feeding on such good quality protein, the IR of metabolic N losses was estimated at 17–30% of dietary N intake. This range may be applied to budgeting in semi-intensive or extensive farming conditions relying majorly on these 'good quality protein' from natural prey in fishponds (e.g. chironomids, daphnia and cyclops having as high as 92% protein digestibility and offering ~7.9% digestible N on dry matter basis; K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). Collating this range with IR of faecal N losses (~10–20% of N intake), up to 50% of dietary N is probably lost in natural systems like fishponds.

Metabolic N excretion via gills and urine is the major source of environmental pollution, and it could be at least theoretically calculated albeit the difficulties in their direct measurement. We theoretically calculated metabolic N losses from the studies that combined growth (protein retention data) and digestibility (faecal losses data) trials with common carp (Takeuchi *et al.* 1989; Pongmaneerat & Watanabe 1991, 1993; Yamamoto *et al.* 1996; Hasan *et al.* 1997; Davies & Gouveia 2010; Kumar *et al.* 2011a,b; Ngoc *et al.* 2016). Across several animal or plant protein-dominated diets and plant–animal mixed diets, the IR of metabolic N losses was estimated at 46.7–58.6% of dietary N intake. The theoretical estimate seems much higher than direct measurements with high-quality protein (presented above); caution needs to be exercised. This might be due to multiple inclusion levels of test ingredients (poor protein profile of some treatments) and/or varying protein levels of diets (inadequate or excessive protein in some treatments) involved in these studies. Combining this with IR of faecal N losses (~10–20% of N intake), it amounts to a total of ~57–79% of N intake probably lost by carps fed exclusively on artificial feedstuffs and raised in indoor, artificial or intensive systems.

Phosphorus

Regarding P, suspended P lost through faeces remains the most dominant pathway. Unlike N, there is lack of quantified data on non-faecal (metabolizable) P losses. Few authors have measured urinary P losses (Sugiura *et al.* 1998, 2000) in fish, but there is limited effort on common carp. Arlinghaus and Niesar (2005) opined carps may excrete minerals (like P) into the aquatic environment through gills or urine, without quantification. In already high P environment, carps were found to excrete more P (Chumchal & Drenner 2004). These metabolic soluble losses, often perceived as negligible or non-quantifiable, have been subject to much less research. They are also rather difficult to assess under laboratory conditions.

Following the above-mentioned approach, we theoretically calculated metabolic P losses from a handful of studies that reported retention and absorption percentage of dietary P intake (Kim & Ahn 1993; Schaefer *et al.* 1995; Watanabe *et al.* 1999; Jahan *et al.* 2001, 2003). All these studies involved artificial feedstuff with high proportion of bone P and/or phytate P occasionally supplemented with inorganic phosphate salts. Unlike N, we could not delineate any study that involved 'good P profile diet' with high P digestibility. Natural diet of common carp (comprising chironomids, daphnids and copepods) has a 'good P profile' with quite high apparent P digestibility (72–99%; K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). Whether such good P profile results in lower non-faecal P losses remains to be explored. The IR of metabolic P losses on artificial feedstuff was theoretically estimated to be 8.6–18.4% of dietary P intake, being quite stable across feed types (unlike N) and miniscule compared to faecal P losses.

Influences of various factors on N and P utilization capacities of common carp

Feed characteristics

From the available data, it could be inferred that (i) feedstuffs dominated by plant protein sources (~50–75% of total protein) or having excess inclusion (by weight, >40%) of anti-nutritional factor rich plant origin feedstuffs are poorly utilized (Chu *et al.* 1991; Hasan *et al.* 1997; Francis *et al.* 2001); (ii) carps exhibit depressed nutrient utilization beyond certain dietary levels of anti-nutritional factors (e.g. 5–6 g kg^{-1} of phytates) present in feedstuffs (Becker & Makkar 1999; Kokou & Fountoulaki 2018); (iii) changes in dietary N content do not deeply impact N digestibility (Ogino & Chen 1973a,b; Grabner & Hofer 1985;

Table 3 Lipid and carbohydrate content and apparent digestibility (interquartile ranges) of common feedstuffs for common carp

Category name	Content		Apparent digestibility	
	Lipid (%)	Carbohydrate (%)†	Lipid (%)	Carbohydrate (%)†
Cereals	2.82–5.38	56.4–82.5	77.7–84.7	44.8–90.1
Oilseeds	1.93–11.0	21.8–34.5	91.6–95.0	41.6–54.1
Fish derivatives	6.35–11.8	2.3–21.3	69.2–91.2	83.1–87.9
Animal proteins (terrestrial)	9.7–14.06	4.7–7.35	83.5–91.6	N.A.
Legumes	2.2–9.9	21.4–55.4	75.6–81.3	N.A.
Brewery wastes	10.2–11.9	18.6–31.8	N.A.	N.A.
Angling ground baits	6.9–11.4	37.1–71.8	83.3–85.9	58.5–79.6
OVERALL‡	3.7–11.8	21.0–58.5	80.3–92.9	52.2–88.8
Gross dietary energy§	–	77.2–99¶		
Non-protein energy (lipid + carbohydrate)§	–	76.3–99¶		

†Carbohydrate = NFE (nitrogen-free extract). Excludes total crude fibre.
‡Across all feedstuff categories in the global metadata; not only restricted to the conventional and alternative feedstuff categories mentioned.
§Calculated by utilizing 'weighted' minima and maxima of 'overall' protein (N), lipid and carbohydrate digestibility. Weights given to carbohydrate (1), protein (1) and lipid (1.25) based on their relative energy density (kcal g^{-1}).
¶Upper limit rounded off to 99, for results > 100%.

Pongmaneerat & Watanabe 1991, 1993; Stankovic *et al.* 2015) supported by our metadata; and (iv) difference in digestibilities of N and total amino acid in a feedstuff is 0.63–3.94% (IR), making them indicative of each other (calculated from Heinitz *et al.* 2016; Yamamoto *et al.* 1998; Kaushik *et al.* 1983a). A detailed account can be found in the Appendix S1.

Carps lack intestinal phytase activity and are unable to digest phytate P from plant ingredients (Ellestad *et al.* 2002). Inorganic phosphate salts are often added in carp feeds to meet dietary P requirements, since the digestibility of bone P is zero (Hua & Bureau 2010). In addition to this, (i) excess supply of dietary P can reduce P digestibility (Satoh *et al.* 1989; Kaushik 1995) supported by metadata; (ii) water-soluble P is easily absorbable by carp (Satoh *et al.* 1997; Watanabe *et al.* 1999; Nwanna & Schwarz 2007), for which inclusion of phytases in plant-based diets was repeatedly advocated; and (iii) primary phosphate salts have higher P bioavailability in carps, than secondary or tertiary P salts (Ogino *et al.* 1979). Our metadata did not reveal any impressive P digestibility from either inorganic phosphate salt or phytase-supplemented carp diets. Contrarily, P digestibility from yeast or brewery wastes was far more impressive than the conventional feedstuffs. Natural food has superior P digestibility (78–95% of cladoceran, chironomid and copepod P; K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). It should be remembered phytases perform optimally at the low pH (3–6 units), which carps lack in their gut. This makes acidic pre-incubation of the plant feedstuffs with phytases necessary to hydrolyse phytate-bound P for the carps (discussed before).

Water temperature

Effect of water temperature on nutrient digestibility in carp seems ingredient specific and at most times, too narrow or non-existent. For example, the effect of temperature on dietary N utilization was prominent for plant protein sources but not prominent enough for animal proteins, especially fish meal (Watanabe *et al.* 1996). The digestibility of cereal-derived N (wheat, corn, rice) indicates a strong temperature-dependent increase (optima: 25°C), better than soya bean-derived N (Watanabe *et al.* 1996). An increase from 15 to 25°C resulted in an 11% improvement of N digestibility in corn gluten meal, whereas not even 1% in soya bean meal (Watanabe *et al.* 1996). N digestibility of corn gluten meal also reportedly increased by 11% with a 10°C increase in water temperature (Yamamoto *et al.* 1998). Kim *et al.* (1998a) found N digestibility from soy protein concentrates at different water temperatures (18 and 25°C) almost similar. Simultaneously, they demonstrated 11–29% improvement in digestibility of cereal N (corn, rice) by increasing water temperature from 15 to 25°C (Kim *et al.* 1998b). On the contrary, Sándor *et al.* (2016) found decreasing utilization of N and P in a cereal-based diet with rising water temperatures (from 20 to 30°C). As with all poikilotherms, feed intake and thus nutrient intake by carp sharply decrease with the decrease in water temperature, being depressed at ≤13°C and resumed at ≥15°C (Yamamoto *et al.* 2001). Interestingly in high protein diets, N digestibility had no dependence on temperature (for 17–25°C) staying almost unaltered (Yamamoto *et al.* 2003). In low protein (high fat) diets, N digestibility decreased slightly at lower temperatures (17°C) compared to 25°C (Yamamoto *et al.* 2007). P digestibility reportedly is not influenced by water temperature

(Yamamoto *et al.* 2007). From the available data, it is inferred that temperatures <15 and >30°C are, respectively, the lower and upper critical thresholds for feeding carps.

Dissolved oxygen

Carps exhibit optimum physiological processes, including feeding and digestion, in water that is over 70% oxygen saturation. It is advised that at levels below 30% oxygen saturation (or <3 mg L^{-1} dissolved O_2), feeding should be either reduced or discontinued until the oxygen conditions improve (Mazurkiewicz 2009). As of now, no specific digestibility studies with respect to dissolved oxygen gradient are available. Under laboratory conditions, carps completely suspended feeding at 1–1.3 mg L^{-1} dissolved oxygen (K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). Matching this with the autumn–winter exclusive, early morning observations of some Czech carp farmers (J. Mraz, personal communication), it was suspected that carps tend to expel out half-digested feed particles following a long anoxic night. The particles usually with a slimy coating, being half-digested, tend to be afloat and accumulate near pond banks, locally termed as '*plogs*'. This claim requires further validation and can also be due to excessive mucosal coating on semi-digested faecal matter passing through carp's long and coiled intestine.

Technological improvements of feedstuffs

Thermal treatment (roasting, cooking, expanding) of cereals allowed better utilization of dietary N (protein) which resulted in better fish weight gains (Przybyl & Mazurkiewicz 2004). This is because improved digestibility of starch from heat treatment increases non-protein energy availability, leading to better utilization of protein (Kaushik 1995). With peas, dry heating treatment seemed more effective than moist heat treatment for improving dietary N utilization by carp (Davies & Gouveia 2010). Compared to untreated cereals, thermally treated or thermally treated and pressed cereals reportedly improved the utilization of P in carp (Hlaváč *et al.* 2015). Contrarily, thermally treated cereals did not significantly improve carp growth in comparison with untreated cereals, although it was significantly higher than no supplementary feeding (Másílko *et al.* 2014; Hlaváč *et al.* 2016b). More than simple thermal processing, hydro-thermal treatments appear to improve the nutritional value of some feedstuffs. Sensitivity of carps to the heat-stable antimetabolic factors contained in the 'autoclaved' mucuna seed meal was demonstrated by Siddhuraju and Becker (2001). When the mucuna seeds were soaked and then autoclaved, that is hydro-thermal treatment, there was significant improvement in nutrient utilization (Siddhuraju & Becker 2001). The presence of free amino acids or peptides can also improve protein utilization, as was found with silage-based (acid hydrolysed) diets (Ramasubburayan *et al.* 2013). Likewise, protein hydrolysates were found to improve growth and N utilization in carp larvae (Carvalho *et al.* 1997). Yamamoto *et al.* (1996, 1998) found that extrusion processing of feeds improved the bio-availability of amino acids from plant protein sources but only to a small extent. Hot extrusion processing of the diets has even been reported to negatively affect N digestibility (Heinitz *et al.* 2016).

Amino acid supplementation

In most plant protein sources, there are some essential amino acids which are inadequate compared to the requirements of carp (Kaushik 1995; Rerat & Kaushik 1995). Supplementation of crystalline amino acids (AAs), mostly the essential AAs like methionine and lysine in formulated feeds, is also known to improve N utilization (Nose 1974; Schwarz *et al.* 1998; Zhou *et al.* 2008; Nwanna *et al.* 2012). DL-Methionine supplementation significantly improved N (protein) digestibility in feed (Nwanna *et al.* 2012). The addition of lysine to practical carp diets reduced dietary protein (N) requirement without affecting growth, through improved utilization of dietary N (Viola *et al.* 1986; Schwarz *et al.* 1998). On the contrary, diet with supplemental essential amino acid showed no improvement in N digestibility over those of non-supplemented diet (Yamamoto *et al.* 1996).

Interactions among dietary components

Imbalances in digestible protein: Digestible energy ratio aggravates metabolic N losses (Takeuchi *et al.* 1979a,b). The recommended dietary digestible protein-to-digestible energy ratio in practical carp diets is between 18 and 20 mg kJ^{-1} (Takeuchi *et al.* 1979a,b; Kaushik 1995). High ash levels in ingredients are known to correlate negatively with N digestibility (Chu *et al.* 1991; Pongmaneerat & Watanabe 1991). High fibre contents and high level of complex carbohydrates are known to negatively interfere with dietary nutrient utilization (Chu *et al.* 1991). Feathers and keratinized fats in poultry meals render nutrients indigestible (Degani *et al.* 1997). Takeuchi *et al.* (1979b) observed no effect of dietary lipid inclusion on N digestibility in carp. But, an increase in dietary lipid content reportedly decreased N retention (Murai *et al.* 1985). Within a dietary IR of 3.7–11.8% crude lipid, 21–58.4% crude NFE and 4.4–10.7% total ash contents, our metadata showed no significant interferences of other dietary components on N or P digestibility.

Feeding practices

Mehner *et al.* (2018) demonstrated that addition of artificial feeds (bait) strongly alters feeding behaviour of carps. Carps spend more time at the feeding (baiting) sites thus utilizing nutrients from feedstuffs, rather than nutrients from natural preys. Increase in ration size has been reported to reduce the nutrient digestibility (Yamamoto *et al.* 1998). With increasing dietary N intake or manual feeding up to satiation, metabolic N losses aggravate (Kaushik 1980). Carps, when self-feeding, have superior dietary utilization of nutrients compared to manual feeding up to satiation (Yamamoto *et al.* 1998). Difference in N digestibility between daytime and nighttime feeding or with increased feeding frequency was negligible (Yamamoto *et al.* 2007), whereas P digestibility either increased (1.6% feeding^{-1}) or decreased (1.5–3% feeding^{-1}) with increasing feeding frequency, depending on dietary fat content (Yamamoto *et al.* 2007).

Effect of body size

Negligible effects on digestibility exist due to age or size of carp, especially once they have reached juvenile stage (Watanabe *et al.* 1996; Arlinghaus & Niesar 2005). Within a size range of 85–475 g, apparent N digestibility of a commercial carp diet fluctuated maximum ±8% that too randomly (K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). However, metabolic N losses are higher in larger carps compared to the smaller ones (Watanabe & Ohta 1995). Hlaváč *et al.* (2015) argued large carps retain more P per unit weight than smaller carps, resulting in lower P concentrations in effluent water from the grow-out carp ponds. They also argued P retention in the fish body improved with an increase in diet P levels. However, no instances in support of this argument were visible from our analysed metadata. Our metadata revealed P content of whole fish (carp) does not change much over growth period; IR of change (whole-body P content) lies within −0.08 to + 0.03%, which is almost constant. IR of carp's whole-body P content was estimated between 0.43 and 0.55% (*data*: Steffens *et al.* 1988; Takeuchi *et al.* 1989; Kim *et al.* 1995a,b; Kim *et al.* 1998a; Watanabe *et al.* 1999; Jahan *et al.* 2001, 2003; Nwanna & Schwarz 2007; Xie *et al.* 2011).

Temporal effects

A period of adaptation is required by carps at times when introduced with new feeds or ingredients. Their digestibility increases with time before attaining maximum digestibility (Appleford & Anderson 1997). Daily variability in digestibility is also observed. A day or two of higher digestibility was generally followed by a day or two of lower digestibility (Appleford & Anderson 1997). Daily variation in N digestibility of carps is quite random (Nandeesha *et al.* 2002). Under laboratory conditions with carps fed on a fixed ration of commercial carp diet, daily fluctuations in faecal P content appeared negligible (±0.07% day^{-1} over a 10-day period; K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data).

Habitat nutrient content

Phosphorus loading (input) into the environment significantly increased the defecation rates of common carp with faeces richer in total P content (Chumchal & Drenner 2004). With decreasing N input (proteins) through artificial diets, carps naturally tend to increase their reliance on natural food (Keshavanath *et al.* 2002). Natural food has superior N and P digestibility (K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data).

Optimum dietary N and P requirement and trade-offs with ecologically relevant levels

Nitrogen

About the optimal dietary N levels recommended for common carp, there is considerable variability (Kaushik 1995). Digestible N levels in common carp diet based on growth stages have been recommended by NRC (2011): 7.2% (for <20 g), 6.1% (20–200 g), 5.1% (200–600 g) and 4.5% (>600 g). Ngoc *et al.* (2016) reported that lower than recommended digestible N in artificial feed is well accepted by farmers doing semi-intensive carp aquaculture, for example, 3.57–3.79% digestible N in Ngoc *et al.* (2016). 'Economically optimum' dietary N levels for carps were standardized at 4.96%, irrespective of developmental stages (Takeuchi *et al.* 1979a, 2002). The N content in carp's natural prey (diet) ranges between 6.72% and 9.49% (Steffens 1986, Kaushik & Dabrowski 1983b), already higher than the artificial feed in per unit dry weight. The cladoceran, copepodite and chironomid-N (crude content: 8.32–11.3% dry matter basis) are many-folds higher than carps' optimum requirement (Bogut *et al.* 2007; K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). A detailed account on optimum dietary N for carps is provided in Appendix S1.

From our metadata analyses, we have observed an IR of 0.58–1.13 thermal growth coefficient (TGC) in common carp within dietary N levels of 5.02–6.36% (IR). Growth curve of common carp (TGC) under different dietary N and feedstuff conditions is depicted in Figure S14. The generalized additive model (GAM) demonstrated a steady increase in carp growth with increasing dietary N levels (*Deviance explained 31.2%, GCV 0.14*, $P < 0.01$), with hints of slowed-down growth beyond 5.5% N (Fig. 11). Rise in growth curve beyond 7% dietary N could not be taken into consideration due to dilated prediction belt, implying low confidence. Peak TGC (at 5.5% N) was not considered for recommendation; rather, upper semi-IR of TGC (0.86–1.12) was considered. Based on the generated models (Figs 11 and S14), we identified a threshold range of dietary N (4.16–4.96%) for common carp that would support reasonable growth and be ecologically relevant. Collating this value with IR of N digestibility, the recommended digestible N from artificial feeding should be ~3.3–4.9%. This was done assuming that natural food will help in peaking (pushing) the growth curve beyond upper semi-IR of TGC, for example, scenario where 30% extra (from the identified thresholds) 'highly digestible' dietary N is being provided by natural food. Usually, there is lack of quantified data on how much percentage of growth is supported through natural food, under semi-intensive conditions. Adámek *et al.* (2012) claim 60–65% of carp growth in Czech fishponds is supported by natural food, rest (35–40%) through supplementary feeding. Under these circumstances, recommendations can be set much lower (<4.16% dietary N, <3.29% digestible N), subject to further calibration.

Phosphorus

Unlike N, there is close agreement on dietary P requirements for carp. P requirement in carp diet has been reported to be around 0.7% (Kaushik 1995). NRC, National Research Council (2011) recommends 0.7% available P in common carp diets. Early growing stages have a higher demand of dietary P (Kim *et al.* 1998a). It should be kept in mind that P content in carp's natural prey (cladocerans, copepods, chironomids) is sufficiently high (crude content: 1–1.3% dry matter basis; availability >80%), more than the carps need (K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). A detailed account on the optimum dietary P for carps is provided in Appendix S1.

Under dietary P levels of 0.7–1.5% (IR), TGC of common carp was observed between 0.64 and 1.21 (IR). The upper semi-IR of TGC was 0.83–1.21. Growth curve of common carp under different dietary P levels and feedstuffs (P-sources) is depicted in Figure S15. The GAM demonstrated an initial increase in growth up to 1% dietary P, beyond which TGC became 'almost' constant (*Deviance explained 28.5%, GCV 0.11*, $P < 0.01$; Fig. 12). Collating this with the 75th percentile of P digestibility, we suspect increasing available P beyond 0.47% may not significantly alter (enhance) growth in common carp. Based on the generated models (Figs 12,S15) and the above-mentioned strategy, we identified a threshold range of dietary P (0.65–1%) that would support reasonable growth and be ecologically relevant. Collating this range with IR of P digestibility, the recommended available P from artificial feeding should be ~0.2–0.5%. Under circumstances where 'highly digestible' dietary P is mainly contributed (>60%, Adámek *et al.* 2012) through natural food, the recommended 'supplementary' P can be set much lower (<0.65% dietary P, <0.18% digestible P) (*discussed above*).

Digestible N:P ratio

From the available data, the digestible N:P in carp feedstuffs appears quite outstretched (IR 7:1–44:1). Recommendations from NRC, National Research Council (2011) for common carp (4.5–6.1% digestible N; 0.7% digestible P) or our generated GAM models supporting reasonable growth at ecologically relevant levels (~3.3–4.9% digestible N; ~0.2–0.5% digestible P) have a much narrower N:P (~6:1 to 17:1). Probably to promote cleaner production, carp feed formulation may be more focused in ways to narrow down digestible N:P ratios around or within the recommendations' range, subjected to future validation.

Faecal eutrophication potential of carps and its controversies

Our preliminary data suggest that freshly defecated carp faeces (fed on compound dry diet; 4–8 h water residence)

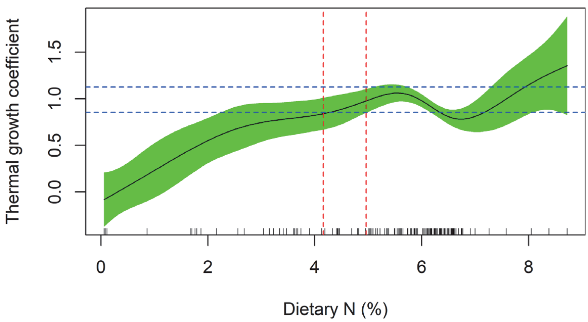

Figure 11 Generalized additive model between thermal growth coefficient (TGC) of common carp and dietary N (%). Recommended dietary N 4.16–4.96% (red vertical dashed lines) against upper semi-interquartile range of TGC 0.86–1.13 (blue horizontal dashed lines). Data from Ngoc *et al.* (2016), Stankovic *et al.* (2015), Davies and Gouveia (2010), Kumar *et al.* (2010, 2011a,b), Nwanna and Schwarz (2007), Nwanna *et al.* (2007, 2008, 2010), Niesar *et al.* (2004), Keshavanath *et al.* (2002), Jahan *et al.* (2001, 2003), Siddhuraju and Becker (2001), Hasan *et al.* (1997), Pongmaneerat and Watanabe (1991, 1993), Yamamoto *et al.* (1996), Kim *et al.* (1995a,b), Nandeesha *et al.* (1995, 2002), Kim and Ahn (1993), Takeuchi *et al.* (1979a, 1989) and Kaushik *et al.* (1983a).

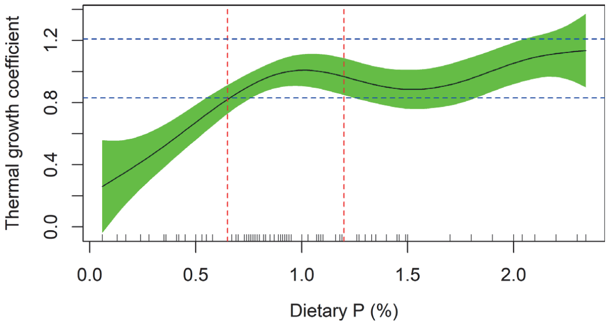

Figure 12 Generalized additive model between thermal growth coefficient (TGC) of common carp and dietary P (%). Recommended dietary P 0.65–1.2% (red vertical dashed lines; restricted to 1% P due to attenuated plateau) against upper semi-interquartile range of TGC 0.83–1.21 (blue horizontal dashed lines). Data from Xie *et al*. (2011), Nwanna and Schwarz (2007), Nwanna *et al*. (2007, 2008, 2010), Niesar *et al*. (2004), Jahan *et al*. (2001, 2003), Kim and Ahn (1993), Kim *et al*. (1995a,b), Schaefer *et al*. (1995), Takeuchi *et al*. (1989), Kim and Oh (1985), Hepher and Sandbank (1984), Ogino *et al*. (1979) and Ogino and Takeda (1976).

can contain 2.08–4.48% N and 1.2–2.66% P (K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). From the global metadata, however, carp faeces estimated to contain as little as 0.47–1.68% N and 0.36–0.86% P, most likely due to nutrient leaching out of faeces during the prolonged water residence prior to their sampling (in most cases, overnight, i.e. ≥12 h). It is beyond the scope of our present attempt to comment on the variation in digestibility data itself, arising due to methodological differences. Interestingly, either in freshly defecated or in metadata-calculated carp faeces, the N:P ratio is common, that is within 0.55:1–4.67:1, estimated from global metadata (see digestibility section). This low N:P ratio in carp faeces falls 'convincingly' within the threshold N:P ratio for triggering eutrophication, that is less than 6:1 (Barica 1990). Combining the branchial and urinary losses (metabolic losses) with faecal losses (digestible losses), the nutrient richness of carp excreta is estimated to be between 0.9 and 6.4% N and 0.4 and 1.1% P with a N:P ratio of 2.1:1–5.8:1, which is still 'eutrophic'. The N:P ratio of carp feeds, however, ranged from 6.4:1 to 10.4:1 lies above this ratio critical for eutrophication (6:1). It indicates that the N:P ratio decreased from food to faeces (i.e. through the process of digestion). Mathematically, the denominator (here, P) has the biggest contribution in decreasing any ratio. This implies that 'faecal eutrophication potential' of carps is directly linked to the P digestibility, followed by poor protein profile of diets prone to metabolic N losses. For addressing environmental concerns, P demands higher priority over N among the nutrients excreted. Our objective should be to increase N retention as well.

Carp's poor digestibility of P from artificial feedstuff stems from their biological limitation, that is absence of an acid-secreting stomach or lack of endogenous phytase activity (Ellestad *et al.* 2002; Hua & Bureau 2010). Defecation trials indicated that carp populations can play a strong role in eutrophication (Lamarra 1975; Qin & Threlkeld 1990; Chumchal & Drenner 2004). Total N and P loadings in pond-based culture systems were estimated at 31–86 kg N and 8.9–26.4 kg P ton^{-1} of carps produced (Watanabe *et al.* 1999; Jahan *et al.* 2003; Hlaváč *et al.* 2014). Half of the excreted P was directly available for algal production (Lamarra 1975). Common carp was directly related to total P content in habitat (Parkos *et al.* 2003) and found to enhance phytoplankton biomass in mesocosm experiments (Chumchal & Drenner 2004). Prikryl (1983) attributed increased concentrations of nutrients in fishponds to carp culture. Petrovici *et al.* (2010) criticized effluent water from carp fishponds polluting downstream watercourses. Butz (1988) pointed out carp fishponds causing greatest nutrient loads to the receiving waters during the annual harvest. Potužák *et al.* (2007) and Pechar (2000) tagged carp production in fishponds as an anthropogenic driver of eutrophication and water pollution.

Contradictory studies exist too, e.g. Kainz (1985) marked the receiving water quality as 'safe' from traditionally managed carp fishponds decades ago. Pursiainen (1988) argued P is not a major problem in carp fishponds, which can be easily reduced by controlling solids output, not by manipulating carps. Knösche *et al.* (2000) disapproved the argument of carp culture critics by concluding fishponds are not a burden on the environment. At production of <1.5 ton ha^{-1}, carp fishponds release 510 g P ha^{-1} less than it receives via incoming water (Knösche *et al.* 2000). A great deal for P loading depends on the choice of supplementary feed (Watanabe *et al.* 1999). Carp diets based on plant protein sources could reduce P loading by 53% (~7 kg P ton^{-1} carps) despite having 25% lesser available P than fish meal-based commercial feed (Jahan *et al.* 2003). Simple reduction of fish meal (15–20%) could yield ~42% reduction in P loading, without even reducing dietary P content (Jahan *et al.* 2001). Hlaváč *et al.* (2015, 2016a,b) found no significant influence on nutrient loads with the different types of supplementary feed used or even suspending feeding. Dulic *et al.* (2010) observed manipulating supplementary feeds in fishponds to alter water quality is a futile decision. However, the stocking density of common carp is an important factor; eutrophication risk is negligible if density is below 1000 kg ha^{-1} (Rahman 2015). Even after deliberately reducing the stocking density of carps for 3 years, eutrophication in fishponds could not be controlled (Pechar *et al.* 2017; Francová *et al.* 2019). The 'bioturbation' activity led to increasing water column nutrients; carps do not contribute to eutrophication in hard substrate systems (Weber & Brown 2009; Kaemingk *et al.* 2017).

Recommended ameliorative measures

Some recommended ameliorative measures drawn from the global literature are listed in Table 4. A detailed list of the ameliorative measures is furnished in Appendix S1. In general, there are three basic principles that must be followed to reduce the dietary nutrient footprints of carp culture: (i) optimize food intake, (ii) decrease feed loss and (iii) improved digestibility of feeds and feed ingredients (Rerat & Kaushik 1995). As discussed above, complying to the 'reasonable growth and ecologically relevant' dietary nutrient supplementation, under semi-intensive culture conditions, may effectively rebut 'eutrophic notions'

Table 4 Recommended ameliorative measures to offset faecal eutrophication risks of common carp in aquaculture (P assigned higher priority over N)

Principle	Strategy	Reference
Reduced P in faeces		
Reduce phytate	Addition of phytase in diet. Pre-incubation of ingredients with phytase, in acidic medium – probably better	Nwanna *et al*. (2005, 2007) and Nwanna and Schwarz (2007)
Reduce dietary P	Minimizing P content and maximizing P digestibility in feedstuffs – adding P supplements/digestibility enhancers	Arlinghaus and Niesar (2005)
Replace fish meal	Reduce/replace fish meal and 1–2% supplementing with inorganic P salts like MCP	Kim *et al*. (1998a,b), Kumar *et al*. (2011a,b) and Ngoc *et al*. (2016)
	Earthworm meal promising replacement to fish meal – without needing to supplement P	
	Plant protein isolates with essential amino acid supplementation – soya bean/ jatropha protein isolates	
Alternative ingredients	Brewer's yeast, malt protein flour and corn DDGS, that is brewery wastes – better P digestibility over conventional ingredients	Yamamoto *et al*. (1996) and Sándor *et al*. (2016)
Feeding frequency	Reduced 'artificial feeding' frequency (1 time per day)	Yamamoto *et al*. (2007)
Low-fat feed	Lower fat, higher starch to meet the dietary energy	Yamamoto *et al*. 2007
Reduced N in faeces		
Energy: protein ratio	Increasing non-protein energy sources in diet. Primarily by raw starch, not crude fat	Kaushik (1995), Keshavanath *et al*. (2002) and Yamamoto *et al*. (2007)
Balanced amino acid profile	Choice of feeds with ideal amino acid profiles	Rerat and Kaushik (1995) and Nwanna *et al*. (2012)
Fixing deficient amino acids	Amino acid supplementation in diets, when necessary	
Reduced both P and N in faeces		
Synchronize with seasonality	Reducing feed provisions with decreasing temperature (≤15–16°C) and at hot temperatures (≥29–30°C)	Yamamoto *et al*. (2003) and Sándor *et al*. (2016)
Synchronize with natural feeding times	Feed application with natural peak feeding times (08:00 and 17:00 h) of carps	Rahman and Meyer (2009)
Ingredient pre-treatment	Practice beyond extrusion and thermal processing, that is water soaking, autoclaving, acid silages/hydrolysis, fermentation	Chu *et al*. (1991), Yamamoto *et al*. (1996, 1998), Siddhuraju and Becker (2001), Davies and Gouveia (2010), and Ramasubburayan *et al*. (2013)
Water stability of pellets	Use of hydrophobic coated or hard coated extruded feeds in carp ponds	Hlaváč *et al*. (2016a,b)
General measures	Optimize feed intake. Decreased feed loss. Improved digestibility	Rerat and Kaushik (1995)

against carp culture. Reducing feed wastage is another crucial aspect. Under field conditions, the use of feed bags (e.g. bag feeding in Andhra Pradesh, India) is an excellent way to manage voluntary feed intake. Floating feeds is another excellent tool for controlling feed intake (Ramakrishna *et al.* 2013; Roy 2019). Some authors advocated the advantage of feeding whole cereals in fishponds, over compounded feed, as the strung hull of cereals resists nutrient leaching (Másílko *et al.* 2014; Hlaváč *et al.* 2016b). Based on our metadata, this recommendation appears partially correct because P digestibility of whole cereal(s) is poor. Feed provisioning in outdoor culture systems should be adjusted according to the prevailing environmental conditions. For example, at temperatures around 13–15°C or dissolved oxygen below 2 mg L^{-1}, the appetite of carps apparently reduces to one-third of that exists at ≥19°C or >3 mg L^{-1} (Füllner 2015, K. Roy, J. Vrba, S.J. Kaushik, J. Mraz, unpublished data). Uneaten feed may lead to nutrient leaching into the pond environment; poor digestibility cannot be blamed.

In addition to this, minimizing or even suspending supplementary feeding coupled with low stocking densities (≤500 kg ha^{-1}) will most likely promote clear-water conditions, as well as improve the carps' reliance on natural preys (such as chironomids, cladocerans and copepods) (Scheffer & van Nes 2007; Sommer *et al.* 2012). In return, faster nutrient mobilization within the ecosystem and both better energy conversion and nutrient uptake should support the optimum carp growth (Potužák *et al.* 2007) with minimized costs, for example for feedstuffs (see the scheme in Fig. S16). The common carp in cooperation with zooplankton and zoobenthos will act for bio-remediating excessive N and P in the hypertrophic fishpond ecosystems. This way, even the legacy of surplus P in the sediments (Pechar 2000) could be 'stepwise excavated' from fishponds via fish harvest. The key approach certainly is to optimize the size of fish stock for allowing cladoceran and chironomid populations to propagate optimally (Sommer *et al.* 2012). This is important for ameliorating the hypertrophic fishponds also facing the effects of climate change-induced eutrophication.

Conclusion

There has been some debate between environmentalists and carp farmers concerning eutrophication of water bodies. Our goal is to moderate the argument between environmentalists and aquaculture enthusiasts surrounding carp culture with the global metadata-driven facts and recommendations. Contradictory studies exist, that is both cursing the common carp aquaculture and not corroborating such allegations. It is naive to ascertain that common carp, despite its poor P digestibility, solely contributes to eutrophication. Carps can digest the N and P locked in their natural preys with an efficiency beyond expectations. On the other hand, our meta-analysis clearly suggested that recent eutrophication of the carp fishponds might have been rather 'management driven' than caused by 'biological limitations' of common carp. Therefore, our study supports science-based solutions for mitigating eutrophication and chances for fishpond remediation. Ameliorative measures have been recognized to reduce primary ecological nutrient footprints of the common carp aquaculture.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Frequency distribution of various classes of feedstuffs tested and/or used in artificial diets of common carp.

Figure S2. Nitrogen content of feedstuff categories/ category dominated carp diets.

Figure S3. Phosphorus content of feedstuff categories/ category dominated carp diets.

Figure S4. Inclusion range of various feedstuff categories in practical carp diets.

Figure S5. Data on apparent digestibility of Nitrogen from different cereals in common carp.

Figure S6. Data on phosphorus availability from different cereals in common carp.

Figure S7. Data on apparent digestibility of Nitrogen from different oilseeds in common carp.

Figure S8. Data on phosphorus availability from different oilseeds in common carp.

Figure S9. Data on apparent digestibility of Nitrogen from different fish derivatives in common carp.

Figure S10. Data on phosphorus availability from different fish derivatives in common carp.

Figure S11. Data on apparent digestibility of nitrogen from different animal proteins (terrestrial) in common carp.

Figure S12. Data on digestibility of Lipid from different feedstuff categories in common carp.

Figure S13. Data on digestibility of carbohydrate (NFE) from different feedstuff categories in common carp.

Figure S14. Thermal growth coefficient of common carp under different feedstuffs and dietary N conditions.

Figure S15. Thermal growth coefficient of common carp under different feedstuffs and dietary P conditions.

Figure S16. Schematic diagram of both simplified plankton food webs and nutrient cycling in fishponds with two alternative (left vs. right) pathways for the two contrasting states (e.g. Scheffer & van Nes 2007): clear-water and turbid conditions with lower and higher fish stock, respectively.

Table S1. Checklist of the feedstuffs (alphabetically arranged) used in experimental and/or practical diets of common carp from the research articles surveyed.

Appendix S1. Supplementary text with further detailed information and additional bibliography.

CHAPTER 4

CIRCULAR RESOURCE USE: PROBLEMS AND PROSPECTS FROM A LENS OF NUTRITION

Lunda, R.*, Roy, K.*, Dvorak, P., Kouba, A., Mraz, J., 2020. Recycling biofloc waste as novel protein source for crayfish with special reference to crayfish nutritional standards and growth trajectory. Scientific Reports 10, 1–10.
*LR and KR contributed equally to the work

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Recycling biofloc waste as novel protein source for crayfish with special reference to crayfish nutritional standards and growth trajectory

Roman Lunda[1,2], Koushik Roy[1,2], Petr Dvorak[1], Antonin Kouba[1] & Jan Mraz[1]✉

Screening of novel feedstuffs, that too for data-deficient (nutritionally) animals, is somewhat ambiguous or problematic. Through systematic meta-analyses, the present study formulated most up-to-date crayfish nutritional standards, against which a recyclable waste (biofloc biomass, BM) from intensive aquaculture systems was assessed as a novel protein source. Growth trajectory dependencies and thermal growth coefficient qualifying for good growth in crayfish (TGC 0.5–0.64 units) were benchmarked. Using these standards and a 7-week growth trial, BM's suitability as a novel protein source for red swamp crayfish *Procambarus clarkii* was evaluated through its graded inclusions in a commercial feed. Results suggest that BM can elevate growth at 33–66% inclusion in existing feed formulations. Beyond 66% inclusion, BM can deteriorate growth in crayfish due to high ash content (exceeding physiological limit > 14%), arginine deficiency (~ 14–20% lower than an optimum requirement), and insufficient non-protein energy: protein ratio (3.7 cal mg^{-1}). Arginine is perhaps the most critical amino acid in dietary protein for crayfish, and deficient in BM. Although no critical bioaccumulation levels of heavy metals were breached by feeding 100% BM to crayfish, a mineral and heavy metal (Hg) stress seemed plausible. Crayfish raised solely on biofloc may not realize full growth potential.

Abbreviations

BM	Biofloc meal (biomass)
BFT	Biofloc technology aquaculture system
TGC	Thermal growth coefficient
EAA	Essential amino acid
IR	Interquartile range
GAM	Generalized additive model
LWG	Live-weight gain
CP	Crude protein
CL	Crude lipid

Freshwater crayfish, mostly endemic to the continents of North America, Australia-Oceania, and Europe[1], account for 1.71 million tons of global aquaculture production with a worth of 14.46 billion € as of 2018[2]. Presently they contribute a negligible fraction in the global aquaculture scenario (~ 3.5% of total freshwater aquaculture production) but having great potential ahead. During the last half-decade alone (2013–2018), freshwater crayfish production, and its commercial valuation have tripled[2]. In terms of crayfish nutrition research, efforts have been quite limited compared to other commercially important crustaceans (like penaeids and palaemonids)[3,4]. Therefore, screening of novel feedstuffs, that too for crayfish, is somewhat ambiguous or problematic. A brief prologue in this regard is provided in the supplementary text. On the other hand, aquaculture nutrition research has focused on developing feed substitution strategies with a minimal supply of fishmeal and fish oil

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in recent times. One potential ingredient could be a microbial biomass meal from biofloc technology systems (BFT)[4]. BFTs are a modern, intensive aquaculture system that evolved from the classic 'activated-sludge based sewage bioremediation' in wastewater treatment plants. The system essentially operates on the rationale of maintaining an optimum C: N ratio (6:1 to 15:1) by daily purging with carbohydrate (carbon) source[5,6]. It is done to support the blooming of microbial biomass (flocs). These microbial flocculants, known as 'bioflocs' bioremediate the nitrogenous wastes generated by fish and uneaten feed into consumable microbial protein for cultured animals[7,8]. Although they are consumed by the fish or shrimp stock, the biofloc biomass (as measured in Imhoff cones) or total suspended solids (TSS) may often exceed the recommended values for fish (25–50 ml L^{-1}; TSS up to 1000 mg L^{-1}) and shrimp (10–15 ml L^{-1}; TSS 400–600 mg L^{-1})—posing problems for the cultured animals[7,9–11]. It is advisable to drain part of the biofloc biomass daily through sedimentation or fractionation of biofloc system water[10,12–14]. Such thinning (filtering) of culture water generates a large amount of biofloc biomass as waste, quite frequently. This drained biofloc is often of limited use. In general, they can be used as an alternative to synthetic polymers for wastewater treatment[15], fertilizer, or inoculum to start a new system[16].

Our research intervenes in recycling this waste for aquatic animal nutrition. Since conventional protein sources in aquafeed (e.g., fishmeal) are becoming expensive and scarce, there has been a growing impetus in testing biofloc as an unconventional protein source for aquatic animals[8,17–19]. Few commercial floc meals are generically marketed under 'single-cell protein (SCP)' or 'microbial protein' category—Profloc (Nutrinsic), FeedKind (Calysta), and Novacq/OBM (Ridley, Maritech) with pricing (as of 2018) between 1.1–3.3 USD kg^{-1}[17,18]. One of these is listed in IAFFD (international aquaculture feed formulation database), with complete nutrient spectrum data, including essential amino acids[20]. So far, crayfish are not included in these mentioned researches. The novelty here is its potential use as a feedstuff (protein source) in the crayfish diet. In general, the protein (12–49%), lipid (0.5–12.5%), and ash (13–46%) contents in biofloc can vary substantially depending on several factors (reviewed by[22]). To the best of our knowledge, nutritional evaluation of biofloc as a feedstuff ingredient for artificial crayfish diets has not been done so far. Although rearing of crayfish in BFT system, where the animals co-fed on commercial feed pellets (primarily) and bioflocs suspended in the system, are recently being explored[23,24]. Our objective was to understand—(a) nutritional optima of freshwater crayfish from the available literature in the absence of centralized recommendations (see supplementary material); (b) growth trajectory and nutritional dependencies in crayfish (supplementary material); (c) response of red swamp crayfish to biofloc meal in their diet, in terms of nutrition, growth, and survivability; (d) the risk of heavy metals bioaccumulation or mineral stress in crayfish from feeding on biofloc, and; (e) evaluate nutritional strengths and bottlenecks associated with using biofloc meal in crayfish diet. The first two objectives (a and b) were rather a methodological and necessary step (placed in supplementary material) to the second part of our research related to the use of biofloc meal for crayfish (objectives c to e).

Results and discussion

Nutritional optima, growth trajectory, and nutritional dependencies of crayfish. Based on our meta-analyses, crayfish' optimum dietary nutritional requirement is tabulated as crayfish standards in Table 1. It is also compared with established standards of penaeid shrimps, often assumed as a template for most crustacean diets. Detailed information in this regard can be found in the supplementary material. In terms of crayfish growth trajectory, their thermal growth coefficient (TGC) may vary from 0.07–1 unit (interquartile range, IR 0.32–0.64 units). Results suggest any TGC in the range of 0.5–0.64 units may be regarded as 'reasonably good growth' in crayfish. Further insights into crayfish growth trajectory and its nutritional dependencies are presented in detail in the supplementary material. The information synthesized and approach used may serve as a template for future researchers exploring three less-established or unknown dimensions simultaneously (as in the present study)—novel feedstuff, optimum nutrition, and data-deficient (nutritionally) animals.

Growth response of crayfish to biofloc protein. Following a 9-week growth trial with graded BM levels in the diet, differential growth response by crayfish was realized (Fig. 1). Except for control and BM_{33} groups, crayfish' final body weight showed a significant deviation from the normal distribution. Further examining the skewness of final body weight distribution in BM_{66} and BM_{100} groups, it was apparent that these groups were dominated by runts (smaller sized individuals) with large size deviations from the handful of bigger individuals. The size heterogeneity showed a significant and negative correlation with BM inclusion in the diet (Pearson's 2-tailed $r = -0.63$, $p < 0.05$). Size heterogeneity in crayfish may aggravate community aggression[25]. However, the diet-driven size heterogeneity was not significantly correlated with mortality. The dietary treatments did not cause significant differences ($p > 0.05$) in survivability, confirmed by *post-hoc* analyses. Overall, the survivability remained > 70% through the experimental period in all groups (Table 2). It implies—BM does not pose a significant mortality risk to crayfish stocks irrespective of inclusion levels, but it has implications on growth (presented below).

The growth in terms of TGC, live-weight gain (LWG), and body weight (BW) were significantly depressed ($p < 0.05$) in the BM_{100} fed group. In contrast, the growth in control, BM_{33}, and BM_{66} groups were higher with insignificant differences among them ($p > 0.05$) (Table 2, Fig. 1, 2). A statistically insignificant dampening of growth rate over time ($p > 0.05$) was observed in groups BM_{66} and BM_{100} (Fig. 2). At the end of culture (63 days), the realized TGC in crayfish fed on BM_{100} was on an average two times lower ($p < 0.05$) than the growth exhibited on control, BM_{33}, or BM_{66} diets (Table 2, Fig. 2). In terms of feed utilization, the feed conversion ratio (FCR) and protein efficiency ratio (PER) were linearly related to increasing BM inclusion in the diet. The FCR increased with increasing share of BM in diet**:** $FCR = 1.156 + 0.006 \times BM$ (Adj. R^2 0.95, $p < 0.05$). The PER decreased with an increasing BM inclusion: $PER = 1.922 - 0.006 \times BM$ (Adj. R^2 0.95, $p < 0.05$). It means, for every 10% inclusion of BM, FCR increased by + 0.06 units, and PER decreased by − 0.066 units (Fig. 3). The results from the growth

Parameter	Crayfish standard (calculated)	NRC[4] standards for penaeid shrimps
Macronutrient and energy (based on *Cherax* sp. and *Procambarus* sp.)—crude		
Crude protein	29–34% (44%)*	33–42%**
Crude lipid	6.5–9%	5–6%
Crude NFE (nitrogen-free extract)	40–47%	–
Dietary fiber	Up to 7%	–
Total ash	7.8–10.8%	–
Gross energy	3590–4205 kcal kg^{-1}	3666–4888 kcal kg^{-1}**
Protein: Energy	72–91 mg $kcal^{-1}$ (113–119 mg $kcal^{-1}$)*	85–90 mg $kcal^{-1}$
Non-protein energy: Protein ratio	5.3–8.5 cal mg^{-1} (4.4–4.8 cal mg^{-1})*	–
Essential amino acids (based on *P. clarkii* only)—digestible		
Leucine	1.8–2.5%	1.8%
Valine	1.2–1.6%	1.4%
Threonine	0.3–1.5%	1.3%
Isoleucine	1.2–1.7%	1.2%
Arginine	**2.1–2.7%**	1.8%
Phenylalanine	0.8–1.5%	1.4%
Lysine	1.2–2.4%	1.8%
Methionine	1.1–4.9%	0.7%
Histidine	0.6–0.9%	0.7%
Tryptophan	0.4%	–
Essential minerals (based on *Astacus* sp., *Ornectes* sp., and *Procambarus* sp.)—available		
Calcium	3000–4000 mg kg^{-1}	–
Phosphorus	164–235 mg kg^{-1}	3000–7000 mg kg^{-1}
Iron	27–125 mg kg^{-1}	–
Zinc	10–14 mg kg^{-1}	15 mg kg^{-1}
Copper	6–9 mg kg^{-1}	10–32 mg kg^{-1}
Manganese	14.2–17.8 mg kg^{-1}	–

Table 1. Optimum dietary nutritional requirement of freshwater crayfish and its comparison with NRC (2011) standards for penaeid shrimps (usually adopted as *status quo*). *In parentheses—proposed reconsideration of calculated standards, based on high TGC obtained in the present trial. **Digestible values converted to crude values assuming 90% apparent digestibility.

trial are summarized in Table 2, and the relationship of feed utilization parameters in response to BM inclusion is depicted in Fig. 3. Interestingly, the calculated FCR(s) of our respective diets, when multiplied with the dietary arginine content, seem to 'hit the target' of arginine requirements by crayfish (e.g., FCR of BM_{100} × Arginine in BM_{100} = Fulfillment of arginine requirement).

As per the crayfish growth trajectory (quantified in the previous section), the group fed on 100% BM failed to show reasonably good growth. They were dominated by smaller-sized runts, poorest of the FCR and PER, but no significant mortality. Among the limited studies testing flocculated microbial meals in crustacean diets [reviewed in 8], BM inclusions were mostly up to 10–30% (of the total diet) or 30% (of fishmeal replacement). Good results in terms of growth were usually obtained at the maximum inclusion levels[8]. Like the present study, two previous studies had tested BM (on *Litopenaeus vannamei*) at a broader inclusion level from 17 to 84% of the total diet[17,26]. Despite different target species, the results seem close to that of the present study. Above 41–53% BM inclusion, the growth advantages were gradually lost[17,26]. Looking deeper into the aspects of our BM_{100}-protein compared to control, BM_{33}, or BM_{66}-protein, the arginine seems to be a bottleneck for reasonably good growth (Tables 2, 4). Other EAAs, which could also be critical (e.g., methionine and lysine), were comparable-to-higher in BM_{100} than in other diets (Table 4). Although methionine and lysine levels in diets fell short of our formulated crayfish nutritional standard (Table 1), at least it fulfilled penaeid EAA standards of NRC[4]. It hints that NRC's penaeid EAA standards cover well for most of the EAA requirements in crayfish, except for arginine (and tryptophan could not be judged). Arginine levels in BM (Table 4) neither fulfilled crayfish nor penaeid standards (Table 1).

Biofloc has been previously criticized for being partly deficient in arginine[27–29]. The arginine coefficient (proportion of total protein, in %) of biofloc meals, be it commercial ones like Novacq (2.38%[19]), FeedKind (2.54%[20]), or in the present study (2.73%) seem to have close resemblance (CV 5.5%). If we consider the mean arginine coefficient of BM from these data (2.55%) and tally it to fulfill the optimum arginine requirement of crayfish (minimum 1.8%), the crude protein level of such BM should be at least 70%. It is beyond the expected range of ordinary bioflocs[22]. BM harvested from high TSS systems (due to infrequent sedimentation or water exchange) can have lower protein content[10]. For example, the crude protein content of a biofloc can drop by – 34.5% if the

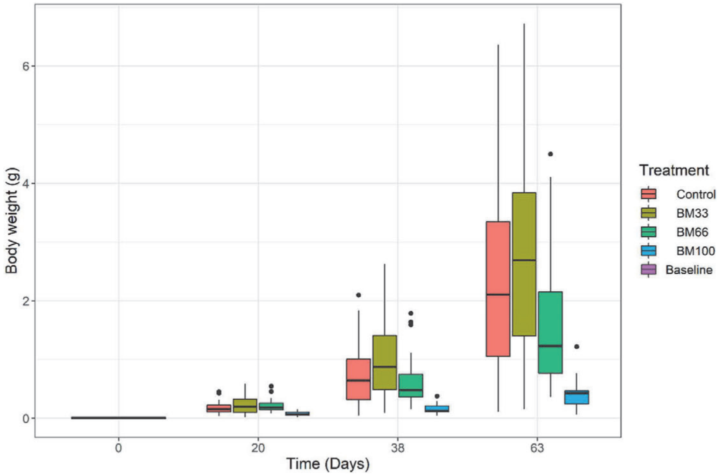

Figure 1. Body weight distribution in red swamp crayfish *Procambarus clarkii* fed graded level of biofloc meal (BM) in diets over 9 weeks of experimental duration. Measured on 20th, 38th and 63rd days post stocking. 'Baseline' indicates stocked stage-3 juveniles (0.007–0.008 g individual^{-1}). Size heterogeneity (measured by coefficient of variance, CV) seems maximum and comparable in control (mean CV = 67%), BM_{33} (mean CV = 67.5%) and BM_{66} (mean CV = 63.4%) groups but significantly suppressed ($p < 0.05$) in BM_{100} (mean CV = 51%). BM_{100} showed poor size throughout the experiment.

Diet group	Survival (%)	Final body weight (g)	Live weight gain (mg day^{-1})	Food conversion ratio	Protein efficiency ratio	Thermal growth coefficient
Control	70^a	1.06–3.34 (2.44 ± 1.79)^a	17–53 (39 ± 15)^a	1.2*	2	0.60–0.94 (0.84 ± 0.14)^a
BM_{33}	70^a	1.40–3.84 (2.80 ± 1.86)^a	22–61 (44 ± 16)^a	1.4	1.6	0.68–0.99 (0.89 ± 0.13)^a
BM_{66}	80^a	0.77–2.15 (1.62 ± 1.19)^a	12–34 (26 ± 9)^a	1.5	1.5	0.53–0.79 (0.72 ± 0.11)^a
BM_{100}	83^a	0.25–0.47 (0.41 ± 0.25)^b	4–7 (6 ± 1)^b	1.8*	1.3	0.32–0.42** (0.40 ± 0.04)^b

Table 2. Response of the red swamp crayfish *Procambarus clarkii* (initial body weight 7–8 mg) under 9-week growth trial (21.8 °C) fed experimental diets. Values presented in interquartile range with mean ± standard deviation in parentheses. ^{a,b}Superscripts denote statistically different ($p < 0.05$) groups. *Pattern: FCR multiplied by Arginine content of feeds ≈ fulfillment of Arginine requirement (as per crayfish or penaeid standards). **Below reasonably good growth (TGC 0.47–0.59) for crayfish standards.

TSS of the system is let to increase from ≤ 200 mg L^{-1} to 800–1000 mg L^{-1}[10]. As such, BM harvested from a low TSS system would have higher arginine (0.72%) compared to a high TSS system (0.47% arginine) (recalculated from[10]; using mean arginine coefficient = 2.55% of total protein). Even with aging biofloc, the content of arginine (also other EAAs) may decline. For example, from the 10th day to the 30th day of a biofloc culture, the arginine levels can decrease by 25–41% (recalculated from[27]). However, some specially produced commercial flocculated meals can have a high arginine coefficient (e.g., 5.3% of the protein in ProFloc[17]). Among all the EAAs, arginine content in red swamp crayfish seems maximum[3,21,30,31], indicating a supposedly higher arginine demand in crayfish. The same is true for marbled crayfish *Procambarus virginalis*[32]. Arginine is perhaps the most limiting EAA in most crustacean diets and is required between 1.6–2.7% of diet[33]. Due to the poor activity of the urea cycle in crustaceans, arginine is indispensable for growth[33,34]. Arginine functions as a phosphagen in crustaceans, being the only amino acid providing amidino group for the synthesis of creatine—a major reserve of high-energy phosphate for ATP regeneration[33,35].

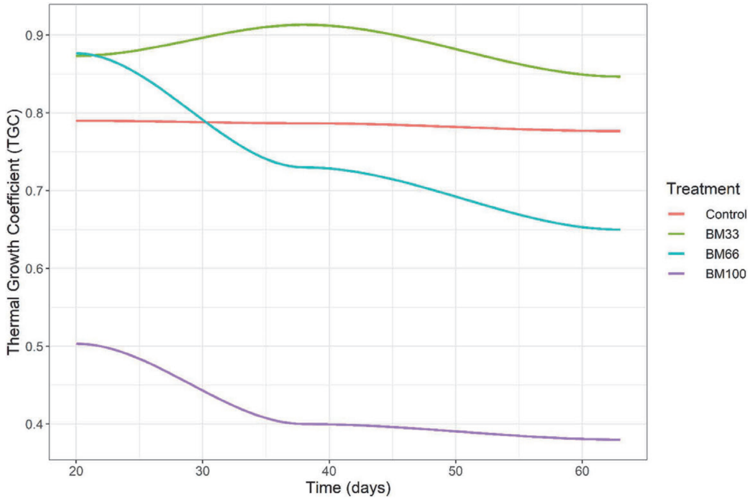

Figure 2. Growth pattern (TGC: thermal growth coefficient) of red swamp crayfish *Procambarus clarkii* fed different experimental diets over 9 weeks. A dampening of growth over time gradually setting-in at higher BM inclusion in the crayfish diet (from BM_{66} to BM_{100}). At the end of culture, BM_{100} resulted in twice less growth ($p<0.05$) than achievable on other diets (control or BM_{33} and BM_{66}—statistically comparable TGC).

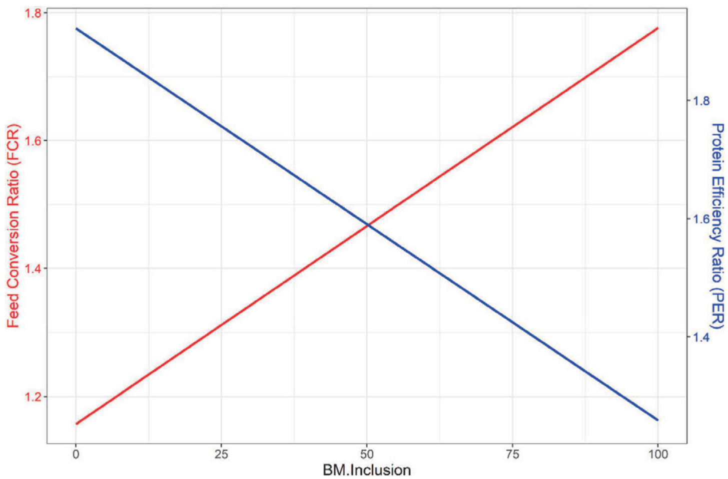

Figure 3. Feed utilization pattern (FCR in red and PER in blue) of red swamp crayfish *Procambarus clarkii* in response to the level of biofloc meal (indicated by BM.Inclusion, in %) in the diet. More feed is required per unit weight gain of crayfish with an increasing share of BM in the diet because protein utilization is lowered at higher BM inclusion.

Group	Hg (μg kg^{-1})	Mn (mg kg^{-1})	Cd (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Muscle					
Control	9.4 ± 0.9^{a}	BDL	0.008 ± 0.01^{a}	11.4 ± 1.4^{a}	4.1 ± 2.7^{a}
BM_{33}	10.5 ± 1^{b}	BDL	BDL	11.8 ± 0.9^{a}	7.0 ± 5.7^{b}
BM_{66}	10.4 ± 1.4^{b}	BDL	BDL	10.4 ± 1.0^{b}	3.2 ± 2.4^{a}
BM_{100}	12.8 ± 1.2^{c}	BDL	BDL	8.5 ± 0.5^{c}	BDL
Hepatopancreas					
Control	4.6 ± 1.0^{a}	2.2 ± 0.1^{a}	0.17 ± 0.05^{a}	46.1 ± 30.0^{a}	54.6 ± 13.0^{a}
BM_{33}	5.4 ± 0.6^{b}	2.9 ± 0.4^{ab}	0.13 ± 0.03^{b}	72.4 ± 26.3^{b}	90.4 ± 13.4^{b}
BM_{66}	5.4 ± 0.7^{b}	3.2 ± 0.8^{b}	0.13 ± 0.01^{b}	67.7 ± 34.8^{ab}	88.0 ± 6.0^{b}
BM_{100}	11.0 ± 1.2^{c}	3.6 ± 2.2^{b}	0.19 ± 0.01^{a}	76.3 ± 28.9^{b}	82.4 ± 12.0^{b}

Table 3. Heavy metals and mineral content (mean ± SE; dry matter basis) in the tail muscle and hepatopancreas of red swamp crayfish *Procambarus clarkii* fed graded levels of biofloc meal. BDL = below detection limit (Mn and Fe: < 2 mg kg^{-1}, Cd: < 0.002 mg kg^{-1}); different letters in superscript denote groups with significant differences as derived from Tukey's HSD multiple range test (α = 0.05).

Proximate fraction	Basal	BM_{33}	BM_{66}	BM_{100}
Crude protein (CP) (%)	44.2	44.1	44	43.9
Crude lipid (%)	7.8	6.7	5.6	4.5*
Crude NFE (%)	35.5	33.8	32.1	30.3
Crude Fibre (%)	2.7	3.4	4.2	4.9
Total Ash (%)	9.8	12	14.2	16.4*
Gross energy (kcal kg^{-1})	3890	3719	3549	3373
Protein: Energy ratio (mg kcal^{-1})	113.6	118.6	124	130.2
Non-protein energy: Protein ratio (cal mg^{-1})	4.8	4.4	4.1	3.7*
Essential amino acids (%)				
Leucine	2.3	2.3	2.2	2.2
Valine	1.3	1.5	1.6	1.8
Threonine	1.1	1.3	1.4	1.6
Isoleucine	1	1.1	1.1	1.2
Arginine	1.5	1.4	1.3	1.2**
Phenylalanine	1.4	1.5	1.7	1.8
Lysine	1.7	1.7	1.8	1.8
Methionine	0.6	0.6	0.7	0.7
Histidine	0.8	0.8	0.8	0.8
Tryptophan	–	–	–	–
Minerals and heavy metals (mg kg^{-1})				
Arsenic (As)	< 0.21	< 0.21	< 0.21	< 0.21
Cadmium (Cd)	0.41	0.6	0.7	0.90
Chromium (Cr)	2.06	3.9	5.8	7.72
Copper (Cu)	11.70	110.1	208.6	310#
Iron (Fe)	185	2437.3	4689.5	7010#
Mercury (Hg)	0.01	0.03	0.04	0.06
Manganese (Mn)	59.60	220.4	381.3	547#
Nickel (Ni)	2.06	4.2	6.4	8.67
Lead (Pb)	2.06	3.5	4.9	6.32
Zinc (Zn)	93.30	306.4	519.5	739#

Table 4. Proximate composition of biofloc meal, basal and treatment diets (dry matter basis). *Matching the values with crayfish standards (Table 1)—hints under-supply (lipid, NPE:P) or excessive supply (ash). **Matching the values with crayfish standards (Table 1) and optimistic assumption of biofloc protein digestibility (~ 90%)—hints under-supply of amino acid. #Matching the values with crayfish standards (Table 1) and most conservative assumption of mineral retention (~ 10% retention)—hints mineral stress due to over-supply.

Risk of heavy metals bioaccumulation or mineral stress from biofloc meal. The contents of heavy metals in BM were below the critical pollution limits. No critical limits were breached in the crayfish body that could qualify BM as a feedstuff capable of inducing unsafe heavy metal biomagnification, rendering them unfit for consumption. Content of Cd and Mn were mostly below the detection limits (Table 3). Except for mercury, hepatopancreas contained a higher amount of heavy metals (and minerals) than muscle. Hepatopancreas of crayfish, like most crustaceans, have been reported to be major storage of minerals, including heavy metals[3,37]. With increasing BM fraction in the diet, the concentration of Hg significantly increased in hepatopancreas (control → BM_{33} and BM_{66} → BM_{100}; $p<0.05$), while other metals did not show any significant trend (Table 3). Except for Cd, all metals were significantly higher ($p<0.05$) in the hepatopancreas of BM_{100} fed crayfish compared to the control group. Such accumulation of heavy metal in hepatopancreas is capable of impairing metabolism in crayfish[37]. The concentration of Fe exhibits a rather 'bell curve' pattern, peaking at BM_{33} and receding thereafter, only in the muscle (Table 3). Cd and Zn did not exhibit any pattern as such. The heavy metal contents in crayfish and BM are given in Tables 3 and 4, respectively.

Globally, the total ash content in biofloc may range between 13–46% (reviewed by[22]), also applicable in our case. The problem of high ash content in most biofloc, limiting its inclusion in diets (despite good protein content), has been briefly discussed in Sabry Neto et al.[38]. One previous study, which studied BM at a high enough inclusion level, attributed high ash and probable toxic effects of trace minerals to retarded growth in *Litopenaeus vannamei* fed > 60% BM in a diet[26]. Owing to high ash content in BM, mineral stress seems plausible in the present study as well (*see* Tables 1, 4). By mineral stress, we imply even if 10% of the ash or minerals from BM are digested by crayfish, it is potentially much higher 'bioavailable minerals' in the body than their optimum physiological limits. Information on this aspect have been limited for shrimps [reviewed in 39, 40] and none for crayfish[3,36]. In shrimps (*Penaeus monodon, P. japonicus*), retarded growth was observed when excessive mineral premixes were supplemented in a practical diet[39], or more specifically, when trace minerals like Fe and Mn exceeded levels of 0.01% each in the diet[40]. The BM_{100} had all these factors (ash, Fe, and Mn) in excess (Table 4). Heavy metal stress could also be plausible. Any significant absorption of Hg in the body (presented above) is capable of impairing crayfish metabolism[37], provoking hyper-osmoregulation in crustaceans[41], with repercussions on aggravated energy expenditure[42]. Our metadata derived models show TGC in crayfish deteriorates at dietary ash levels > 14% (also in BM_{100}), during which the retention of ash is merely < 10% of total dietary intake (see supplementary material and Fig S2, S3). Thus ≥ 90% of the ingested ash (exceeding physiological limits) are excreted through digestive and osmoregulatory (metabolic) pathways. It has its own energy cost, which could have been utilized for protein-sparing or growth[42].

Recycling biofloc waste as a novel feedstuff for crayfish: Strengths and bottlenecks. Comparing the nutritional standards for crayfish with observed performance in growth trials, few strengths and bottlenecks of BM were realized (Tables 1 and 4). In terms of advantages: (a) BM has a high crude protein content (43.9%); (b) crude fiber content in BM (4.9%) was in the optimum range for crayfish, and; (c) BM is a rich supplier of minerals. However, there are more bottlenecks than limited advantages. BM has excessive total ash detrimental to crayfish growth, with probable manifestations on hyper-osmoregulation and energy expenditure (discussed above). A mediocre crude lipid content (4.5%) is another bottleneck for supplying non-protein energy. These, in combination, render the non-protein energy: protein ratio (NPE: $P=3.7$ cal non-protein energy per 1 mg protein) in BM insufficient for effective protein sparing (≈growth). At such low NPE:P, the proteins are catabolized for meeting energy demand (even after oxidizing carbohydrates and lipids), rather than building biomass[42]. It is further compounded by arginine deficiency in BM (~ 14–20% less than an optimum requirement)—probably the most critical essential amino acid for crayfish (discussed above).

A retrospective evaluation of BM_{100} or BM (as a feedstuff for crayfish) applying our metadata derived 'growth-retention models' (supplementary Fig S3, S4, S6) could explain few nutrient utilization scenarios behind low growth in BM_{100}. The ash, protein, and lipid retentions from BM should be less than 5%, 10%, and 3% of dietary intakes, respectively (predicted). For control, $BM_{33,}$ and BM_{66} diets, these retentions were well above the identified thresholds qualifying for reasonably good growth in crayfish (refer to supplementary material). Comprehensively, the retarded growth problem with solely feeding on biofloc biomass could be a synergistic effect of— (a) arginine deficiency, (b) mineral and heavy metal stress, and, (c) low non-protein energy to protein ratio.

Methods

Calculation of crayfish nutritional standards, growth trajectory, and its nutritional dependencies. In the absence of centralized nutrition recommendations for freshwater crayfish species, unlike other commercially important crustaceans (e.g., penaeid shrimps, *see* NRC[4]), available literature was meta-analyzed. Peer-reviewed and published articles (in English or at least with English abstract) were searched online (search engines: Web of Science, Scopus, and Google Scholar) using keywords like 'growth trials', 'crayfish', 'nutrition', 'proximate composition', 'body composition', 'amino acids', 'heavy metals', 'optimum requirement' were used in different combinations (depending on target information). Altogether 27 articles were sourced and data extracted for meta-analyses. Detailed methodology on each meta-analysis (*i.e.,* formulation of nutritional standards, calculation of growth trajectory and feed utilization parameters, quantification of nutritional dependencies on growth) are provided in the supplementary material.

Collection of biofloc biomass. Biofloc biomass was obtained from a well-established indoor, freshwater biofloc system, stocked with Nile tilapia *Oreochromis niloticus* at a stocking density of 35 kg m^{-3}. Commercial pellets (TILAPICO 3 mm, Coppens, The Netherlands) were used as standard feed for fish. Fish feed was given twice daily based on a feed amount equivalent to 2.5% of the fish body weight. Wheat flour (35.56% C; 2.38% N) served as a carbon source which was applied daily with feed (22.05% C; 7.07% N) in a ratio of 1:0.6 (feed: flour). Assuming a 30% retention of nutrients from feed to fish, the projected C: N ratio was ≈6:1. Such a low C: N ratio favored frequent harvest of young and N-rich wet biofloc biomass[6,10] to be converted to dry matter for the ensuing experiment. Biofloc biomass was drained daily through a pump and a vortex separation device so that the suspended solids level stayed between 25 and 50 ml L^{-1} in the system. After separation, biofloc was filtered through a nylon screen (mesh size 60 µm) to drain the excess water. The filtrate was then dried at 80 °C to obtain a material of solid consistency. After obtaining enough dried biofloc, the samples were grounded by a hammer mill to yield finer particles and hereinafter referred to as the biofloc meal (BM).

Preparation of experimental feed. Commercial pellets (TILAPICO 3 mm, Coppens, The Netherlands) were used as the basal diet due to its similar protein content with our test ingredient (BM). The commercial 'fish feed' was chosen due to a lack of established 'crayfish feeds' in the market. Even the available ones appeared to be random feed mixtures targeted for ornamental crayfish keeping. Inclusion of BM by replacing basal diet was done on a weight by weight basis. All feeds were isonitrogenous. The graded inclusion levels were 0% (basal diet = control diet), 33% (67% basal + 33% BM; diet BM_{33}), 66% (34% basal + 66% BM; diet BM_{66}) and 100% (only BM; diet BM_{100}). Feed pellets (pellet size 2 mm) were cold extruded, dried (12 h; 45 °C), vacuum sealed, and stored at 4 °C till further use. The diet samples were analyzed in an accredited third-party laboratory (AGRO-LA, spol. s.r.o., https://www.agrola.cz/zemedelske-a-potravinarske-sluzby/) employing analytical methods (ISO verified and certified protocols in the Czech Republic) for proximate composition, essential amino acids (EAAs; except tryptophan due to analytical error), heavy metals, and essential mineral contents. Detailed composition of basal diet, treatment diets and the biofloc meal are summarized in Table 4.

Crayfish keeping. A total of 120 juvenile red swamp crayfish (*Procambarus clarkii;* conservation status: least concern) having a mean weight of 7.8 ± 0.7 mg at the onset of exogenous feeding (developmental stage 3), were used as experimental animals (10 individuals per tank; 4 group x triplicate). The experiment lasting for nine weeks was conducted in a series of indoor glass aquaria (54 × 36 × 30 cm, volume 46 L) with aeration and attached to a recirculating aquaculture system. Two baked clay bricks (28.5 × 13.5 × 6.5 cm), each with 39 cross holes (26 and 13 holes with a profile of 1 × 3 cm and 1 × 1 cm, respectively), were placed in each aquarium to provide shelters/refugia for the stocked crayfish[43]. After three weeks, a block of joined polypropylene tubes containing five tubes (length 10 cm, inner diameter 35 mm) was added to each aquarium as an additional shelter for on-growing animals. The bases were represented by three longitudinally joined tubes with a further two tubes positioned pyramidal in the second layer[44]. Altogether, 12 tanks were used and subjected to stable indoor climatic conditions with natural photoperiod (12L:12D).

Growth trial and feed utilization parameters. Crayfish were fed twice a day to apparent satiation (roughly corresponding 5–6% of the body weight) with the abovementioned diets for nine weeks. Uneaten feed, feces, and other wastes were siphoned out manually every morning. Dissolved oxygen (7.9 ± 0.3 mg L^{-1}), pH (7.6 ± 0.2), and temperature (21.8 ± 0.3 °C) were measured daily using Oxi 3205 and pH 720 m (WTW GmbH, Weilheim, Germany), respectively. Every three weeks, the body weight was measured using an electronic balance (lowest sensitivity 1 mg) and the number of survivors counted. The feed rationing was revised accordingly. Body weight measurements were taken before feeding. After the trial, final body weight and total length were recorded, including the number of survivors. The animals were not fed before the day of the final measurement.

The food conversion ratio (FCR, units), protein efficiency ratio (PER, units), and survivability (%) were determined for each diet following the formulas in Cortes-Jacinto et al.[45]. Live weight gain (LWG) was calculated applying the formula, LWG = final—initial weight (in mg)/ days reared. Coefficient of variance (CV) of body weight (standard deviation × 100 ⁄ mean) was calculated as a measure of size heterogeneity. To eliminate statistical biasedness in the data due to hierarchical size distribution in crayfish groups, other measures of central dispersion like interquartile range (IR) and median were included besides the mean. The abovementioned parameters were calculated from the IR, median, and mean estimates of each treatment. All graphical models were generated using the ggplot2 package in R. Statistically significant differences (α level set at 0.05) in body weight, growth, and survivability of crayfish fed on different dietary treatments were tested. The grouped data were first subjected to a Shapiro–Wilk's normality test; then following the *p value*, either one-way ANOVA with *post-hoc* Tukey HSD (parametric test), or, Kruskal–Wallis *post-hoc* Dunn's test with Bonferroni correction (non-parametric test) was selected. The tests were performed using default commands in RStudio v1.2.5042.

Assessment of heavy metals risk from biofloc biomass. At the end of the experiment, tail muscle and hepatopancreas samples from representative crayfish of each group were collected and frozen (−20 °C). Selected heavy metals (Hg, Cd, Zn; following high bioaccumulation affinity realized in Kouba et al.[46]) and some additional minerals (Fe, Mn) were analyzed from these samples in the same accredited third-party laboratory. Body (muscle + hepatopancreas) heavy metal levels were compared with maximum permissible limits (Cd or Hg 0.5 mg kg^{-1} wet weight basis) given in the European Commission[47] for aquatic meat products (in the context of safety for consumption). In the context of agricultural use safety (as fertilizers), the heavy metal content of biofloc meal was determined and compared with Czech EPA limits (Cd 5 mg kg^{-1}, Hg 4 mg kg^{-1} dry matter basis) (Decree of Ministry of Environmental of the Czech Republic No. 437/2016 on the Code, 2016).

Ethics approval. All procedures performed in studies involving animals (*Oreochromis niloticus* and *Procambarus clarkii*) were in accordance with the ethical standards approved by the institutional ethics committee (Jihočeská univerzita v Českých Budějovicích Fakulta rybářství a ochrany vod).

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Author contributions

R.L. and K.R. (contributed equally to the work): Conceptualization, Methodology, Investigation, Data Curation, Formal analysis, Visualization, Writing—original draft. P.D.: Investigation, Resources, Project administration. A.K.: Conceptualization, Resources, Investigation, Funding Acquisition, Validation, Writing—review & editing. J.M.: Conceptualization, Resources, Supervision, Funding Acquisition, Validation, Writing—review & editing.

Competing interests

The authors declare no competing interests.

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CHAPTER 5

SUSTAINABLE PRODUCTION: NUTRIENT FOOTPRINT, ECOSYSTEM SERVICES OF FISH FARMING

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Nutrient footprint and ecosystem services of carp production in European fishponds in contrast to EU crop and livestock sectors

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ABSTRACT

There have been some arguments concerning supplementary feed (cereals) based common carp production in fishponds and water pollution, mostly in Central Europe. Using Czech Republic (top producer in EU) as a benchmark and combining data on nutrient digestibility of feedstuffs used combined with analyses of literature data, we have assessed – nutrient footprint (~9.4–10.8 kg N ha^{-1}, ~2.7–3.2 kg P ha^{-1}; 1.5–4 × < EU crop-livestock sectors); nutrient utilization efficiencies (NUE$_N$ ~36%, NUE$_P$ ~50%; 1.5–1.7 × > EU livestock average); autochthonous nutrient removal (~8–9.2 kg N ha^{-1}, 1.4–1.6 kg P ha^{-1}); eco-cost burden (13–29 × ≪ positive services); eco-services (~74.5–100.6 million € country^{-1}; ~2375 € ha^{-1}) of carp production in Central Eastern European Region (CEER). Digestible nutrients offered by natural prey (7.9% N, 1% P on dry matter basis) to carp are ~5–8 times higher than those provided by cereals and remains the key determinant for production. Despite this, 70–90% of nutrient footprint from feeding is contributed by cereals. Neutral footprint (~374 kg ha^{-1}) and exclusively natural (up to 300 kg ha^{-1}) carp production intensities were identified, following which, commercial interest of carp farming may falter (costing intangible losses >56.5 million € in CEER), despite achieving 'greener-goals'. Per production cycle, carp aquaculture in CEER fishponds offer at least 579 million € worth of services. Our results show that carp production in ponds have lesser nutrient burden than crop and livestock productions in EU. Existing management of fishponds 'barely meet' optimum P requirements of common carp and present production intensity should not be vilified as a pollution causing activity. Risks and solutions for achieving both environmental (minimized footprint) and aquaculture goals (uncompromised production) are discussed.

1. Introduction

For decades, the 'land-locked' central European countries have been relying mostly on carp culture for fisheries production (Adámek et al., 2012; Gál et al., 2015; Woynarovich et al., 2011). Common carp (*Cyprinus carpio* L.) farming in fishponds has remained the mainstay, both traditionally and commercially (Gál et al., 2015). About 80–88% of the aquaculture production in these countries come from carp farming in fishponds (Eurostat fish_aq2a 2017). Czech Republic followed by Poland, Hungary and Germany (ranked in order of production) support ~80% of carp production in the European Union (EU) (Eurostat fish_aq2a 2017). The apparent per capita consumption of carp in the region varies between 0.6 and 1.2 kg (EUMOFA, 2016). Since the late 1960s, carp farming in Europe has undergone intensification with yield <190 kg ha^{-1} to >450 kg ha^{-1} (Pechar, 2000). The higher stocking density corresponded higher input of supplementary feed. Today, about 86% of Czech fishponds involved in production are fed with supplementary feed, mostly cereals (CZ-Ryby, 2019). Present practices include semi-intensive farming with a low to moderate stocking density (0.2–0.4 ton ha^{-1}) and having a production ceiling of ~0.5–1 ton ha^{-1}, partly supported by supplementary feeding (Sterniša et al., 2017). In most of these fishponds, ~50–60% of carp growth (protein growth) is believed to be supported by natural food while cereals (rich source of energy) are provided as

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Abbreviations

FCR	Food conversion ratio (= dietary intake / biomass gain) used in relative sense (in the presence of other food component in fishponds *i.e.* natural food or cereals)
$FCR_{cereals}$	Relative FCR of cereals in the presence of carp's natural food in fishponds
$FCR_{natural\ prey}$	Relative FCR of carp's natural food in the presence of cereals as supplementary feed
CEER	Central Eastern European region
EU	European Union
NUE	Nutrient Utilization Efficiency
NUE_N	NUE of Nitrogen
NUE_P	NUE of Phosphorus
LCA	Life Cycle Assessment
GHG EI	Greenhouse gas Emission Intensity (kg CO_2-equivalent per kg consumable weight)

supplementary feed (Adámek et al., 2009, 2012). This co-feeding by carps on natural prey and cereals require at least two growing seasons to reach marketable table-sizes (>1.5–2 kg) under temperate conditions in Western and Central Europe (Gál et al., 2016; Pechar, 2000). Unlike Asia (e.g. Indian major carp production, up to 10–11 tons ha^{-1} $year^{-1}$ (ICAR, 2011)), the carp farming in Europe is occurring at far lesser intensity, with state and/or EU ratified environmental legislations in place (reviewed in O'Hagan et al., 2017).

Unlike Asian fishponds, fertilizing fishponds in Europe have already different levels of restrictions among different countries (Gál et al., 2015), e.g. prohibited in the Czech Republic. Most carp farmers therefore regard their pond sediment as the only fertilizer they need and are anxious not to flush it out (Knösche et al., 2000; Potužák et al., 2016), while some perform green manuring on dried pond beds and later filling them (Hartman et al., 2015). This narrows it down to a more regular practice *i.e.* supplementary feeding; probably the only major, 'deliberate' allochthonous nutrient source. The leading role played by feed and feeding efficiency on the environmental impact of any aquaculture practice is well recognized (Aubin et al., 2009; Henriksson et al., 2015; Papatryphon et al., 2004). Likewise, a great deal for nutrient loading from carp dominated systems depend on the choice and proportion of supplementary feed used (Biermann and Geist, 2019; Jahan et al., 2002, 2003; Watanabe et al., 1999). Common carp (*Cyprinus carpio*) can lose about 50–79% of N intake through metabolizable and faecal losses (Kaushik, 1995; Roy et al., 2019). Apart from its natural prey, carps lose quite a lot of dietary P (53–73% of dietary P intake) from most of the artificial feedstuffs (Hua and Bureau, 2010; Roy et al., 2019), including cereals. Half of the excreted P from carps was reported to be directly available for algal production (Lamarra, 1975), probably corresponding to the fractions of ortho-phosphate which is readily assimilated.

The present water directive of EU insists carp waters (waters for/from cyprinid culture) to maintain ≤0.4 mg L^{-1} PO_4 and ≤1 mg L^{-1} NH_4 (EU Directive, 2006/44/E Article 3 & 5, Annex I). There have been concerns surrounding the impacts of carp culture in fishponds on eutrophication of associated water bodies (reviewed in Roy et al., 2019). It has resulted in arguments and lobbying between environmentalists and carp farmers regarding fishpond-environment legislations (e.g. Czech Republic: Duras and Potužák, 2016, 2019, Duras, 2019; Germany and Hungary: Knösche et al., 2000; Poland: Kufel, 2012, Mazurkiewicz, 2009). Amidst these arguments, even the supplementary feeding gets tagged as a 'harmful substance' applied to fishponds (Duras and Potužák, 2019). Such stringent measures or presumptions restricting the intensity of carp farming in European fishponds, in order to reduce environmental footprint, have impacts on commercial viability too. The market prices of common carp have in fact come down significantly in most European countries (FAO Globefish, 2018; Gál et al., 2015). Present farm-gate prices of carp in the Czech Republic and Germany are ~2–2.5 € kg^{-1} live weight (EUMOFA, 2016; O'Hagan et al., 2017) or even lower (1.9 € kg^{-1} live weight) in Hungary (FAO Globefish, 2018). Although the concerns of environmentalists are in good faith, however, being too harsh on carp farming without 'clarified' knowledge is unfair.

In order that sustainable management strategies in aquaculture be based on environmental impact analyses, life cycle assessment (LCA) often is the first choice (Aubin et al., 2009; Mungkung et al., 2013; Philis et al., 2019). Albeit the advantages (Biermann and Geist, 2019), ambiguities in inventory creation, methodological incompleteness and limited comparability across production systems or studies exists (reviewed in Philis et al., 2019, Biermann and Geist). The supply chain of agriculture-livestock sector, for example cereals supply chain, is also important in achieving cleaner production goals. Novel approaches in supply chain assessment and inventory management already exists (Duan et al., 2018; Hoseini Shekarabi et al., 2019; Gharaei et al., 2019a,b,c,d). To the best of our knowledge, the environmental impact of carp farming has been subject to only three LCA case studies – Indonesian net cage system (Mungkung et al., 2013), Indian carp polyculture system (Aubin et al., 2011) and German fishponds (Biermann and Geist, 2019). These LCAs were more focused on 'percentage contribution' of various management parameters towards multiple threat categories (e.g. climate change, eutrophication, toxicity, energy use, etc.). Employing an alternative approach, we rather focused on quantifying the key parameters itself (*i.e.* primary nutrients, N and P) in the dominant pathway (feeding activity) of the core production stage (fishponds) driving a threat category (freshwater eutrophication). The LCA and supply chain concepts were beyond the scope of our present, already extensive exercise.

In our present attempt, we have assessed the primary environmental macronutrient (N and P) footprint of carp farming in Czech Republic. By the term 'footprint', we imply nutrients excreted (faecal and metabolic losses) into the aquatic environment by the carps. The aim is to have an objective assessment of eutrophication incriminated by carp farming in the region. The objectives were to assess – (a) nutrient footprint of carps feeding on supplementary feed (cereals) and natural prey in fishponds, employing different methodologies; (b) nutrient footprint of carp production in comparison to EU crop and livestock production; (c) nutrient utilization efficiencies by carps in fishponds and comparison with other EU food production sectors; (d) autochthonous nutrient removal by carps; (e) environmental cost burden worth of nutrient footprint in contrast to total ecosystem services offered by carp production in fishponds; (f) required production intensity in fishponds to neutralize nutrient footprint and its practicality; (g) trade-offs between good growth (optimum digestible nutrient supply) and reduced footprint. We have further extrapolated our findings onto the production scenarios of Germany, Hungary, Poland and Russian Federation to generate a comprehensive picture of the central-eastern European region (CEER) – a complimentary fit to existing assessments on EU crop-livestock sectors (Buckwell and Nadeu, 2016, Csatho et al., 2007, Gerber al. 2014, Kronvang et al., 2007, Leip et al., 2011, 2014, 2015, Richards and Dawson, 2008, Rosendorf et al., 2016, van Dijk et al., 2016, Velthof et al., 2007). The managerial implication of the present study is discussed at the end.

2. Materials and methods

2.1. Collection of baseline statistics for carp production

Carp production statistics (18460 tons from 41080 ha of fishponds; yield 449.4 kg ha^{-1}) was obtained from CZ-Ryby (2019). Relative feeding coefficient (*i.e.* relative food conversion ratio in the presence of natural food) of cereals supporting carp production in fishponds of the region have been estimated at 2–2.5 (Woynarovich et al., 2010, Jan Mraz, IAPW FROV Ceske Budejovice – *unpublished data*, Martin Oberle, LfL-Bayern Bavaria – *unpublished data*). Collating higher nutrient richness and digestibility of carp's natural prey over cereals (Table 1), natural food was found to be 6–8 times superior in terms of digestible nutrient supply per unit dry matter. Therefore, FCR of natural prey was back calculated from standardized FCR of cereals and estimated at 0.3–0.4. Here, the term 'FCR' implies food conversion ratio (= dietary intake / biomass gain) in relative sense. $FCR_{cereals}$ imply FCR of cereals in the presence of carp's natural food in fishponds. $FCR_{natural\ prey}$ imply FCR of carp's natural food in the presence of cereals as supplementary feed.

In the absence of supplementary feeding with cereals (*i.e.* exclusively natural production), the annual yield in temperate Czech fishponds (thermal cycle 6.9–26.8 °C; Řezníčková et al., 2016; Kopp et al., 2016) is around 250–300 kg ha^{-1} (Pechar, 2000; Duras and Dziaman, 2010, Mraz – *unpublished data*). In this case, absolute FCR of natural food was estimated at least ~0.7 to fulfill the optimum digestible nutrient supply for growing carps.

2.2. Assessment of nutrient availabilities from supplementary feed (cereals) and natural food

Apparent digestibility of N and P of commonly used cereals in Czech fishponds (wheat, corn, triticale) and carp's natural prey (daphnia, chironomid larvae, cyclops) were determined, following standard procedures (NRC, 2011; Glencross et al., 2007). Digestibility trials were conducted in a 12 tank Guelph system (6 control + 6 treatment; 120 L capacity each; Cho and Slinger, 1979) for facilitating passive collection of faeces from carps (*Cyprinus carpio*) weighing 150–475 g (mixed assortment of sizes; 6–7 kg carp biomass per tank). Trials were conducted under species optimum conditions: temperature 19–21 °C, dissolved oxygen >4 mg L^{-1}, pH 6.8–7.3 and unionized ammonia <0.05 mg L^{-1}. The procedures entailing experimental feed preparation, feeding, faeces collection and sample processing have been detailed in supplementary text. Apparent digestibility coefficients of N (ADC_N) and P (ADC_P), both diet and ingredient level, were calculated following the formula given in NRC (2011). All calculations were done on 100% dry matter basis. In total, the entire experiment lasted for 7 months.

2.3. Collection and use of reference metadata

From the online databases, literature metadata were compiled for the following categories: (a) N:P balances, NUEs of EU agriculture-livestock sectors (*data* from Buckwell and Nadeu, 2016, Csatho et al., 2007, Gerber al. 2014, Kronvang et al., 2007, Leip et al., 2011, 2014, 2015, Richards and Dawson, 2008, Rosendorf et al., 2016, van Dijk et al., 2016, Velthof et al., 2007); (b) cost of removing 1 kg N or P from wastewaters (freshwater origin) (*data* from Bashar et al., 2018; Huang et al., 2015; Mangi, 2016; Mackay et al., 2014; Molinos-Senante et al., 2011; Vinten et al., 2012); (c) valuation of regulatory eco-services by fishponds of CEER origin (meta-analysed by Frélichová et al., 2014; Czech Republic), and; (d) farm-gate prices of common carp, live-weight basis (EUMOFA, 2016; O'Hagan et al., 2017). All these metadata were used for further comparison or calculation (indicated below).

3. Calculation

3.1. N and P losses from carp's feeding in fishponds

N and P losses from carp's feeding in fishponds involved the following calculations in sequence: (a) total input of feed, dietary N and P; (b) estimating digestible, metabolic and total losses; (c) calculation of nutrient balances from diffused losses – approach A; (d) calculation of net nutrient balances from feed (cereals) losses – approach B; (e) calculation of net nutrient balances from cumulative losses – approach C, and; (f) representative footprint merging all approaches and comparison with other sectors. Considering the space limitations, these sub-chapters are explained in the supplementary text.

3.2. Nutrient utilization efficiency and comparison with other sectors

N and P retentions in carp were back calculated by assuming 2.88% N and 0.76% P content on whole body basis (Ramseyer, 2002; Roy et al., 2019, Mraz et al. *unpublished results*). These values were multiplied with harvested biomass of carp to estimate N and P harvested. Harvested values were subtracted from total dietary N or P (cereals and natural prey combined) and expressed in percentage (NUE_N, NUE_P). For comparison, we used published estimates on NUE_N, NUE_P from crop and livestock production sector(s) within EU region.

3.3. Autochthonous nutrient extraction by carps

There is inherent complexity in determining nutrients of autochthonous origin extracted by carps from fishponds (Potužák et al., 2016), especially in the presence allochthonous input like

Table 1
Results from the digestibility trials with common carp (data on dry matter basis).

Food	Crude N (%)	ADC_N (%)	Digestible N (g 100 g^{-1})	Crude P (%)	ADC_P (%)	Digestible P (g 100 g^{-1})
Corn	2.14	70.9	1.52	0.38	24	0.09
Triticale ↘	2.5	37.8	0.95	0.36	1	–
Wheat ↗	3.24	75.7	2.45	1	36	0.36
Average $_{cereals}$	**2.62**	**61.5**	**1.61**	**0.58**	**20.3**	**0.12**
Chironomid larvae ↗	8.46	91.9	7.77	0.99	99	0.98
Cyclops ↗	11.3	74.9	8.46	1.24	72.1	0.89
Daphnia ↗	8.95	80.5	7.2	1.34	72.2	0.97
Average $_{natural\ prey}$	**9.57**	**82.4**	**7.89**	**1.19**	**81.1**	**0.97**
Skretting® Carpe-F 3.5 mm™ (commercial carp feed)[a]	5.93	85.2	5.05	1.05	40.6	0.43

Intra-group comparison (cereals or natural prey): ↗ *Comparatively good;* ↘ *Comparatively poor.*
[a] Control diet. Results given for reference purpose. ADC = Apparent digestibility coefficient.

supplementary feeding. We attempted to grossly indicate the nutrients of autochthonous origin withdrawn by carps. The portion of retained nutrients from natural prey in carp body was grossly budgeted (in the absence of stable isotope approach). It was calculated by subtracting total losses of natural prey origin from total dietary intake (nutrient) of natural prey. The terms autochthonous and allochthonous refer to nutrients either originating from within the fishponds or introduced to the fishponds from outside, respectively.

3.4. Environmental cost burden and ecosystem services of carp production in fishponds

With the existing water treatment technologies, cost of removing 1 kg N or P from wastewaters (freshwater origin), were meta-analysed. The inter-quartile ranges of costs were 3–5 € kg^{-1} N removed and 19–35 € kg^{-1} P removed. These costs were multiplied with calculated nutrient footprint and regarded as environmental cost burden. Under ecosystem services offered, following aspects were summed up: (a) non-production or regulatory services offered by fishponds in Czech Republic (1257 € ha^{-1}); (b) commercial production services offered by fishponds (~2–2.5 € kg^{-1} live weight), and; (c) valuation of autochthonous nutrient removed (cost mentioned above). All valuations were made on 'per ha fishpond' basis.

3.5. Neutral-footprint carp production scenario

The required cereals-based production intensity in fishponds to neutralize existing footprint to 'near-zero' levels was coined as 'neutral footprint' production. For its mathematical derivation, median values between 'exclusively natural' and 'existing' production scenarios were calculated for certain variables, *i.e.* $FCR_{natural\ prey}$, $FCR_{cereals}$, yield (kg ha^{-1}), NUE_N and NUE_P. Nutrient balances from feeding within this 'median scenario' was calculated and validated for sub- or near-zero values.

3.6. Trade-offs between nutrient supply, good growth and reduced footprint

An exercise was done with different relevant combinations of cereals and natural prey ($FCR_{cereals}$ 0–4.3; $FCR_{natural\ prey}$ 0.1–0.7) covering 'exclusively natural' to 'completely cereals dominated' production scenarios. Digestible N and P (g kg^{-1} fed basis) from cereals and natural prey were multiplied with their respective FCRs and summed up for total diet. NRC (2011) recommendations on optimum digestible nutrient requirement of common carp were used as baseline, *i.e.* 49.6 g digestible N kg^{-1} of diet and 7 g digestible P kg^{-1} of diet. The instances of FCR combinations which successfully 'hit the target' (*i.e.* fulfilled baseline) were demarcated from the ones that failed. Multiple linear regression models were generated to aid such budgeting.

Similar exercise was repeated with footprint (faecal losses in g kg^{-1} diet basis) from cereals and natural prey under different FCR combinations (same range as above). Complimentary contribution curves of faecal footprint under different FCR combinations were plotted in ggplot2 using linear fitting (Wickham, 2016; R Development Core Team, 2015). The FCRs at the intersection was designated as trade-off point to reduce faecal footprint without deviating from optimum digestible nutrient supply. By the term 'trade-off', we imply a balanced compromise where we accept some degree of disadvantage (reduced footprint) to retain a benefit (uninterrupted production), which otherwise are two incompatible features.

3.7. Data application in Central and Eastern European Region (CEER) production scenario

Values obtained on Czech carp production were upscaled and applied for Germany, Hungary, Poland and Russian Federation to derive figures representing Central and Eastern European Region (CEER). The strategy is detailed in supplementary text. In addition to the text above, infographics on the methodological framework are provided in Supplementary Figs. S4–S5 for better clarity.

4. Results

4.1. Nutrient availabilities from cereals and natural prey

On dry matter basis, the average N and P contents in cereals commonly used in Czech fishponds (corn, triticale, wheat) is 2.62% and 0.58%, respectively. Carp's natural prey (chironomid larvae, cyclops, daphnia) have much higher N (9.57%) and P (1.19%) contents. Apparent digestibility of N in cereals and natural prey were 61.5% and 82.4%, respectively. Natural prey-N is therefore ~1.3 times more digestible than cereal-N. Likewise, apparent digestibility of natural prey-P (81.1%) is ~4 times superior to cereal-P which is only 20.3% digestible. The digestible nutrients offered by natural prey (N: 7.89 g 100 g^{-1}; P: 0.97 g 100 g^{-1}) are ~5–8 times higher ($p < 0.05$) than cereals (N: 1.61 g 100 g^{-1}; P: 0.12 g 100 g^{-1}). Detailed results are summarized in Table 1.

4.2. N and P losses from carp's feeding in fishponds

4.2.1. Cereals

It was estimated about 36920–46150 tons of cereals (~967.3–1209.1 tons N, 214.1–267.7 tons P) supported carp production in Czech fishponds (Table 2). Combining the global metadata and our digestibility results, the N and P digestibility of cereals usually range between 61.5–71% and 20.3–25% respectively. It implies 29–38.5% of cereal-N and 75–79.7% of cereal-P are not digested by carps. Considering the metabolic N losses through gills and urine, another 17–30% of N intake is lost. Faecal and metabolic losses from feeding on cereals was estimated at 24.1–44.9 kg N and 8.7–11.6 kg P ton^{-1} of carp produced or, 10.8–20.2 kg N and 3.9–5.2 kg P ha^{-1} fishpond. The N:P ratio of cereals derived losses is ~3:1–4:1 (Table 2).

4.2.2. Natural prey

About 5538–7384 tons of natural prey dry matter (~530–706.6 tons N, 65.9–87.9 tons P) was supposedly consumed by the carp production in Czech fishponds (Table 2). Due to lack of pre-existing data on N and P digestibility of natural prey, only results obtained from our digestibility trials were used. About 17.6% of natural prey-N and 18.9% of natural prey-P are not digested by carps. Another 17–30% of N intake is lost as metabolic losses. Carp's digestive losses from grazing on natural prey was estimated at 9.9–18.2 kg N and 0.7–0.9 kg P ton carp $produced^{-1}$ or, 4.5–8.2 kg N and 0.3–0.4 kg P ha^{-1} fishpond. The N:P ratio of natural prey derived losses is ~14:1–20:1 (Table 2). Compared to cereals, the losses of natural prey origin are far less and with better N:P ratio. If the sum of losses from cereals and natural prey is considered, cereals has the major share of total footprint (>70% of N and >90% of P footprint).

4.3. Nutrient footprint through the production cycle and comparison with other sectors

Using multiple approaches, the nutrient balance from carp's feeding activity in fishponds were calculated (Table 2). The spatial footprint (footprint expressed per unit farmed area) of common

Table 2
Nutrient footprint from natural and supplementary feeding supporting 18460 tons of common carp production from 41080 ha of fishponds (yield 449.4 kg ha^{-1}) in Czech Republic.

Cereals (FCR 2–2.5)		Natural food (FCR 0.3–0.4)	
Dietary input			
Requirement: 36920–46150 tons (dry matter)		Requirement: 5538–7384 tons (dry matter)	
Avg. N: 2.62% and P: 0.58% (dry matter)		Avg. N: 9.57% and P: 1.19% (dry matter)	
52.4–65.5 kg N ton $carp^{-1}$		28.7–38.3 kg N ton $carp^{-1}$	
23.5–29.4 kg N ha^{-1} fishpond		12.9–17.2 kg N ha^{-1} fishpond	
11.6–14.5 kg P ton $carp^{-1}$		3.6–4.8 kg P ton $carp^{-1}$	
5.2–6.5 kg P ha^{-1} fishpond		1.6–2.1 kg P ha^{-1} fishpond	
Faecal and metabolic losses			
Faecal losses: 29–38.5% N; 75–79.7% P		Faecal losses: 17.6% N; 18.9% P	
Metabolic losses: 17–30% of N intake		Metabolic losses: 17–30% of N intake	
24.1–44.9 kg N ton $carp^{-1}$		9.9–18.2 kg N ton carp $produced^{-1}$	
10.8–20.2 kg N ha^{-1} fishpond		4.5–8.2 kg N ha^{-1} fishpond	
8.7–11.6 kg P ton $carp^{-1}$		0.7–0.9 kg P ton carp $produced^{-1}$	
3.9–5.2 kg P ha^{-1} fishpond		0.3–0.4 kg P ha^{-1} fishpond	
N:P ~3:1–4:1		N:P ~14:1–20:1	
Spatial footprint on environment (per ha fishpond)			
Approach A (diffused)	**Approach B (allochthonous)**	**Approach C (cumulative)**	**Representative footprint (merged)**
7.6–14.2 kg N ha^{-1}	2.4–11.2 kg N ha^{-1}	6.9–19.3 kg N ha^{-1}	7.08–13.45 kg N ha^{-1}
2.1–2.8 kg P ha^{-1}	2.6–3.5 kg P ha^{-1}	2.9–3.9 kg P ha^{-1}	2.65–3.35 kg P ha^{-1}

carp production in Czech fishponds was estimated at 7.08–13.45 kg N and 2.65–3.35 kg P ha^{-1} (equivalent to 15.8–29.9 kg N and 5.9–7.5 kg P ton^{-1} of carp produced). In terms of N footprint, carp production in European fishponds appear ~4–6 times less burdening than other food production sectors. Regarding P, carp production is ~1.5–2.4 times less burdening than other sectors (Fig. 1a and b).

4.4. Nutrient utilization efficiency and comparison with other sectors

Comparing the total nutrient input (cereals + natural prey) with output through harvested carp biomass (12.9 kg N and 3.4 kg P ha^{-1} fishpond), NUE_N in fishponds was estimated at 27.7–35.4% and NUE_P at 39.1–50% of dietary intakes. In case of completely natural carp production (input from natural prey: ~20.7 kg N and ~2.6 kg P ha^{-1} fishpond; output carp biomass: ~8.6 kg N and ~2.3 kg P ha^{-1} fishpond), the NUE_N and NUE_P are ~41.5% and ~88% respectively. A marked improvement in NUE_P is evident. Inter-sectoral comparison of NUEs, with cereal-fed (present regime), fully natural and neutral footprint production scenarios are depicted in Fig. 2a, b.

4.5. Autochthonous nutrient extraction by carps

Under the present production regime, about 18.8–20.1 kg N and 2.9–3.9 kg P of autochthonous origin (*i.e.* from live prey) is withdrawn per ton of carp produced. It is equivalent to 8.4–9 kg N and 1.3–1.7 kg P ha^{-1} of fishponds. It should be noted that despite this nutrient removal, the above-mentioned nutrient footprint is a spin-off product of the production cycle. Hence, it should not be double subtracted while comparing. If the production scenario is assumed 'exclusively natural', autochthonous nutrient removal is ~19.2 kg N and ~5.1 kg P ton^{-1} of carp produced, or, ~8.6 kg N and ~2.3 kg P ha^{-1} of fishponds. In this case no nutrient footprint occurs, and the autochthonous nutrients removed by carps contributes to positive ecosystem service. The present cereal-based production regime seems only ~2.2 times or ~1.5 times less efficient in terms of autochthonous N and P removal respectively, compared to natural production.

4.6. Environmental cost burden and ecosystem services of carp production in fishponds

The environmental cost burden, under the present production regime, was estimated at ~72–184 € ha^{-1}. Whereas, ecosystem services offered by carp production and fishponds amount to ~2206–2485 € ha^{-1}. It is obvious that environmental cost burden ≪ ecosystem services. Environmental cost burden of carp production amounts to <10% of its positive services to the environment and commerce combined. Present carp production regime is already inclined towards positive ecosystem services with 'net worth' of 2134–2300 € ha^{-1}. Under completely natural carp production, with zero environmental cost burden, the service amounts to ~1926–2130 € ha^{-1}. It is apparent that cereals-based carp production delivers ~8–10% higher services than completely natural production. This difference is driven by saleable amount of carp from fishponds, realized by the application of cereals. A comparative and self-explanatory account has been depicted in Fig. 3 and Fig. S7 respectively.

4.7. Assessment of neutral footprint carp production scenario

Neutralizing existing footprint to negligible levels might require $FCR_{cereals}$ 1–1.3 and $FCR_{natural\ prey}$: 0.5–0.6 with a yield limitation of 374.7 kg ha^{-1}. In this scenario, NUE_N and NUE_P is expected to be in the range of 34.6–38.5% and 63.6–69% respectively. The nutrient footprint under such circumstances is estimated to be −0.8 (removal) to 2.4 kg N ha^{-1} fishpond and 0.2 (negligible) to 0.5 kg P ha^{-1} fishpond. Although theoretically proposed, some application bottlenecks might render its practicality questionable (clarified later).

4.8. Trade-offs between nutrient supply, good growth and reduced footprint

4.8.1. Digestible nutrient supply

Digestible N requirement is easily met under semi-intensive rearing conditions. However, meeting the digestible P demand remains a concern under low natural prey availability – might be even inadequate (red zones; Table 3). Increasing supplementary feed inputs (cereals, from FCR 2 to 2.5) under low support from

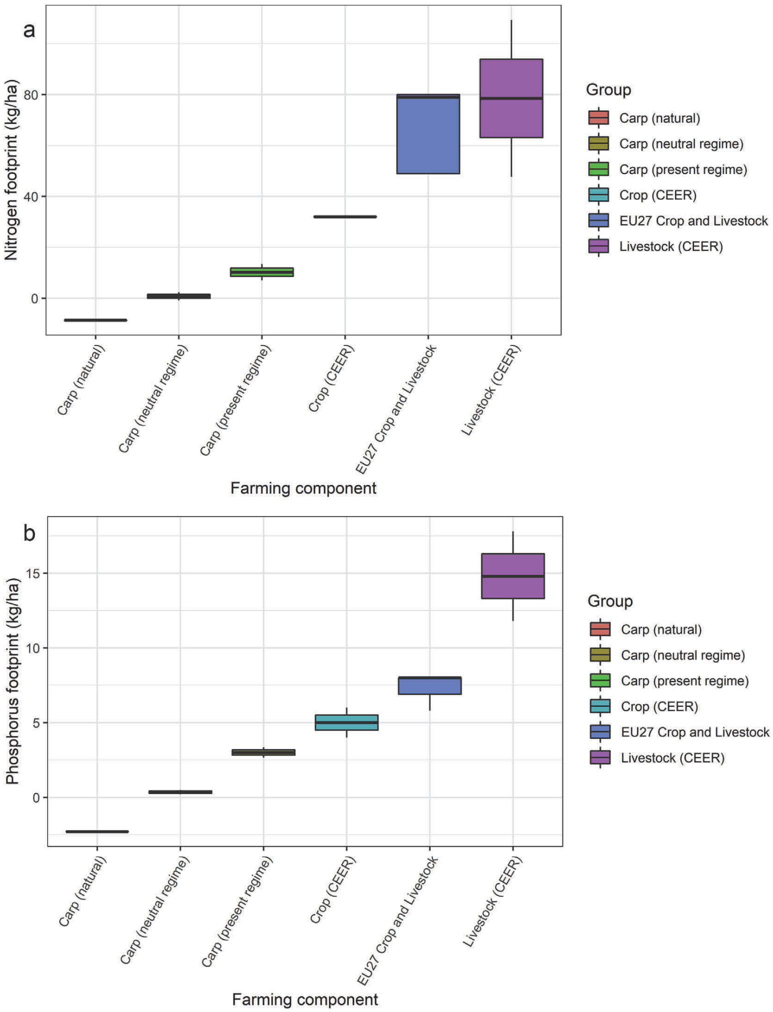

Fig 1. (a, b): Spatial nitrogen (a) and phosphorus (b) footprints of different farming sectors within EU or Central Eastern European Region (CEER). Data from Buckwell and Nadeu (2016), van Dijk et al. (2016), Rosendorf et al. (2016), Leip et al. (2015), Richards and Dawson (2008), Csathó et al. (2007), Kronvang et al. (2007), Velthof et al. (2007) and present study. Carp production in fishponds, in general, have the least nutrient burdens to environment than any other food production sector in Europe. Nutrient footprint below zero indicates nutrient removal from fishpond ecosystem.

natural prey ($FCR_{natural\ prey}$: $\leq$0.3) does not necessarily help. The nutritionally fulfilling combinations of relative FCRs have been identified as 'green zones' in Table 3. To reduce the use of cereals (by −15% to −25%) in fishponds, the minimum support from natural prey must be pushed by +0.1 units (or, +25%), *i.e.* $FCR_{natural\ prey}$ should be $\geq$ 0.4 for supporting carp production (modified scenario; Table 3). Although this 25% (+0.1 FCR) increase of dependency on natural prey appears theoretically promising, it is difficult practically (discussed below). Multiple linear models for calibrating digestible nutrient supply in fishponds have been generated (Table 3).

4.8.2. Footprint of fecal origin (excluding uneaten feed)

Faecal nutrient losses progressively increase with relative increase in $FCR_{cereals}$ while decrease with relative increase in $FCR_{natural\ prey}$ (Fig. 4). It means higher dependency on cereals has

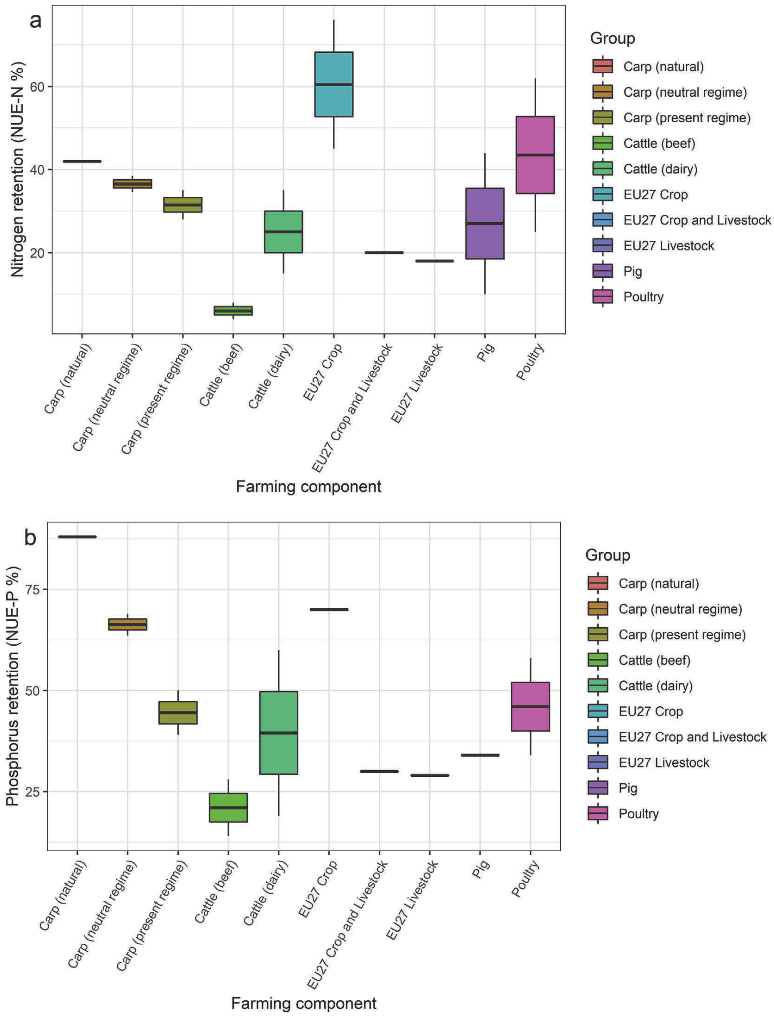

Fig 2. (a, b): Animal or plant level nutrient utilization efficiencies for nitrogen (a) and phosphorus (b) of different farming components within EU. Data from Buckwell and Nadeu (2016), Gerber et al. (2014), Leip et al. (2011) and present study. In terms of NUE_N and NUE_P, common carp is superior than EU27 livestock or EU27 crop and livestock average but inferior to EU27 crop sector average.

inevitable consequences on magnification of nutrient footprint; indicated by the red line in Fig. 4a, b. Increased reliance on natural food have positive environmental consequences; blue line in Fig. 4a, b. The trade-off FCRs for minimizing footprint and yet supplying optimum digestible nutrient were identified at $FCR_{cereals} \leq 2.2$ and $FCR_{natural\ prey} \geq 0.35$ (Fig. 4). Compliance to these relative FCR recommendations may result in ~10% reduction in existing footprint without compromising growth (digestible nutrient supply) or production (discussed below).

4.9. Central and Eastern European Region (CEER) carp production scenario

Data on nutrient footprint, nutrient removal, eco-cost burden and eco-services of carp production in Europe are provided in Table 4. The profile is based on five major European producers of common carp (Czech Republic, Poland, Hungary, Germany and Russian Federation) producing >72% of the total carp in Europe. The yield, nutrient footprint and removal, eco-burden and services are

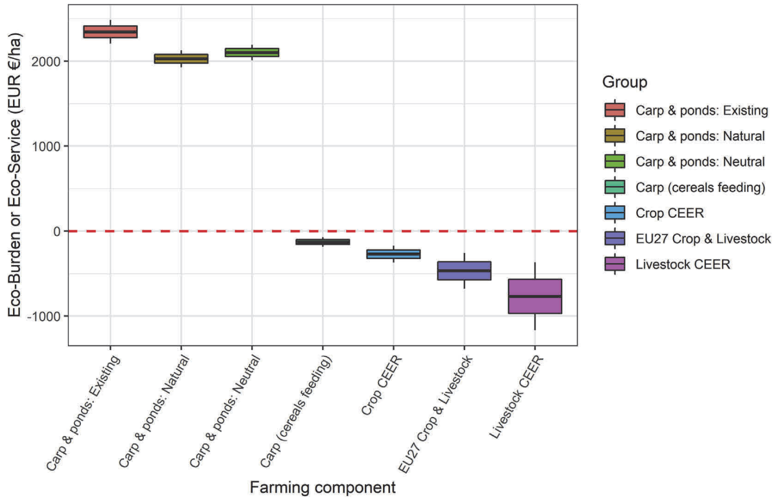

Fig. 3. Comparative account of ecosystem services (*above red line*) and environmental cost burden (*below red line*): Carp production in fishponds has far greater positive services compared to miniscule negative effect of supplementary feeding through cereals. Crop and livestock sectors in EU or CEER (Central Eastern European Region) have greater environmental cost burdens than carp farming. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

comparable among Czech Republic, Germany, Hungary and Poland ($p > 0.05$); Germany being slightly on a lower side than others. Russian Federation has significantly higher figures in all aspects ($p < 0.05$).

With a yield of 488.8 kg carp ha^{-1}, the N and P footprint from CEER is currently estimated at ~7.7–14.6 kg N and ~2.9–3.7 kg P ha^{-1} respectively. This amounts to ~19.7–50.9 million € of eco-cost burden in the region. The autochthonous N (8–9.2 kg ha^{-1}) and P (1.4–1.6 kg ha^{-1}) bioremediated by carps from fishponds in CEER, coupled with production value and regulatory services of fishponds is worth ~578.9–656.2 million € on regional scale (Table 4). The European country level averages of spatial footprint are ~9.4–10.8 kg N and ~2.7–3.2 kg P ha^{-1} with an average eco-cost burden of ~3.5–5.3 million €. The autochthonous nutrient

Table 3
Digestible nutrient supply (g kg^{-1} diet) from cereals (supplementary feed) and natural prey under different FCR (relative feeding coefficient) combinations for optimum carp growth in fishponds.

FCR (cereals)	FCR (natural prey): 0.7[d] N	0.7[d] P	0.6[c] N	0.6[c] P	0.5[b, c] N	0.5[b, c] P	0.4[a, b] N	0.4[a, b] P	0.3[a, b] N	0.3[a, b] P	0.2[e] N	0.2[e] P	0.1[e] N	0.1[e] P
0[d]	55.2	6.8												
1[c]			64.7	7.1	56.8	6.1								
1.3[c]			69.9	7.5	62	6.5								
1.5[b]					65.5	6.8	57.6	5.8	49.7	4.9				
1.7[b]					68.9	7.1	61.1	6.1	53.2	5.1				
2[a]							66.3	6.5	58.4	5.5				
2.5[a]							74.9	7.1	67.1	6.2				
3.5[e]											76.5	6.5		
4.3[e]													82.5	6.6

Good	Bad	**Recommended supply** (NRC 2011) ~49.6 g digestible N and ~7 g digestible P kg^{-1} diet.

Multiple linear regression models

Digestible N supply = 0.11 + 17.33*FCR$_{cereals}$ + 78.72*FCR$_{natural\ prey}$ (*Adj. R^2 0.99, $p<0.01$*)

Digestible P supply = −0.01 + 1.3*FCR$_{cereals}$ + 9.67*FCR$_{natural\ prey}$ (*Adj. R^2 0.99, $p<0.01$*)

[a] Present production regime.
[b] Modified production scenario. Reduced supplementary feed and increased reliance on natural food.
[c] Neutral footprint carp production scenario.
[d] Completely natural production scenario. Exclusive reliance on natural food.
[e] Almost complete reliance on supplementary feed (cereals) for production. Low natural food.

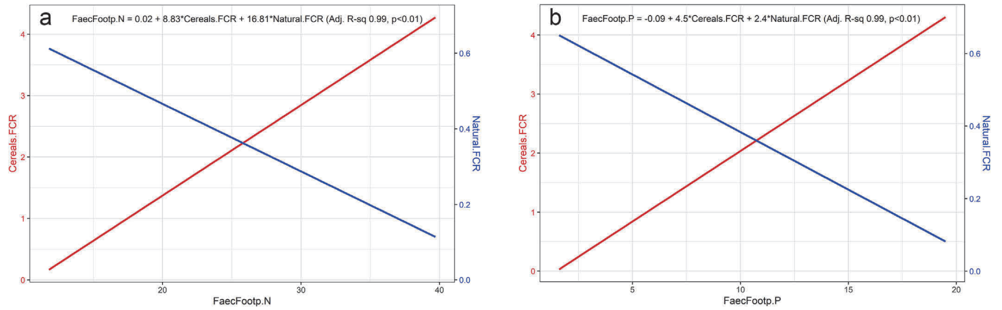

Fig 4. (a, b): Complimentary footprint (faecal) curve under relative proportions of cereals and natural food in fishponds. Point of inter-section denote trade-off FCRs (cereals ≤2.2 and natural prey ≥0.35) to reduce faecal nutrient losses in fishponds (FaecFootp.N, FaecFootp.P; in g kg^{-1} diet) without compromising optimum digestible nutrient supply for good growth. Red line and blue line correspond relative feed efficiency of cereals (supplementary feed) and natural prey, respectively. Nutrient footprint from feeding increases with relative increase in cereals input and relative decrease in natural food availability.

removal (average 9.2–9.8 kg N and 1.4–1.9 kg P ha^{-1}), coupled with production value (average ~1042.9 € ha^{-1}) and regulatory services by fishponds (~1257 € ha^{-1}) is worth ~74.5–100.6 million € on national scale (Table 4). The positive services of carp farming in European fishponds is many folds higher (~13–29 times) than any cost burden through nutrient footprint (Figs. 5 and 6).

5. Discussion

5.1. Nutrient availabilities from cereals and natural prey

The apparent protein (*i.e.* N) digestibility of various cereals by common carp have been well studied over last six decades (Roy et al., 2019), whereas data are sparse as regards to the availability of P. The existing studies have been listed in supplementary text. From the global metadata (Roy et al., 2019), the inter-quartile range (IR) of N digestibility for corn and wheat is 74–80% and 62–92% respectively. No data on P digestibility of corn and wheat for common carp was encountered in the reviewed literature (Roy et al., 2019). The present results are possibly the first ones. To the best of our knowledge, N and P digestibility of triticale (a hybrid between corn and wheat) by *Cyprinus carpio* is reported here for the first time. Generally, 71–93% of cereals-N and 25–57% (IRs) of cereals-P are digested by common carp (Roy et al., 2019). Our digestibility results agree with this general range but near the lower end of IRs (*see* supplementary text). The reason behind the poor P digestibility is predominantly phytate bound P fractions in cereals that are indigestible by carps (Hua and Bureau, 2010). N digestibility of cereals is moderate to good in nature, depending on their amino acid (AA) profile. Deficiencies in certain AAs render lower N digestibility (Kaushik, 1995; Nwanna et al., 2012; Schwarz et al., 1998).

To the best of our knowledge (Roy et al., 2019), digestibility of natural preys (chironomid larvae, cyclops and daphnia) by *C. carpio* are reported here for the first time. No prior data existed on digestible N and P supply, although their superior nutrient contents have been discussed before (Bogut et al., 2007; Steffens, 1986). Here, we have observed ~5–8 times higher digestible N, P supply from natural prey than cereals.

5.2. N and P losses from carp's feeding in fishponds

Within Europe, especially from the Central region, only a handful of 'published' estimates on carp fishpond nutrient balances exists: e.g. Austria (Kainz, 1985), Czech Republic (Duras et al., 2018; Potužák et al., 2016; Prikryl, 1983), Germany (Knösche et al., 2000) and Hungary (Gál et al., 2016; Knösche et al., 2000; Oláh et al., 1994). From these studies it could be summarized that: (a) average balance of N is ~23 kg ha^{-1} or ~24 kg ton^{-1} of carp produced; (b) maximum balance of P is ~6.7 kg ha^{-1} or ~2.7 kg ton^{-1} of

Table 4
Environmental footprint and bio-remediation services of carp production in Europe. Profile based on major European producers of common carp.

Country/Region [a]	Yield (kg ha^{-1})	Footprint N (kg ha^{-1})	Footprint P (kg ha^{-1})	N removed (kg ha^{-1})	P removed (kg ha^{-1})	Eco-burden (million €) [b]	Eco-service (million €) [b]
Czech Republic	449.4	7.1–13.4	2.7–3.4	8.4–9	1.3–1.8	2.9–7.6	90.6–102.2
Germany	250	4–7.5	1.5–1.9	4.7–5	0.7–1	1.6–4.1	71.4–77.7
Hungary	470.8	7.4–14.1	2.8–3.5	8.9–9.5	1.4–1.8	2–5	58.5–66.2
Poland	410	6.5–12.3	2.4–3.1	7.7–8.2	1.2–1.6	2.9–7.5	94.9–106.3
Russia	638.8	10.1–19.1	3.8–4.8	12–12.8	1.9–2.5	10.3–26.6	263.5–303.9
Central Eastern European Region [c]	488.8	7.7–14.6	2.9–3.7	9.2–9.8	1.4–1.9	19.7–50.9	578.9–656.2
Country average [d] (European)		9.4–10.8	2.7–3.2	8–9.2	1.4–1.6	3.5–5.3	74.5–100.6

[a] *Carp Production/carp fishpond area* (as of 2017; in parenthesis): Czech Republic (18460 tons/41080 ha), Germany (10000 tons/40000 ha), Hungary (12240 tons/26000 ha), Poland (18325 tons/44700 ha) and Russia (64587 tons/101100 ha).
[b] Eco-burden: cost burden due to nutrient footprint. Eco-service: regulatory services of fishponds, autochthonous nutrients bioremediated by carp, farm-gate sale value of harvested carps. All values in million € – on national scale.
[c] Derived from total carp production (123612 tons) and total carp pond area (252880 ha) in the region (sum of countries).
[d] Inter-quartile range of medians. Median value derived from the minima-maxima span of top five common carp producing countries in Europe.

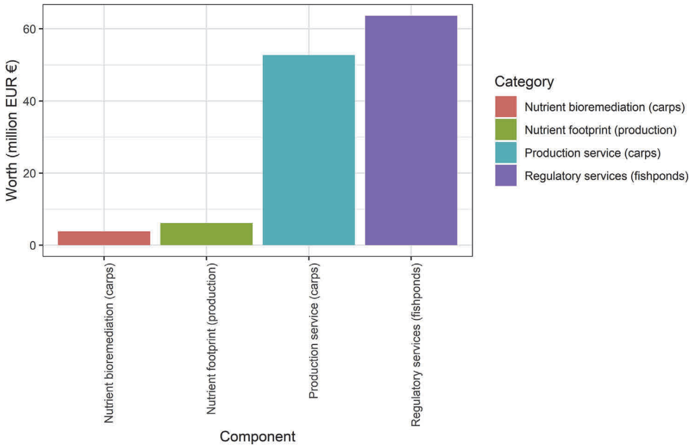

Fig. 5. Breakdown (million €) of different eco-services associated with carp production in fishponds on national scale. Figure depicts national scale average from top 5 producers in Europe (Russian Federation, Czech Republic, Poland, Hungary and Germany; contributing >70% production in Europe). Per hectare averages of top producers: Nutrient bio-remediated by carp worth ~75.3 € ha^{-1}, nutrient footprint of production (*negative service*) worth ~120.8 € ha^{-1}, production value of harvested biomass worth ~1042.9 € ha^{-1} and regulatory ecosystem services by fishponds worth ~1257 € ha^{-1}.

carp produced; and, (c) fishponds have special benefits of acting as a sink for P, trapping ~0.5–78 kg P ha^{-1} (average ~34 kg P ha^{-1}). Although most of them emphasized the non-polluting nature of carp production in fishponds through mass balance approach, no attempt pin-pointed the nutrients left behind by the growing carps through their feeding activity per production cycle. The most dynamic fluctuation of nutrients in fishponds is perhaps through the type and quantity of food consumed (Biermann and Geist, 2019; Knösche et al., 2000; Pechar, 2000; Watanabe et al., 1999). N or P balance of fishponds beyond carp's excretory losses from feeding

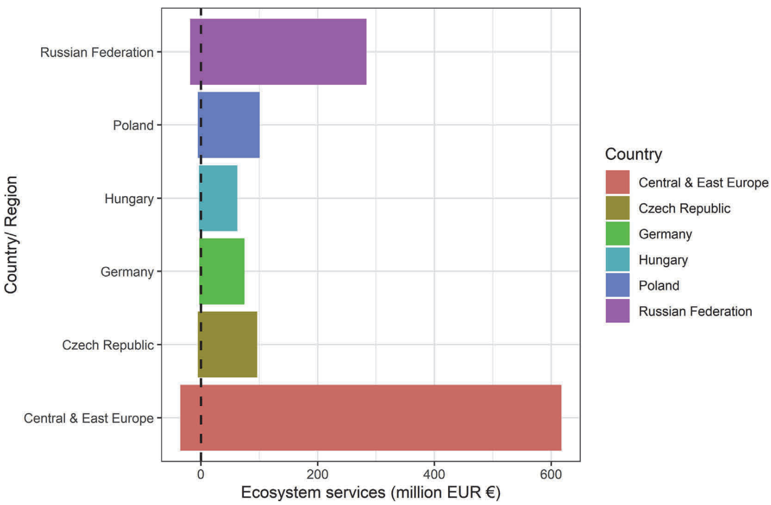

Fig. 6. Worth of positive (right of dotted line) and negative (left) ecosystem services from carp production in fishponds on national/regional scale. On country scales, Czech Republic and Poland almost have 100 million € of total services. *Scale for comparison*: total budget of EU spent on aquaculture during 2000–2014 amounts to 1170 million € (Guillen et al., 2019), 50% of which appears to be intangibly paid back by carp production alone in CEER fishponds per production cycle. Assuming 5 carp production cycles during 2000–2014, carp aquaculture 'alone' might have intangibly paid back ~2.9 billion € which is 2.5 times over the invested budget.

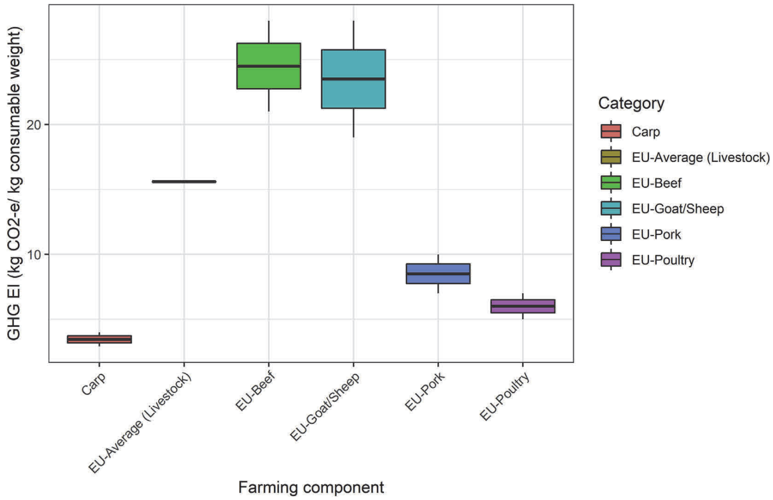

Fig. 7. GHG EI (kg CO_2-equivalent per kg consumable weight) of European livestock produce in comparison with farmed carp. Maximum GHG EI of carp production is ~4 times less than the average GHG EI of livestock sector (big/small ruminants, poultry). Carp farming in fishponds is cleaner than most terrestrial animal farming. Carbon emission of EU/CEER carp production was recalculated from dataset in MacLeod et al. (2019), then corrected with slaughter yield range for common carp (Prchal et al., 2018) to arrive at carp level GHG EI values. For inter-sectoral comparison, data were taken from Weiss and Leip (2012).

on natural prey and cereals (*i.e.* beyond our estimated footprint), might have been the nutrients received through inflow water or catchment fertilization. The present work highlights this overlooked interference in most fishpond nutrient budgeting results.

Potužák et al. (2016) earlier validated the results derived through the traditional methodology *i.e.* mass balance equations between input and output of fishponds. They demonstrated 'mass balanced' results when validated under practical conditions seldom make any sense. Alternative nutrient budgeting methods more appropriate for Central European fishponds were proposed (Hejzlar et al., 2006; Potužák et al., 2016). Our results, if compared with the 'mass-balanced' results, appears to be on a conservative side; probably more realistic. Interestingly, our results are in close agreement with an independent LCA by Biermann and Geist (2019) on conventional and organic carp farming in Germany. The footprint from carp and feed combined was estimated ~10.5–50.5 kg N and 5.7–6.3 kg P ton^{-1} of carp produced (recalculated from Biermann and Geist, 2019); reinforcing our findings.

5.3. Nutrient footprint through the production cycle and comparison with other sectors

The EU crop and livestock (terrestrial) production sectors, together, have spatial footprints in the range of 32–80 kg N and 4–8 kg P ha^{-1} farming area (Buckwell and Nadeu, 2016; Csatho et al., 2007; Kronvang et al., 2007; Leip et al., 2015; Richards and Dawson, 2008; Rosendorf et al., 2016; van Dijk et al., 2016, Velthof et al., 2007). Hence, the spatial footprint of European agriculture and livestock production is at least 1.5 times (for P) to 4 times (for N) higher than fishpond-based, cereals-fed carp production (European average: 9.9–11.4 kg N ha^{-1}; 2.2–2.6 kg P ha^{-1}). Linking the estimated footprint with existing observations on nutrient trapping by fishponds (e.g. outflow water-P < inflow water-P; Gál et al., 2016; Knösche et al., 2000; Potužák et al., 2016; Všetičková et al., 2012), we suspect the quantified footprint might not always end-up enriching downstream waters. Long term water-residence period is known to precipitate P into fishpond sediments (Hejzlar et al., 2006; Potužák et al., 2016), only a part of which is released during harvesting through sludge (Duras et al., 2018; Knösche et al., 2000; Potužák et al., 2016). It can be avoided, provided careful harvesting measures are adopted (Knösche et al., 2000; Potužák et al., 2016).

We have further hinted a neutral footprint production intensity in fishponds, following which, the commercial interests of 'profitable' carp production may falter – despite fulfilling 'greener-goals'. Downscaling the existing production to 'neutral' or 'natural' modes may reduce earning by at least –170 € ha^{-1} or –223 € ha^{-1} respectively. This view, from environmentalist's perspective, is a traditional argument 'sold' by the producers. Present production regime, with 'still intact' commercial interests, is close to the neutral footprint zone (Fig. 1a and b). However, compliance to the trade-off FCRs (discussed below) and better pond management practices (listed in supplementary text; Woynarovich et al., 2011) is recommended. Present supplementary feeding provisions in fishponds for supporting production should not be incriminated as an anthropogenic driver of eutrophication.

Beyond eutrophication, two additional analyses on greenhouse gas emission (e.g. CO_2-equivalent and CH_4) are presented for additional clarity: (a) carbon emission from European carp production in contrast to EU livestock sectors (illustrated in Fig. 7), and; (b) methane emission from Czech fishponds in contrast to Asian carp ponds, Czech agricultural farms and livestock units (Fig. S6). The greenhouse gas emission intensity (GHG EI) of EU livestock products (range 5–28 kg CO_2-e, average 15.6 kg CO_2-e kg^{-1} consumable weight) appear much higher than farmed carp (2.9–4 kg CO_2-e kg^{-1} consumable weight) (Fig. 7). Overall, the results reinforce European carp farming in fishponds as relatively 'cleaner' way of production than other food production sectors.

5.4. Nutrient utilization efficiency (NUE) and comparison with other sectors

Under controlled conditions and with good quality protein diet, common carp may retain up to ~50% of dietary N intake (Kaushik, 1980, 1995; Roy et al., 2019). Metabolic losses (as soluble NH_4–N), predominantly through branchial pathway and little through urine, are the major N losses in carps (Kaushik, 1980). In Czech fishponds, carps feeding on natural prey and cereals overall have mediocre NUE_N (up to 36% of dietary N intake). This might be attributed to endogenous obligatory losses (NRC, 2011) to meet energy expenditure, especially during survival through the ice-covered winter months (90–120 days), in the absence of adequate food. This is a situation unlike experimental or indoor aquaculture systems where optimum temperature is maintained with uninterrupted food supply. Carps even suspended feeding in our indoor systems when water temperature dropped below 13 °C. Concerning P, suspended losses through faeces remains the most dominant pathway (Kaushik, 1995; Roy et al., 2019). Present estimates indicate ~50% of dietary P intake are likely retained by the carps in Czech fishponds; little better than NUE_N. Carps excrete more P in already high P environment (Chumchal and Drenner, 2004); a phenomenon which might coincide with spring thawing (and blooming) of fishponds. During late spring to summer, Czech fishponds are known to release the highest amount of P from sediments due to internal loading (Pokorný and Hauser, 2002; Vystavna et al., 2017).

The EU livestock sector (dairy cattle, beef cattle, pigs, poultry) has animal level NUE_N and NUE_P in the range of 4–62% (average 18%) and 14–60% (average 29%) respectively (Buckwell and Nadeu, 2016; Gerber et al., 2014; Leip et al., 2011). Hence, the average NUEs of EU livestock sector appears 1.5–1.7 times inferior than cereals based common carp production in European fishponds. Plant level NUE_N and NUE_P in the EU crop sector is 45–76% and 70% respectively (Buckwell and Nadeu, 2016); superior to both livestock and carp production. With increasing reliance on natural prey and decreasing production intensity (existing → neutral footprint → natural regime), a progressive improvement in NUE_P has been predicted. In fact, the achievable NUE_P for common carp under neutral or natural production regime are comparable or superior than the maximum NUE_P of crop and livestock sectors (Fig. 2a and b). Hence, presumptions surrounding inferior NUEs of common carp, at animal level, should be reconsidered; a lot depends on man-made choices.

5.5. Autochthonous nutrient extraction by carps

Our present estimate highlights the amount of autochthonous nutrients carp extract from fishponds through retention in body (European average: 8–9.2 kg N and 1.4–1.6 kg P ha^{-1}). Like in the case of footprint, our estimate of extracted nutrients is also on conservative side compared to 'mass balanced' results (explained in supplementary text). In terms of autochthonous nutrient extraction, present production regime is only ~1.5–2.2 times less efficient than natural carp production. A more precise estimation would require stable-isotopes approach; conveniently for N but difficult for P. Nonetheless, greater retention of dietary N and P by farmed fish is the key to balance aquaculture and environmental sustainability goals (Rerat and Kaushik, 1995).

5.6. Environmental cost burden and ecosystem services of carp production in fishponds

Carp production in European fishponds has been 'qualitatively' attributed to various positive services (Szücs et al., 2007; Bekefi and Varadi, 2007, Popp et al., 2019). Ecosystem services include flood control, biomass production, nutrient remediation, biodiversity support, groundwater recharge, oxygen production, micro-climate regulation, carbon sequestration, aesthetics, etc. (Pokorný and Hauser, 2002, Popp et al., 2019). Even the maximum production service (up to ~1123 € ha^{-1}) comes after average eco-service (1257 € ha^{-1}; Frélichová et al., 2014) offered by regional fishponds. In addition, the production benefit (+298.8–373.5 € ha^{-1}) over natural yields due to use of cereals (as supplementary feed) outweighs the little environmental cost burden caused (72–184 € ha^{-1}). This advantage (Fig. S7) only applies given that weed fish biomass does not select-out mature stages of zooplankton (natural prey) and result in their population collapse (Musil et al., 2014; Zemanová et al., 2019).

In the Czech Republic, present carp production regime offers positive services of net worth ~2134–2300 € ha^{-1} (European average: ~2375 € ha^{-1}); almost 100 million € on country scale. On regional scale (CEER), total net worth of services is at least ~579 million €. If we consider the total budget of EU spent on aquaculture (1.17 billion €) during 2000–2014 (Guillen et al., 2019), carp production in CEER fishponds appears to have intangibly paid back half of it 'per production cycle'. Assuming 5 production cycles (average 3 years per cycle; Gál et al., 2016; Pechar, 2000) during the EU investment period (2000–2014), carp aquaculture 'alone' might have intangibly paid back ~2.9 billion € *i.e.* ~2.5 times over the invested budget. The positive services of carp farming in European fishponds is many folds higher (~13–29 times) than any cost burden caused through nutrient footprint; little-bad compared to the greater-good. This situation may be reversed to 'greater-bad, lesser-good', losing >1118 € ha^{-1} or >56.5 million € worth of services in CEER, if production regime is adjusted to purely environmentalists' interests (explained in supplementary text).

5.7. Trade-offs between nutrient supply, good growth and reduced footprint

Over the last four decades in Europe, there have been reports alleging carp production in fishponds as polluting and studies not corroborating such allegations (listed in supplementary text; reviewed in Roy et al., 2019). From a nutritional point of view, the 5–8 times superior digestible nutrient supply of natural prey over cereals is not as straightforward as it seems. For example – digestible N or P in one corn grain kernel (weighing ~0.38 g) is available from ~0.05 to 0.08 g natural prey dry matter, but in fishponds, it is equivalent to ~0.38–0.6 g natural prey biomass (wet weight) roughly amounting to ~1230–1969 Daphnids or ~258–414 Chironomids (*data* from Bezmaternykh and Shcherbina, 2015; Řezníčková et al., 2016, Simčič and Brancelj, 1997). One must imagine the differences in energy allocation by carps in fetching one static corn grain versus filtering equivalent numbers of active natural prey(s) in fishponds. Cereals itself are rich and easy source of digestible energy for carps (~2759.4 kcal kg^{-1}; our data) having an energy profile slightly below their optimum requirement (~3200 kcal kg^{-1} diet; NRC (2011)). On the other hand, production solely on natural food has its own limitations. High value proteins or lipids in natural prey, in the absence of cereals, are utilized for energy rather than acting as building blocks for biomass gain (Füllner, 2015). Here, the importance and role of cereals must be recognized before including it in legislative discussions concerning fishpond environment (e.g. Czech Republic, Duras and Potužák, 2019). Importance of a good balance between natural food availability, supplementary feed application and nutrient footprint is discussed below.

5.8. Managerial implications

Conclusions from previous life cycle assessments (LCAs) highlight the feed and feeding efficiency as fundamental to the environmental impact of most aquaculture production systems (e.g. Aubin et al., 2009; Biermann and Geist, 2019; Henriksson et al., 2015; Mungkung et al., 2013; Papatryphon et al., 2004). In a recent LCA assessment on German carp production in fishponds (Biermann and Geist, 2019), feed contributed almost unanimously to the impact category: eutrophication. The feed types and amounts were proposed as point-of-action to improve environmental sustainability of carp production atop other parameters. Any reduction in supplementary feeding alone greatly lowers the freshwater eutrophication threat scenario posed by fishpond effluents (Biermann and Geist, 2019). Here, using an alternative approach, we highlighted the same and quantified it. To the best of our knowledge, this is the first data-driven effort to demarcate possible trade-offs in relative FCR combinations ($FCR_{cereals}$ and $FCR_{natural\ prey}$) for balancing environmental and commercial goals of carp production.

The existing feeding regimen ($FCR_{cereals}$ 2–2.5; $FCR_{natural\ prey}$ 0.3–0.4) in European fishponds already has its own bottlenecks; detailed in the supplementary text. On both sides of the proposed trade-off FCRs, it is either forcing farmers to reduce carp production (e.g. maintaining $FCR_{natural\ prey}$ of 0.5 in fishponds), or inadequate supply of digestible nutrients for carp's optimum growth (e.g. if $FCR_{natural\ prey}$ is below 0.3, increasing cereals will only cause footprint, not production). In the former case, at least eco-subsidies should be offered to the farmers for their environmental contribution. In the latter case, better supplementary feed *i.e.* options beyond cereals should be availed (discussed below). In the present study, we have mostly dealt with N while discussing about protein. Fish need all 20 amino acids in adequate quantities for protein growth (Kaushik, 1995; Rerat and Kaushik, 1995). Cereals alone under low natural food availability cannot provide that. Poor protein quality or amino acid profile of carp's diet in fishponds, caused by lower natural food availability (*i.e.* abundant, high-quality protein) and excess cereals application (*i.e.* scarce, low-quality protein), can aggravate metabolic N losses up to 46.7–58.6% of dietary N intake (Roy et al., 2019). This will most likely manifest into lower NUE_N and higher N footprint than presently estimated. In this situation, both N and P might be of equal concern.

5.9. Future suggestions

Feeding management decisions in European carp farming should involve – (a) further LCAs of arable cereals (Biermann and Geist, 2019), including supply chain concepts (mentioned above); (b) efforts toward lowering the overall FCR (Mungkung et al., 2013; Biermann and Geist, 2019, present study) for improving environmental performance; (c) validate our proposed trade-off FCRs under practical conditions; (d) calibrate the stocking densities (lower carp heads feeding on natural food) for reducing existing footprint without compromising production; (e) changing frequency, timing and dosages of feed application depending on environmental conditions (Roy et al., 2019); (f) 'supplementing the supplementary feed' under low natural food availability – e.g. use of commercial carp feed (not cereals at critically low natural food availability), partial replacement of cereals with pulses-legumes having ~2.7 times higher digestible P, or, brewery wastes offering ~4–5 times higher digestible P than parent cereals (Roy et al., 2019, Vlastimil Stejskal, JCU-FROV Ceske Budejovice, *personal communication.*). If reduced production intensity is still imposed, at least the farmers should be compensated with 'eco-subsidies' for their environmental contribution. To some extent, this would offset their decreased farm-gate income.

6. Conclusion

The present study revealed that carp production in fishponds has the least nutrient burdens to environment compared to other food production sectors in Europe. Existing feed provisioning in carp ponds and production intensity cannot thus be considered as a pollution causing activity. Focus should be on actual management of the fishponds. The ecosystem and production services offered by carp farming in fishponds have immense societal and economic advantages. Majority of nutrient footprint from carp's feeding activity is contributed by supplementary feeding with cereals. Monetary benefit of improved production over natural yields, by using cereals, out-weighs the slightly increased environmental burden caused. Reducing the production intensity to neutralize footprint might cause rural societal disturbances and intangible economic losses in the region. In such a case, at least eco-subsidies should be offered to the farmers for their environmental contribution. Carp production exclusively based on natural productivity has its own limitations; high value protein from natural prey is utilized for energy supply, rather than building biomass. Here the role of cereals, as rich source of energy, must be recognized. For producers, over-relying on cereals for growth under low natural food availability is most likely futile – only aggravates environmental footprint. Yet, opportunities exist to calibrate the present feeding practices for achieving both environmental (minimized footprint) and aquaculture goals (uncompromised production).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Koushik Roy: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Visualization. **Jaroslav Vrba:** Validation, Writing - review & editing, Funding acquisition, Project administration. **Sadasivam J. Kaushik:** Validation, Writing - review & editing. **Jan Mraz:** Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

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CHAPTER 6

OPTIMIZING SYSTEMS, MINIMIZING LOSSES, AND ECO-FARMING: USE OF FISH NUTRITION

Roy, K., Vrba, J., Kajgrova, L., Mraz, J., 2021/2022. The concept of balanced fish nutrition in temperate European ponds to tackle eutrophication: further cleansing of an already cleaner production. Manuscript.

My share on this work was about 60%.

The concept of balanced fish nutrition in temperate European ponds to tackle eutrophication: further cleansing of an already cleaner production

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Abstract

This novel work brings forth a new lens to look at the problem of eutrophication in temperate shallow-lake ecosystems – combining animal nutrition and PEG principles. We show aggravated N, P loading by fish may occur both under high (≈beginning of the vegetative season) and low (≈end of season) zooplankton-zoobenthos availability. At beginning-season, the following phenomenon occurs in feeding fish: (a) high bioavailable P (P-homeostasis and digested-P urinated), (b) high PPR (renal stress≈ urinary PO_4^{3-} excretion), (c) renal EAA biosynthesis, e.g., arginine from abundant precursor NEAA proline (by-product≈ PO_4^{3-}), (d) low protein-sparing by low digestible carbohydrates (more protein catabolized≈ NH_4^+ excreted). Fish exhibit high but 'inefficient' N and P-retention under beginning-season diets. At end-season, the following phenomenon occurs: (a) insufficient, poor-quality protein (limited in lysine, isoleucine; rich in glutamic acid) results in poor N-deposition and aggravated NH_4^+ disposal, (b) highest P losses (poorest digestibility and discarding of already-digested P) in tandem with poorest N-deposition, (c) *de-novo* lipogenesis due to excessive starch, limitation of branched-chain amino acids for carbohydrate metabolism, obesity-inducing high omega-6: omega-3 fatty acids ratio in digested lipids. Protein accretion or growth almost ceases, fishes become fatty, and worst environmental loading of N, P happens in algae-reactive forms (NH_4^+, and PO_4^{3-}), under end-season diets. These novel observations were successfully validated against field metadata. We conclude highest ecosystem resource utilization efficiency and least N, P loading by fish are related to a balanced nutrition and managing fishes' satiety to graze (or spare) zooplankton-zoobenthos, enabling maintenance of clear-water phase and ecosystem services.

Keywords: *Temperate shallow lake ecosystem; plankton ecology group model; animal nutrition; nutritional bioenergetics; feeding and excretion; amino acids and fatty acids; carbohydrates and energy; protein and phosphorus metabolism; nitrogen and phosphorus loading.*

Highlights

- Optimizing ecosystem resource utilization efficiency (RUE)=mitigating eutrophication.
- Mitigating eutrophication=bio-manipulating European ponds towards balanced fish nutrition.
- Balanced nutrition=Optimized *in-vivo* RUE cascaded to an improved *in-situ* RUE.
- Improved RUEs=highest eco-services, minimized N, P footprint=cleaner production.

Introduction

Temperate, shallow lakes like European large-sized ponds (typical area from 2 to 300 ha; depth 1–4 m) are the predominant forms of standing water in land-locked, temperate parts of continents, e.g., Central Eastern European Region. They offer regional heritage, food security and economy (≥50% of inland protein or PUFA production), and ecosystem services (~74.5–100.6 million € country^{-1}; ~2375 € ha^{-1}); that too with the least nutrient footprint (1.5–4× < EU crop-livestock average) (Roy et al., 2020b). Since the Middle Ages, ponds in Europe were exploited (Adámek et al., 2012). Historically, the production relied on natural resources and seasonal plankton dynamics (Fott et al., 1980). After the rapid intensification of fish farming during 1960–1990 (Knösche et al., 2000; Pechar, 2000), the ponds currently represent hypertrophic, turbid ecosystems resembling an over-stocked (high-density fish) scenario of the revisited Plankton Ecology Group (PEG) model (Sommer et al., 2012). From a limnological perspective, the ponds resemble shallow lake ecosystems (Fott et al., 1980; Scheffer, 1998; Šimek et al., 2019). Though clear-water and turbid states may alternate (Scheffer, 1998; Scheffer and van Nes, 2007), as they did in the past (Fott et al., 1980), present ponds largely remain in the turbid state. It is mainly due to synergistic top-down and bottom-up effects of fish stock and nutrient legacy, respectively (reviewed in (Roy et al., 2020a)). Ponds are also among the most biodiverse and ecologically critical freshwater habitats, making an immense contribution through their ecosystem services (Frélichová et al., 2014; Hill et al., 2018; Roy et al., 2020b). Besides eutrophication, biodiversity losses or lost ecosystem services are additional concerns prevailing in Europe (Roy et al., 2020b).

Environmental laws protect present-day ponds (Roy et al., 2020a,b). However, the problem is far from being solved. The latest ‘fitness check’ of the European Union Water Framework Directive (EU-WFD; EU Directive 2006/44/E Article 3 & 5, Annex I) revealed that >70% of central European standing waters failed to achieve a good ecological status which EU-WFD envisaged 20 years ago (Freyhof et al., 2021). Over recent decades (1992–2018), the average total phosphorus concentrations in these water bodies have only decreased by 0.0003 mg P L^{-1} year^{-1} or 0.8% year^{-1} (European average). Diffused P runoff from agricultural land and sediment P legacy have prevented water quality improvement despite reducing inputs (e.g., carp farming) (EEA, 2020). Optimizing N and P cycles in these water bodies are among six planetary boundaries that EU-WFD prioritizes to achieve good ecological status (EEA/FOEN, 2020). The *status quo* nutrition management and nutrients flow of present-day CEER ponds, dominated by omnivorous cyprinids (carp), is detailed in Roy et al. (2020). From ecosystem-based management perspective, after spring thawing (April–May) (Vystavna et al., 2017), due to bountiful availability of carp’s natural food in fishponds (cyclops, daphnia, chironomid larvae; (Roy et al., 2020a,b), the supplementary feeding is felt unnecessary. With progress of vegetative season (June to September), carp’s natural food decreases (Füllner, 2015). Zoobenthos (including chironomids) which are mostly 3rd–5th instar larvae of aquatic insects metamorphose to have wings and emerge out of the aquatic system (Kajgrova et al., 2021). Whereas big zooplankton like cladocerans (mostly egg bearing adults; (Zemanová et al., 2019)) are heavily grazed upon by carps and/or weedy fishes (*Pseudorasbora parva*; (Musil et al., 2014)); zooplankton population is gradually supressed or even collapsed (as predation rate >regeneration rate). Such collapse breaks the links of aquatic food web and disrupts ecosystem functioning. As such, to support carp’s growth and keep them satiated, mostly cereal grains (corn, wheat, triticale, or rye; (Roy et al., 2020a,b)) are applied in fishponds to supplement the missing natural food from the carp’s diet. The relative feeding coefficient or relative feed conversion ratio of cereals in the presence of natural food ($RFC_{cereals}$; (Roy et al., 2020b)) in CEER fishponds is between 2–3 kg cereals applied per kg of fish yield (Füllner, 2015; Roy et al., 2020b; Woynarovich et al., 2010).

The present study is the final chapter that builds on our last key findings (Roy et al., 2020b) which was eventually recognized and adopted for the EU policymaking (AAC, 2021). The context of this study builds on the promised future directions (Roy et al., 2020b) that even a cleaner production can be further cleansed. Nutrients excretion by fish indeed support a large portion of aquatic primary productivity (Sharitt et al., 2021). However, their 'central role' in aquatic nutrient translocation is somewhat under-represented (Sharitt et al., 2021; Vanni, 2002; Vanni et al., 2013; Villeger et al., 2012). It is recently emphasized that fish can be limited by C (energy), N or P even in natural ecosystems, with implications for nutrient excretion (Schiettekatte et al., 2020). With the progress of vegetative season in temperate European ponds, natural prey (zooplankton-zoobenthos) share in carp (cyprinid; principal species) diet decreases while plant matter increases. Thus, fishes endure a counter-moving stoichiometry of food sources, thereby shifting micro-nutrient densities (e.g., amino acids, fatty acids, different forms of phosphorus, carbohydrates) reaching their gut per unit of ingested mass. Till now, ecologists have seen the problem of fishpond eutrophication through various lenses. We present a 'new' lens, *i.e.,* how nutrient footprint from fish depends on balanced-imbalanced nutrition and eventually shapes eutrophication. Our core hypothesis was that such changing dietary scenarios across vegetative seasons have implications on carp's nutritional bioenergetics (Bureau et al., 2003) and raised the following questions: (a) Is there a pattern in nutrient retention, fecal and metabolic excretion with the seasonally shifting diet components? (b) What are the deeper nutritional anomalies that aggravate or suppress nutrient losses from fish, making them source or sink nutrients? (c) Whether excretion products (ammonia or phosphate) reflect in water during nutritionally deficient windows of vegetative season? (d) Is it possible to achieve a balanced diet and suppress fishes pumping out nutrients in reactive forms? The managerial implications of the study are that ponds in central Europe are clearly important ecosystems, and how supplemental feeding might be changed (compared to the *status quo*) to improve nutrient use efficiency and reduce nutrient release by fish.

Materials and Methods

Ponds metadata on specific markers

Monthly raw data on specific hydrobiological parameters were collected from published articles and routine environment monitoring surveys in Central Eastern European region (CEER) ponds (Kajgrova et al., unpublished). Data were mostly from the recent decade (years 2007–2018) from 19 ponds (majority from the Czech Republic) having average area and depth range of 1.7–228 ha and 1.2–4 m, respectively; located at altitude range 190–680 m asl. They can be deemed as typical (representative) for CEER (Roy et al., 2020b). The effective vegetative season mainly spans from April to September and frozen from November/December till February. Dissolved ammonia and phosphate were taken as markers of fish excretion after protein, phosphorus metabolism respectively (Roy et al., 2020a, Villeger et al., 2012). Cladocerans, copepods (zooplankton), and chironomids (zoobenthos) were taken as markers of natural nutrition (Roy et al., 2020b). Data on plant-based food intake, in the absence of sufficient natural prey, were taken from (Füllner, 2015) and (Woynarovich et al., 2010). The specific growth rate (SGR) of carp stock recorded in those ponds was used as a growth marker. The data were further coded into three parts of the vegetative season: start (April–May), middle (June–July), and end (August–September).

Experimental design – Phase I (pre-requisite of phase-II)

11-month long digestibility experiments were conducted with lab-trained *Cyprinus carpio* (120–400 g) in a 12 tank (120 L $tank^{-1}$) Guelph-RAS system (19–21 °C, >4 mg L^{-1} DO, 6.8–7.3 pH) with provisions of six replicates per group (to obtain enough feces dry matter for complete nutrient analyses) per trial. Digestibility trials (6–7 kg carp $tank^{-1}$) on cereals (corn, wheat, triticale; commonly used in CEER ponds) and lyophilized natural prey dry matter (daphnia, cyclops, chironomids; freshwater origin) were conducted. Apparent digestibility coefficients of nutrients in test ingredients were calculated following established methods and formulas (Glencross et al., 2007; NRC, 2011). The further detailed methodology can be found in the supplementary material.

Intuitive feed formulation mimicking vegetative season relevant fishpond diets

In simpler terms, carps in present-day CEER ponds start on a 'keto diet' (in spring), have a balanced diet in between (shortly), and end up on a long, starchy diet before over-wintering (Füllner, 2015; Roy et al., 2020b; Woynarovich et al., 2010). Three diet scenarios were considered that naturally exist in CEER ponds through the vegetative season: 'HIGH' natural food relative to cereals in April-May → 'BALANCED' natural food with cereals in June–July → 'LOW' natural food relative to cereals in August-September. The logic and formula of the three experimental diets or nutrition scenarios are explained in Fig. S1 and Fig. S2, respectively. Details about experimental feed formulation are provided in the supplementary material.

Experimental design – Phase II (nutritional bioenergetics trial)

The first part was a digestible losses trial where the digestible intake and fecal (suspended) losses were assessed for the three abovementioned experimental diets (*i.e.,* High, Balanced, and Low). Details about this part are provided in the supplementary material. The next part was a 5-week growth trial to assess metabolizable (reactive) losses of already digested nutrients and retention of nutrients in the body for the three diets. Details about this part are provided in the supplementary material.

Mapping of nutritional bioenergetics (partitioning) under different diet scenarios

Nutritional bioenergetic or partitioning was quantified in three levels: (a) digestibility (and fecal losses), (b) metabolic losses (and total losses), and (c) retention (and its efficiency) (Bureau et al., 2003). In terms of 'excretory losses', we have considered both indigestible (faecal) and metabolizable (non-faecal) losses of nutrients (e.g., N, P); loaded to the environment by the fish. Non-faecal losses are in readily assimilable or reactive forms for algae, while faecal losses are organic-matter bound forms that enters microbial loop for mineralization. The calculation and formulas are elaborated in supplementary text and Tab. S2, respectively. Retention was deemed as a proxy for resource utilization efficiency (RUE; (Hodapp et al., 2019)), while losses were deemed as a proxy of nutrient footprint (Roy et al., 2020b). The retentions and losses were further analyzed against different nutritional indices. A systematic list of some state-of-the-art nutritional indices used in the present study is provided in the supplementary material. Observed anomalies in nutritional physiology of carps under different nutritional scenario(s) simulated in the laboratory were validated against actual observations in the field (ponds metadata).

Results

Nutritional markers in regional large-sized ponds

Carp's natural food in ponds (chironomids, cladocerans, and copepods) decreases naturally through the vegetative season while supplementary plant feed increases artificially (Fig. 1). Within these counter-moving feed components in the carp diet, the dissolved nutrient levels in ponds also fluctuate. Dissolved ammonia (NH_4^+) and phosphate (PO_4^{3-}) are also excretory end-products of actively feeding carp stocks in ponds: (a) either from feces (after microbial decomposition) or (b) metabolic losses in reactive forms (non-fecal excretion). The fluctuation of NH_4^+ and PO_4^{3-} are in such a way ('V'-shaped pattern) that it is somewhat suppressed when natural food and supplementary feed are in a transitional balanced state in mid-season. At both start (high natural food) and end (high supplementary feed) of the vegetative season, the levels of dissolved NH_4^+ and PO_4^{3-} appear to be high in ponds (Fig. 1). This 'V'-shaped pattern (from field data) has a striking resemblance to the metabolic excretion (non-fecal losses) pattern of carps under different diets (experiment data; next section). The growth rate of carps also decreases through the vegetative season, almost comparable ($p>0.05$) between the start and middle of the vegetative season, but significantly reduced ($p<0.05$) at the end-season (Fig. 2). This pattern is precisely superimposed or nearly superimposed with carp's protein and phosphorus retentions under different diets, respectively (next section).

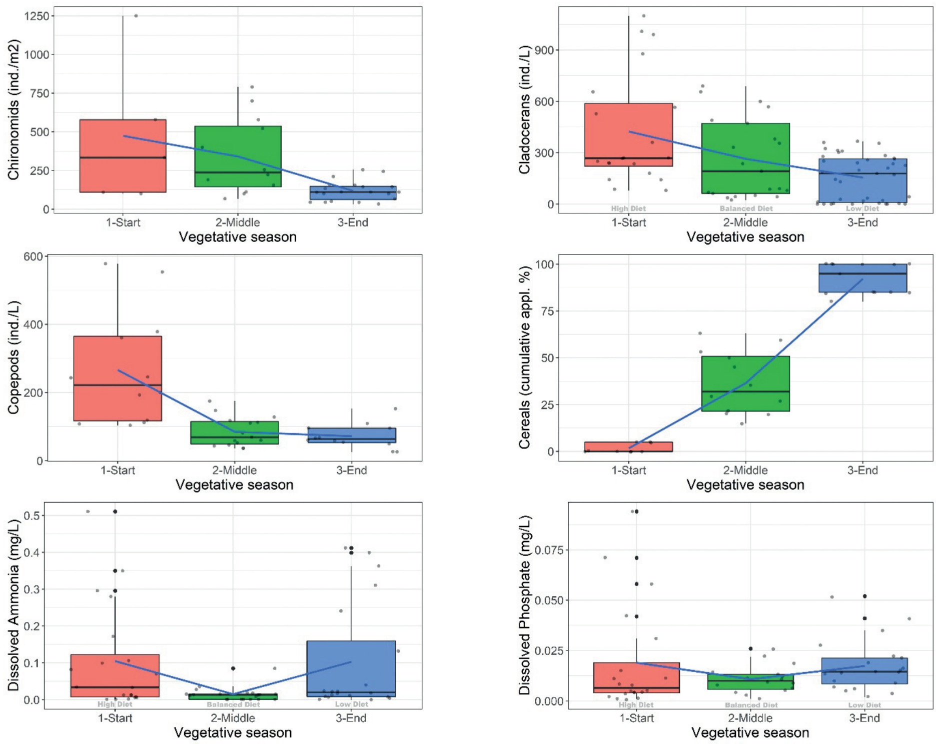

Figure 1. *Carp's nutritional markers in CEER ponds through the vegetative season. Natural food like chironomids (individuals m*$^{-2}$*), cladocerans and copepods (individuals L*$^{-1}$*) naturally decreases over vegetative season. Cereals application (cumulative; % of annual dose 1,000–1,500 kg ha*$^{-1}$ *year*$^{-1}$*) is purposively increased over season. Dissolved forms of N (*NH_3*; mg L*$^{-1}$*) and P (*PO_4^{3-}*; mg L*$^{-1}$*) i.e., excretory end-products of carp stock, is suppressed when food is 'balanced' in the middle of season. This trend has striking resemblance with Fig. 6 and 7.*

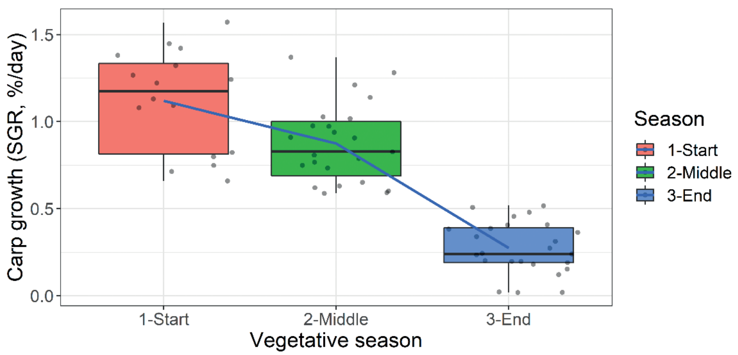

Figure 2. *Growth of carps in regional ponds through the vegetative season. Pattern observed (from field metadata): carp growth at 'start' is almost comparable (*$p>0.05$*) with the 'middle' of vegetative season, but significantly poor (*$p<0.05$*) in the 'end' of season. This pattern is super-imposable with the carp's protein and phosphorus retention pattern observed under different diets (depicted in Fig. 3).*

Base food (natural prey)

The crude and digestible nutrient contents of natural food, *i.e.,* chironomids, cyclops, and daphnia with special reference to their nutritional traits (protein quality/ DIAAS, phosphorus-to-protein ratio (PPR), protein sparing potential) can be found in Tab. 1 and 2. All values are on 100% DM basis. For carps, natural food is the main source of macro-nutrients (55.6–61% protein, 4.6–14.6% lipid) and essential micro-nutrients. The essential micronutrients include: (a) 0.6–1.2% bioavailable P (chironomids poorest); (b) digestible PUFA (0.76–2.36%) and digestible ω–3 FAs (0.36–1.84%; cyclops best); (c) top 5 EAAs comprising 65% of all EAAs (n=10) in carp's body, *i.e.* digestible lysine (1.81–2.33%) followed by leucine (2.42–2.63%), arginine (1.81–2.07%), threonine (1.70–1.97%), and valine (1.82–2.04%); (d) some important non-essential (functional) amino acids (NEAAs) like digestible glycine (1.61–1.94%), proline (0.66–1.51%), and glutamic acid (4.31–6.09%). The DIAAS revealed natural prey have good protein quality for carps with average value >75% (Tab. 2). The first limiting EAA in chironomid larvae is methionine, while in cyclops and daphnia it is lysine (Tab. 2). Especially copepods have the highest protein and lipid quality for carps (Tab. 1, 2). Natural prey items, irrespective of zooplankton or chironomids, had low protein sparing potential with values below 1 (Tab. 2). However, zooplankton (cyclops, daphnia) have high PPR.

Supplementary feed (plant feedstuffs, e.g., cereals)

Except digestible carbohydrate (average 58%), digestible NPE (217 kcal 100 g^{-1}), cereals are deficient in everything else for carps (Tab. 1). Cereals have 3–10′ lower protein, EAAs, P and PUFA than natural food. For example, the 14.9% crude protein of wheat is particularly deficient in digestible lysine (0.13%), leucine (0.54%), arginine (0.36%), threonine (0.23%), and valine (0.30%) mentioned above; also low in digestible NEAAs glycine (0.3%) and proline (0.43%) (Tab. 1). DIAAS revealed wheat protein has no quality claim with average value below 75%. Lysine is the first limiting EAA in wheat protein, followed by isoleucine, for carp (Tab. 2). Cereals are merely carbohydrate or NPE fillers (~26 cal digestible NPE per mg digestible protein), offering very high protein sparing potential for carps with values >> 1 (Tab. 2). Cereals also have low PPR. Altogether, high protein sparing potential and low PPR in cereals help to spare the protein and phosphorus from natural prey respectively, from being metabolized (non-faecal losses). Cereals are also bulk contributor of undigested P (ADC 24–36%) into fishpond environment despite being ~3′ less P-rich than natural food.

Overall diet

Through the vegetative season, supplies of digestible protein (for muscular growth) and digestible P (for skeletal growth) to carps gradually decrease from beginning to end-season ($p<0.05$) (Tab. 1). Supplies of two critical EAAs required in carp body, *i.e.,* digestible lysine and isoleucine, decrease most drastically (2.7–3.0 times) from beginning to end of the season (Tab. 1). This trend explains the declining growth rate of carp stocks from the beginning- to the end season until the growth is almost suppressed ($p<0.05$; Fig. 2). The poorest growth rate of carp stock is observed during the end-season (SGR ≈0.25; Fig. 2). In terms of functional NEEAs, digestible glutamic acid remains somewhat stable through the vegetative season ($p>0.05$), while digestible glycine gradually decreases from the beginning- to end season diet ($p<0.05$).

Digestible proline remains high only in the beginning-season (*$p<0.05$*), compared to mid- or end-season (*$p>0.05$*) (Tab. 1).

Digestibility of P markedly deteriorates at the end-of-season, whereas N digestibility is relatively stable throughout the season. As a result, the digestible intake N: P ratio significantly increases at the end-season (*$p<0.05$*) compared to the beginning- or mid-season diets (*$p>0.05$*) (Tab. 1). This pattern seems inversely superimposable on the growth pattern of carps in regional ponds (Fig. 2). The dietary PPR (Tab. 3) gradually decreases from the beginning- to end-season diet (*$p<0.05$*), concomitant with the general SGR dampening of carp stocks through the vegetative season (Fig. 2). Especially at the beginning of the season, PPR is near the upper critical limit (~16 mg digestible P per g digestible protein), potentially stressful for kidneys and triggering high urinary P excretion. So, carps grow faster at the beginning of the season and aggravate the renal (urinary) P excretion pathway. The trend of lipid and energy is presented in the supplementary text; because not directly related to eutrophication.

Table 1. *Nutritional composition of natural food (chironomids, cyclops, daphnia), supplementary feed (cereals) and dietary scenarios considered. Note: icons indicate relative strength of digestible nutrient/ energy supply, after conditional formatting in spreadsheet (default mode). Units are expressed as g nutrient or kcal energy per 100 g fed. Abbreviations: CHI=chironomids, CYC=cyclops, DAP=daphnia, WHE=wheat, COR=corn, TRI=triticale, Cru=crude content, Dig=digestible content (for agastric carp), NPE=non-protein energy. All values are 100% DM basis.*

Parameter (A)	Natural food*						Cereals*						Season/ Diet scenarios					
	CHI		CYC		DAP		WHE		COR		TRI		Start/ High[1]		Mid/ Balanced[2]		End/ Low[3]	
	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig
Macronutrient																		
Protein	56.8	▲52.1	73.2	▲54.8	64.6	▲52.0	15.8	▼11.9	13.4	▼9.50	15.6	▼5.9	24.6	▲21.3	21.1	▬18.0	18.0	▼14.9
Lipid	4.7	▼3.1	12.4	▲12.4	15.9	▲15.9	0.4	▼0.4	4.1	▼2.5	1.8	▼0.9	4.9	▲4.2	4.3	▲3.6	3.1	▼2.4
Carbohydrate	14.3	▼1.1	0.02	▼0.0	4.95	▼1.0	79.4	▲44.1	77.8	▲62.1	77.6	▲48.1	63.1	▼56.2	67.9	▼58.2	73.2	▲64.2
Fibre	3.2	▼0.2	8.2	▲0.7	5.1	▲0.4	2.5	▼0.0	3.3	▼0	3.2	▼0	2.7	▲0.6	2.5	▼0.0	2.3	▼0.0
Ash	21.3	▲5.3	6.2	▬1.8	9.4	▬3.2	1.9	▼0.5	1.4	▼0	1.8	▼0	4.7	▲1.7	4.2	▼0.4	3.4	▼0.0
Phosphorus	0.61	▬0.44	1.25	▲0.95	1.32	▲0.95	0.35	▼0.13	0.38	▼0.09	0.36	▼0	0.5	▲0.34	0.48	▬0.25	0.4	▼0.15
N: P ratio	14.9	▲19.0	9.4	▼9.3	7.8	▼8.7	7.2	▬15.2	5.7	▲16.7	7	–	7.9	▼10.1	7.1	▼10.6	7.2	▲15.9
Energy																		
Gross energy	326.2	▼241.2	404.7	▲331.0	421.4	▲355.2	384.7	▼227.9	401.5	▬308.5	388.8	▼224.1	394.7	347.8	394.9	337.3	393.2	338.4
NPE	99.2	▼32.7	111.8	▬111.7	163	▬147.2	321.7	▬180.2	348.1	▲270.5	326.6	▲200.5	296.3	▼262.4	310.3	▼265.6	321.2	▲278.7
NPE: Protein	1.7	▼0.6	1.5	▼2.0	2.5	▼2.8	20.4	▬15.1	26.1	▲28.5	20.9	▲34.0	12	▼12.3	14.7	▬14.8	17.8	▲18.7
Essential amino acids																		
Arginine	1.95	▲1.81	2.73	▲2.07	2.44	▲1.99	0.53	▼0.36	N.D.= Not determined, only wheat selected (see methods)				0.79	▲0.63	0.71	▲0.56	0.47	▼0.37
Histidine	1.28	▲1.19	1.26	▲0.96	0.90	▬0.74	0.30	▼0.20					0.46	▲0.35	0.42	▲0.33	0.30	▼0.23
Lysine	2.06	▲1.92	3.07	▲2.33	2.22	▲1.81	0.27	▼0.13					0.50	▲0.35	0.43	▲0.29	0.25	▼0.13
Isoleucine	2.28	▬2.12	4.73	▲3.59	3.25	▲2.65	0.37	▼0.18					0.80	▲0.66	0.61	▬0.42	0.36	▼0.22
Leucine	2.77	▲2.57	3.46	▲2.63	2.96	▲2.42	0.76	▼0.54					1.12	▲1.12	1.02	▲1.02	0.76	▼0.62
Methionine	0.66	▬0.62	1.43	▲1.09	1.23	▲1.00	0.25	▼0.17					0.39	▲0.36	0.32	▬0.28	0.30	▼0.24
Phenylalanine	2.50	▲2.33	2.02	▬1.53	1.68	▬1.37	0.55	▼0.40					0.82	▲0.66	0.76	▲0.61	0.59	▼0.50
Threonine	1.83	▲1.70	2.48	▲1.89	1.97	▲1.61	0.35	▼0.23					0.66	▲0.58	0.60	▬0.51	0.58	▼0.46
Tryptophan	0.46	▲0.43	0.53	▲0.40	0.49	▲0.40	0.25	▼0.15					0.55	▲0.43	0.45	▲0.37	0.25	▼0.18
Valine	1.96	▲1.82	2.69	▲2.04	2.24	▲1.83	0.45	▼0.30					0.75	▲0.58	0.59	▬0.43	0.43	▼0.34

Parameter (B)	Natural food*						Cereals*						Season/ Diet scenarios					
	CHI		CYC		DAP		WHE		COR		TRI		Start/ High[1]		Mid/ Balanced[2]		End/ Low[3]	
	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig	Cru	Dig
Non-essential (functional) amino acids																		
Glycine	1.74	▲1.61	2.56	▲1.94	2.06	▲1.68	0.48	▼0.30	N.D.				0.77	▲0.56	0.66	▬0.45	0.48	▼0.33
Proline	0.70	▼0.66	1.51	▲1.15	1.31	▲1.07	0.52	▼0.43					1.72	▲1.54	1.23	▼1.07	1.32	▼1.18
Glutamic acid	6.55	▲6.09	6.12	▬4.65	5.28	▬4.31	3.71	▼3.19					4.11	▲3.70	3.92	▼3.57	3.97	▲3.70
Fatty acids																		
MUFA	1.71	▼1.17	4.60	▲4.60	6.10	▲6.10	0.07	▼0.07	N.D.				1.03	▲0.88	0.67	▼0.57	0.60	▼0.54
PUFA	1.12	▼0.76	2.36	▲2.36	1.39	▬1.39	0.29	▼0.29					1.88	▬1.66	1.91	▲1.72	1.78	▼1.60
ω-3	0.53	▼0.36	1.84	▲1.84	0.86	▬0.86	0.02	▼0.02					0.40	▲0.33	0.33	▬0.29	0.23	▼0.20
ω-6	0.59	▬0.40	0.39	▬0.39	0.47	▲0.47	0.26	▼0.26					1.47	▼1.32	1.57	▲1.43	1.54	▲1.40
ω6: ω3 ratio	1.10	▼1.10	0.21	▼0.21	0.55	▼0.55	11.01	▲11.01					3.69	▼3.96	4.70	▬4.99	6.67	▲6.89

[1]High diet= mimicking a beginning-of-season diet (April-May) = 80% wheat grain + 20% natural food DM (share by mass in carp gut).

[2]Balanced diet= mimicking a middle-of-season diet (June-July) = 87% wheat grain + 13% natural food DM (share by mass in carp gut).

[3]Low diet= mimicking an end-of-season diet (August-October) = 95% wheat grain + 5% natural food DM (share by mass in carp gut).

*Natural food: cyclops/ daphnia= ~10% DM, chironomid= ~15% DM. Cereals: wheat/ corn/ triticale= ~90% DM

Table 2. *Nutritional traits of different dietary components for fish (carps) in ponds. Abbreviations: EAA=Essential amino acids. DIAAS=Digestible Indispensable Amino Acid Score (%). CHI=chironomids, CYC=cyclops, DAP=daphnia, WHE=wheat. Notes: bars indicate relative strength, after conditional formatting (default mode; maximum=100%, minimum=1%). Icons are self-explanatory. Tryptophan purposively omitted due to non-accredited values.*

EAA/ Protein/ Parameter	Supply potency to fish[1]				Protein quality by DIAAS[2]				P, N (protein), energy balance[3]			
	CHI	*CYC*	*DAP*	*WHE*	*CHI*	*CYC*	*DAP*	*WHE*	*CHI*	*CYC*	*DAP*	*WHE*
Arginine (%)	107	122	117	21	65	71	72	57				
Histidine (%)	237	192	147	40	72	55	45	53				
Lysine (%)	87	106	82	6	43	50	41	12				
Isoleucine (%)	212	359	265	18	119	192	149	45				
Leucine (%)	184	188	173	38	82	80	77	75				
Methionine (%)	88	155	143	24	41	69	67	49		–		
Phenylalanine (%)	179	118	105	31	122	76	72	93				
Threonine (%)	113	126	107	16	75	80	72	45				
Tryptophan (%)	–	–	–	–	–	–	–	–				
Valine (%)	130	146	131	22	86	91	86	62				
Overall Protein (%)	163	171	162	37	78	85	76	55				
PPR (mg P g protein^{-1})									8.4	17.2	18.3	10.6
Protein sparing potential									✗ 0.2	✗ 0.6	✗ 0.8	✓ 4.5

[1]Strength of a dietary component to be able to fulfill the optimum requirement of fish (per unit ingestion). Supply potency (%) = [(digestible content in % of DM)/(optimum requirement in % of diet)]*100. Optimum requirement= NRC (2011) specifications for *Cyprinus carpio* (see, Table S1). Values >100% imply 'bountiful supply'.

[2]For DIAAS calculation: Test protein= apparent digestible values (protein, EAA) in dietary components (Table 1); Reference protein= carp body protein (average protein 52.71%, Arg 2.80%, Hist 1.67%, Lys 4.51%, Ileu 1.80%, Leu 3.17%, Met 1.52%, Phe 1.93%, Thr 2.28%, Tryp 1.10%, Val 2.15% DM basis). Average/ overall protein DIAAS >75-99% = good protein quality; <75%= no quality claim protein. EAAs highlighted in red= first limiting EAA/ bottleneck.

[3]PPR= digestible phosphorus-to-protein ratio (value >16 exert renal stress to P excretion; see methods). Protein sparing potential= digestible non-protein energy: protein ratio (NPEP) in food item, divided by NPEP in 72 hours starved carp body (~3.34 cal/mg). Value <1 indicate poor to no protein sparing potential.

Protein (nitrogen) and amino acids

The digestibility of protein ($ADC_{protein}$) remains comparable across vegetative season (average ADC 82.9–86.8%, *p>0.05*) (Fig. 3). The $ADC_{protein}$ closely follows the average ADC of all 10 EAAs (arginine, histidine, lysine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine) combined (ADC_{EAA}). ADC_{EAA} is 82% in beginning-season, 80% in mid-season, and 74.6% in end-season diets (*p>0.05*). However, in the end-season diet, the difference between $ADC_{protein}$ and ADC_{EAA} becomes large (by ~9%); compared to any other season. It means some EAAs in cereals are less digestible than natural prey, creating such large differences. In end-season diets, four such EAAs include lysine, isoleucine, methionine, and threonine, which are less digested (*p<0.05*).

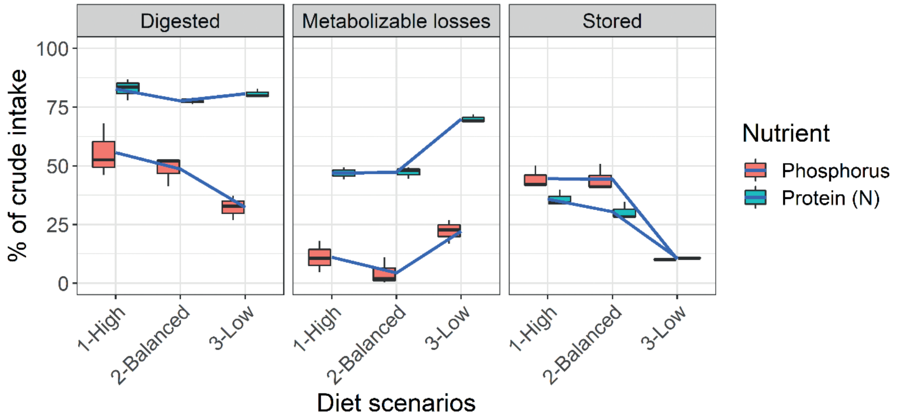

Figure 3. *Nutrient utilization pattern in carps under different dietary scenarios in ponds. Pattern observed (from experiment data): In terms of nutrient retention (=stored), low diet scenario (mimicking end-season diet) is significantly worse (p<0.05) than balanced or high diet scenario (insignificant differences among them, p>0.05). This pattern is superimposable with the pattern depicted in Fig. 2 (i.e. two blind, independent datasets).*

Metabolic losses of protein occur as branchial excretion of reactive NH_4^+ (readily assimilable by algae). It is somewhat stable from the beginning- to mid-season (~44–49% of crude intake; *p>0.05*) but aggravate significantly under end-season diet (69–49% of crude intake; *p<0.05*) (Fig. 3). Aggravated metabolic-N losses under end-season diets may be attributed to low protein quality. Such insufficient and poor-quality protein particularly deficient in lysine and isoleucine, but under high glutamic acid availability, probably aggravate metabolic N losses in the 'low diet' scenario (Fig. 3). Metabolic N losses under beginning-season diet seem low when expressed relatively (*i.e.,* in % of crude intake), but it is quite deceptive. When converted to absolute amounts, the metabolic N losses under beginning-season diet is nearly as bad as end-season diet (Fig. 6). It is simply because the digestible intake of protein in the beginning-season diet is originally high (Tab. 1). Metabolic EAA losses pattern (of all 10 EAAs combined) can be related to protein losses (Fig. 4 and 5). They are inversely relatable to the observed growth pattern of carps in regional ponds (Fig. 2). It was also apparent from the dip of metabolizable losses below 0% (Fig. 4) and retention reaching up to 100% (Fig. 5) that *de-novo* (renal) biosynthesis of arginine and histidine might occur in beginning-season diet; probably from NEAA proline. A supressed proline coefficient in carp body protein under a 'high' diet reinforces the observation (Tab. 3). Such renal biosynthesis of amino acids has repercussions on renal phosphate excretion (presented below).

Table 3. *Nutritional traits of different dietary scenarios existing through the vegetative season in ponds. High=beginning-season diet (~April–May); Balanced=mid-season diet (~June–July); Low=end-season diet (~August–September). PER and P:N retention ratio together are proxy markers of RUE (ecosystem) by fishes in ponds. Notes: bars indicate relative strength, after conditional formatting (default mode; maximum=100%, minimum=1%). Icons are self-explanatory.*

Parameters	Diet		
	High	Balanced	Low
Fishes' efficiency in assimilating ecosystem N (protein) and P			
Protein efficiency ratio[1]	1.4	1.7	0.9
P: N retention ratio[2]	159	205	134
Carp body amino acid body deposition (% of body protein)			
Arginine	6.14	6.04	5.01
Histidine	3.60	3.29	2.94
Lysine	8.89	8.10	7.09
Isoleucine	3.78	3.39	3.04
Leucine	7.49	6.90	6.01
Methionine + Cysteine	3.37	4.20	3.76
Phenylalanine	4.08	3.80	3.30
Threonine	4.56	4.37	3.75
Tryptophan	1.96	2.26	2.30
Valine	4.63	4.05	3.61
Glycine	8.42	9.68	7.70
Proline	0.50	0.72	0.55
Glutamic acid	15.44	14.56	12.23
P, N (protein), energy balance			
PPR[3] (mg P g protein^{-1})	15.9	14.1	10.1
Protein sparing potential[4]	3.7	4.4	5.6
[1]PER= protein efficiency ratio (g gained per g fed)			
[2]Expressed as mg P stored (maximum) per g N retained. Carp body average (fed or starved)= 204 mg P per g N (see methods)			
[3]PPR= digestible phosphorus-to-protein ratio (value >16 exert renal stress to P excretion; see methods)			
[4]Protein sparing potential= digestible non-protein energy: protein ratio (NPE:P) in food item, divided by NPE:P in 72 hours starved carp body (~3.34 cal/mg). Values >>1 indicate excessiveness			

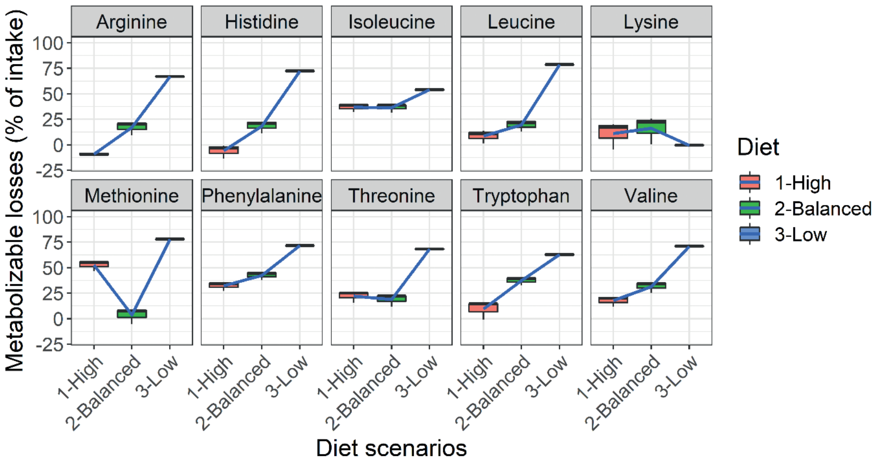

Figure 4. *Metabolic losses of essential amino acids (non-faecal/ metabolic losses) in carps under different dietary scenarios in regional ponds. Pattern observed (high → balanced → low diet axis): keeps aggravating=4 (arginine, histidine, tryptophan, valine); stable initially but aggravate sharply=4 (isoleucine, leucine, phenylalanine, threonine); stable and critical=1 (lysine); aggravates on either side=1 (methionine). Dip below 0% (y-axis) hint de-novo (renal) biosynthesis of arginine and histidine at high diet, probably due to excess of precursor (proline; Tab. 1) and reflected in reduced body storage (arginine, histidine versus proline; Tab. 2).*

Retention or storage of protein (N) remain high under the beginning- (~34–40% of crude intake; slightly better) and mid-season diets (~28–35% of crude intake), but significantly deteriorates ($p<0.05$) by the end of the season (~11% of crude intake) (Fig. 3). Four out of 10 EAAs are decreasingly retained ($p<0.05$) from the beginning- to end-season diet (arginine, histidine, tryptophan, valine) (Fig. 5). Specifically, in beginning-season diet, arginine and histidine are retained to such levels (up to ~90–100% of crude intake), which is virtually impossible without 'supplementation' from *de-novo* biosynthesis because faecal losses were already ~21–24% of crude intake. Other 4 EAAs (isoleucine, leucine, phenylalanine, threonine) were retained comparably higher in the beginning- or mid-season ($p>0.05$), but retention deteriorated sharply at end-season ($p<0.05$) (Fig. 5). The lysine retention remained critical and stable through the vegetative season (Fig. 5), probably because lysine is the first limiting EAA in fishpond diets (Tab. 2). Retention of methionine is suppressed on either side ($p<0.05$) of a balanced diet (Fig. 5). However, the highest protein (or N) retention in the beginning-season diet does not necessarily mean it is most efficient. The efficiency of protein deposited into biomass, *i.e.,* protein efficiency ratio (PER), is maximum only under a balanced diet scenario (PER 1.7 unit). Such high PER in a balanced diet scenario is evidence of protein sparing. Note that the protein-sparing potential of the mid-season (balanced) diet was better than the beginning-season diet (Tab. 3). Since PER drops on either side of the balanced diet, it hints that the protein-sparing mechanism acts most efficiently during the mid-season only. A drop in PER happens most significantly ($p<0.05$) under end-season 'low diet, (Tab. 3). The protein retention pattern (Fig. 3) is superimposable with that of the growth pattern of carp in regional ponds (Fig. 2).

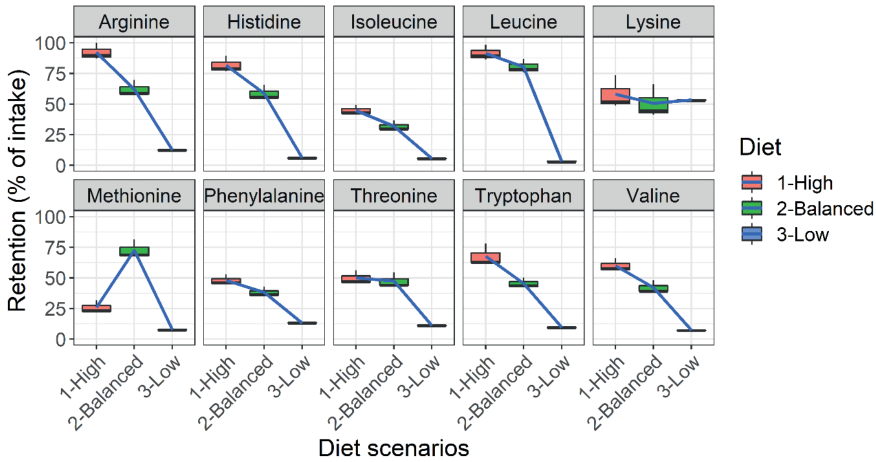

Figure 5. *Essential amino acids retention pattern in carps under different dietary scenarios in ponds. Pattern observed (high → balanced → low diet axis): keeps decreasing=4 (arginine, histidine, tryptophan, valine); stable initially but sharp fall=4 (isoleucine, leucine, phenylalanine, threonine); critical and stable=1 (lysine); supressed on either side=1 (methionine). Altogether, the average picture is superimposable on protein retention (depicted in Fig. 3) and growth pattern in ponds (Fig. 2).*

Concomitant with protein retention pattern, all EAAs and functional NEAAs storage in carp body decrease from beginning- to end-season; except for sulphur containing amino acids (methionine+ cysteine), and proline (Tab. 3). Lower proline storage in beginning-season diet could be linked to its highest digestible intake in 'high' diet which could have triggered renal biosynthesis of arginine and histidine (presented above), leading to its over-utilization. The case of sulphur containing AAs (Met+Cys) is perhaps related to their storage restrictions and toxic metabolites accumulation at higher digestible intake. Met+ Cys coefficient dropped when digestible methionine supply was the highest under beginning-season diet (Tab. 1 versus Tab. 3). Nonetheless, highest storage of these 2–3 AAs combined could have favoured maximum PER under balanced diet, relative to 'high diet'.

Phosphorus (and other minerals)

The digestibility of P (ADC_P) is initially highest in the beginning-season (46–68%) and gradually decreases with the progression of the vegetative season (ADC_P mid-season 41–53% to end-season 27–37%; $p<0.05$) (Fig. 3). The enhanced ADC_P in the initial part of the vegetative season (beginning- or mid-season) is attributed to the higher bioavailable forms in the natural food (ADC_P natural food 72–76% versus wheat 36%). Interestingly when natural food and plant items are eaten together, there is a synergistic digestibility effect imparted by natural food on difficult-to-digest P fractions in plant items (Tab. 4). The distance of 'observed values' ADC_P, ADC_{fiber}, ADC_{ash} of wheat from wheat's originally 'expected values', with increasing natural food share demonstrate the synergistic digestibility effect (Tab. 4). The P from wheat was better, and even better digested from low to high diet (Tab. 4); probably through enzymes preserved in lyophilized zooplankton-zoobenthos. In the high diet, even the indigestible fibers were broken down, which probably released the previously trapped minerals (≈ash) for bio-absorption (ADC_{fiber} versus ADC_{ash} values; Tab. 4).

Table 4. *Synergistic digestibility effect of zooplankton-zoobenthos on difficult-to-digest plant matter (here, cereals). Note: bars indicate relative strength, after conditional formatting (default mode; maximum=100%, minimum=1%). Thick horizontal lines separate individual dietary scenarios; compare 'expected' versus 'observed' values (=bars) under each fraction.*

Season/ Diet	ADC (%)	Fibre	Ash	Phosphorus
Start/ High	$ADC_{Expected}$	0	26	36
	$ADC_{Observed}$	23.9	38	65
Mid/ Balanced	$ADC_{Expected}$	0	26	36
	$ADC_{Observed}$	0	27	48
End/ Low	$ADC_{Expected}$	0	26	36
	$ADC_{Observed}$	0	26	35
$ADC_{Expected}$= normal digestibility of wheat 'alone'.				
$ADC_{Observed}$= digestibility of wheat 'in presence of' natural food (=enzymes).				

Metabolic P loss (as urinary excretion of reactive PO_4^{3-}; readily assimilable by algae) is usually a negligible and much less-studied pathway than faecal loss. This negligibility is not always true. Urinary P losses are negligible (~4.4% of crude intake) only during the mid-season but remain aggravated on either side of a balanced diet, *i.e.*, beginning- (11.1% of crude intake; $p<0.05$) or end-season (22.1% of crude intake; $p<0.05$) (Fig. 3). The high metabolic P loss in the beginning-season coincided with excess PPR (Tab. 2) and probable renal biosynthesis of arginine from proline in 'high diet' mentioned above. Similarly, high urinary P losses in end-season also coincided with slowest growth (Fig. 2), inadequate protein quality (Tab. 2) and poorest protein retention in low diet (Tab. 3, Fig. 3). This pattern of metabolic P loss (Fig. 3) is quite superimposable on phosphate concentration trends in regional ponds through the vegetative season (Fig. 1).

The retention of P remains comparably high in the beginning- and mid-season diet (41–51% of crude intake; $p>0.05$) but significantly deteriorates ($p<0.05$) at end-season diet (10–11% of crude intake) (Fig. 3). The P:N retention ratio reveals that P retention is most efficient in balanced diet scenario (~205 mg P stored per g N retained; $p<0.05$) compared to the beginning- (~22% less efficient) or end season diet (~35% less efficient; expected) (Tab. 3). Lower efficiency in P retention under beginning-season diet, despite the most superior P digestibility, may be attributed to higher urinary losses. The lowest efficiency of P retention in the end-season diet may be attributed to both poor digestibility and high urinary losses (Tab. 3 versus Fig. 6). The P retention pattern occurs in tandem with N retention (Fig. 3) and approximately relatable to the growth pattern of carps in regional ponds (Fig. 2).

Lipid and energy

The case of lipid and energy is detailed in the supplementary text.

Environmental loading of nitrogen and phosphorus under different dietary scenarios

Excretory losses of N and P from fish can happen both via faecal pathway (=suspended losses; faeces-bound N or P) and non-faecal pathway (=reactive losses; branchial NH_4^+ or urinary PO_4^{3-}). Altogether they comprise total losses, hereinafter referred to as environmental loading. Suspended losses of N remain comparable through the vegetative season (interquartile range, IR: ~0.5–0.75 g N per 100 g diet-fed); lowest at end-season due to initially low N-intake ($p<0.05$; Fig. 6). However, on either side of the balanced diet, reactive N losses remain aggravated ($p<0.05$; Fig. 6). Reactive losses are also the major pathway of environmental

N loading (reactive-to-suspended losses ratio >>1; Fig. 7). Thus, the environmental loading of N by carps (Fig. 6) seem to remain aggravated at both beginning- and end-season diets ($p<0.05$). This 'V'-shaped pattern is roughly relatable to the ammonia concentration trends in regional ponds (Fig. 1; presented above).

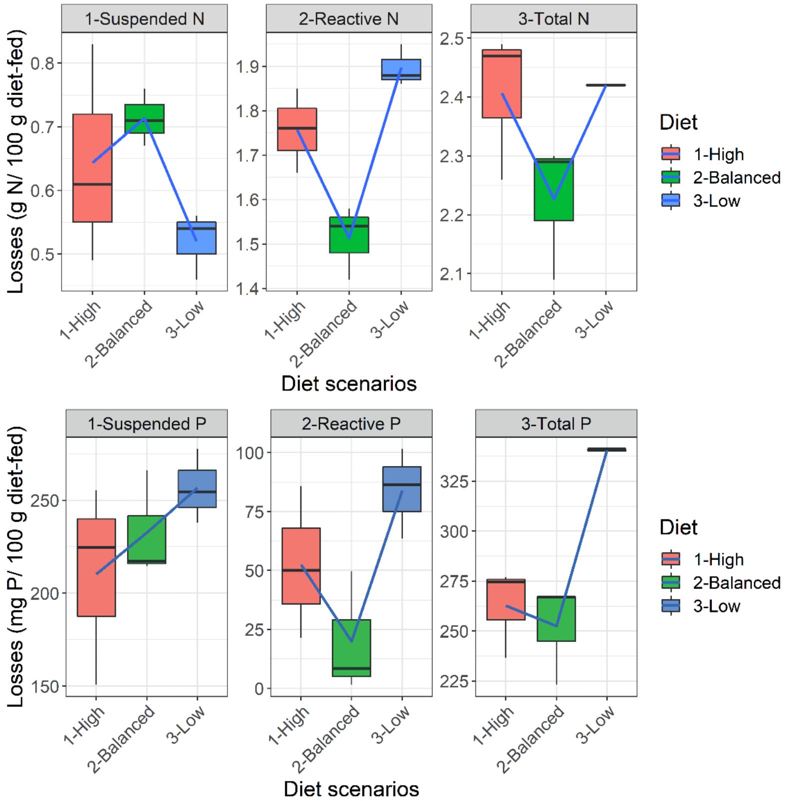

Figure 6. *Nitrogen and phosphorus loading pattern of carps under different dietary scenarios in ponds. Reactive N/P=branchial or urinary losses. Suspended N/P=faecal losses. Pattern observed (high → balanced → low diet axis): already digested N/P losses, in reactive forms, aggravate on either side of the balanced diet (mid-season). This effect ('V' shaped pattern) is strong enough to be reflected in total N/P losses and in ponds (see, NH_3/ PO_4 patterns; Fig. 1). Least environmental loading is achievable only under balanced nutrition (see, middle season; Fig. 1).*

Suspended P losses gradually increase through the vegetative season (IR ~187–263 mg P per 100 g diet-fed); significantly higher in end-season ($p<0.05$; Fig. 6). Suspended losses are the dominant pathway of environmental P loading (reactive-to-suspended losses ratio < 1; Fig. 7). Even reactive P losses can be ignored if the diet is balanced (ratio ≈0; Fig. 7). However, on either side of the mid-season (balanced diet), reactive P losses remain aggravated ($p<0.05$; Fig. 6). The effect of this 'V'-shaped pattern is so strong that it is almost reflected in the total P losses (Fig. 6), despite suspended losses being the primary environmental loading pathway. This 'V'-shaped pattern is approximately superimposable on the phosphate concentration trend observed in regional ponds (Fig. 1; presented above). The environmental loading of P by carps significantly happens ($p<0.05$) under end-season diets (325 mg P per 100 g diet-fed). However, the reactive P losses under beginning-season diet may not be ignored either (Fig. 6).

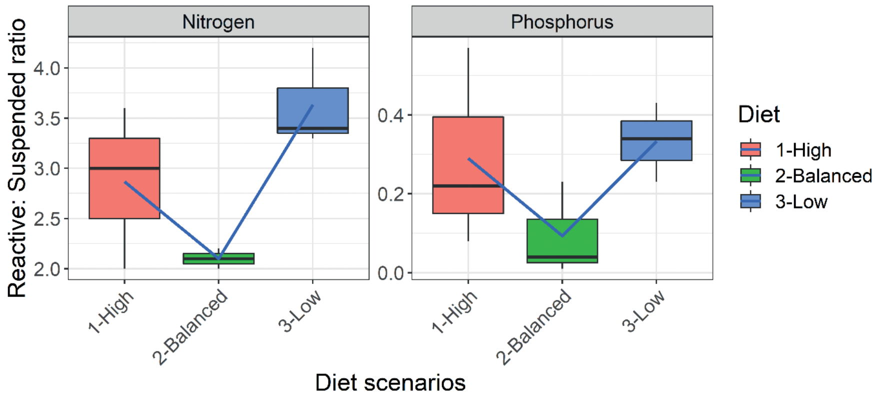

Figure 7. *Excretory product proportions (reactive-to-suspended losses ratio) of carp under different dietary scenarios in ponds. Reactive=metabolic losses of already digested nutrients (branchial NH_3/ urinary PO_4). Suspended=faecal losses of undigested organic bound N/P. Suspended forms enter microbial loop before reaching algae – slower process. Reactive forms, readily assimilable by algae (≈liquid fertilizer), aggravate on either side of the balanced diet. Reactive P release spike in high diet may be connected to de-novo (renal) biosynthesis of arginine and/or histidine (Fig. 5), under high bioavailable P (Tab. 1), high PPR (Tab. 2), P-retention saturation (Tab. 2, Fig. 3), where the by-product of EAA biosynthesis (PO_4^-) is not re-absorbed by renal tubule; part of P homeostasis (?).*

Discussions

Perspectives so far to look at fishpond eutrophication

Till now, ecologists have mostly seen the problem of fishpond eutrophication through various perspectives or lenses. Detailed discussion in this regard can be found in the supplementary material. We have presented a new lens to look at the problem. In line with the ecological stoichiometry theories considering nutrient assimilation efficiencies over simplistic nutrient homeostasis models (Schiettekatte et al., 2020; Sterner, 1990), we show how *in-vivo* nutritional bioenergetics (≈nutrient partitioning and *de-novo* bioconversions) can strongly modulate nutrient translocation (*in-situ* → *in-vivo* → *in-situ*) in aquatic systems. Especially how imbalanced stoichiometry of amino acids, digestible fractions of P and carbohydrate energy (in ponds) aggravate internal nutrient loading by carps and trigger eutrophication.

Nutritional profile of different dietary components and connection to nutrient loading

To the best of our knowledge, 'digestible' nutrient composition, and nutritional traits of carp's natural food in ponds were critically evaluated here for the first time. The protein quality of natural food is good for growing carps, as revealed by the latest DIAAS index (Herreman et al., 2020; Kim et al., 2019). Except for partial insufficiency of S-containing AAs (e.g., methionine) in chironomid larvae (≈zoobenthos; also supported in (Hepher, 1988)), natural food in general supplies some of the most critical, high-requirement EAAs for fish (e.g., lysine; (Kaushik and Seiliez, 2010; NRC, 2011)). Lysine and sulfur-containing AAs (EAA methionine+NEAA cysteine) are widely recognized as the first two limiting AAs for fish (Dabrowski and Guderley, 2003; Kaushik and Seiliez, 2010; Wilson, 2003). The ideal protein concept for fish revolves mainly around lysine and, sometimes, methionine (Bureau and Encarnação, 2006; Rollin et al., 2003; Turchini et al., 2019). In the fishpond environment, lysine seems to be the first limiting amino acid, irrespective of zooplankton or zoobenthos. Next to lysine, the limiting amino acids in natural food are methionine (in zoobenthos) or histidine (in zooplankton ≈cyclops, daphnia).

Interestingly, zoobenthos is rich in histidine, while zooplankton is rich in methionine (present study). Therefore, such amino acid limitations in ponds could be easily balanced by feeding alternatively on zoobenthos or zooplankton, which carps naturally do (Anton-Pardo et al., 2014). Just maintaining their abundant population should be the key, especially zooplankton, because zoobenthos (3rd to 5th larval instars) naturally metamorphose, develop wings, and emerge out of ponds (Kajgrova et al., 2021). Most likely, lysine remains a critical bottleneck through the vegetative season. The central function of lysine in the animal body is protein tissue deposition (Chiba et al., 1991; NRC, 2011, Wilson, 2003). Fortunately, lysine is utilized efficiently even at suboptimal intakes as it is used almost exclusively for protein synthesis and does not take part in other metabolic processes (Heger and Frydrych, 2019; Heger et al., 2002); also presently observed. Therefore, insufficient natural food (or lysine) in ponds would lead to inefficient protein deposition. Besides, an imbalanced AA profile of dietary protein could exacerbate metabolic losses of N (Kaushik, 1995), and eventually losses of P too in tandem (Sugiura et al., 2000); also presently observed.

A recent paradigm shift of focus on some functional NEAAs (He et al., 2021; Li and Guoyao, 2020; Rong et al., 2020; Wu et al., 2011) and pre-existing doubts on the established requirements of EAAs (Bureau and Encarnação, 2006; Dabrowski and Guderley, 2003) has opened new gaps in understanding protein and amino acids metabolism in fish (Kaushik and Seiliez, 2010; Turchini et al., 2019). Besides EAAs, natural food is also a uniquely high supplier of these functional NEAAs to fish, e.g., proline and glycine (He et al., 2021; Rong et al., 2020; Wu et al., 2011). Presently the national water laws or EU's water framework directive restricts input use in ponds, even for supplementary feeding. Only locally sourced and plant-derived feedstuffs are labeled 'ecological,' not animal-derived or complete pelleted feed (Füllner, 2015; Hlaváč et al., 2016). Most plant-derived feedstuffs (e.g., cereals) are deficient in lysine and functional NEAAs like proline and glycine (Li and Guoyao, 2020). On the other hand, plant feedstuffs, especially cereals (e.g., wheat), are a uniquely high supplier of a functional NEAA, glutamic acid (Gorissen et al., 2018; Kumar et al., 2017). Opposite to lysine (≈protein deposition), glutamic acid's central role in animals is N disposal (Cooper and Jeitner, 2016), e.g., ammonia excretion in fish (Huang et al., 2020). Through the vegetative season, lysine deficiency on the one hand (decreasing natural food) and glutamic acid abundance (increasing cereals) on the other hand is bound to cause imbalances in protein metabolism and N, P excretion by fish.

Fortunately, non-carnivorous freshwater fish (e.g. common carp) have the capability to desaturate and elongate shorter (C-18) chain ω−3 or ω−6 FAs (=precursors) to highly unsaturated, long chain (C-22) PUFA (Bláhová et al., 2020, Glencross, 2009, Xu et al., 2020). Carps can selectively preserve or metabolize their body FAs to best suit their metabolic needs under specific dietary situations (Zajic et al., 2013). Common carp is also more inclined to require greater amounts of ω−6 FAs than ω−3 FAs for maximum growth (Turchini et al., 2009), and the *status quo* management of fishponds favor it. High levels of ω−3 PUFA (relative to ω−6 FAs) might not be even useful for carps (Turchini et al., 2009); ω−3 FA rich lipids in beginning-season diets are liberally metabolized (present study). In this light, fatty acid constraints in fishpond diets are perhaps less serious than amino acid problem(s); not directly connected to eutrophication. However, impaired energy metabolism and *de-novo* lipogenesis (also contributed by high ω−6: ω−3 FAs ratio (Simopoulos, 2016)) are two risks worth considering.

Nutrient utilization, environmental loading under different dietary scenarios, and eutrophication

Although natural food is optimum in terms of protein, amino acids, lipids, fatty acids, and phosphorus requirements for carp ((NRC, 2011); summarized in Tab. S1), there are some inherent bottlenecks with natural food itself. Considering the PPR pyramid for human nutrition and kidney health (D'Alessandro et al., 2015), natural food's high PPR can be considered stressful even for the developed mammalian kidneys (Noori et al., 2010; Nouri et al., 2010). A high PPR diet triggers higher urinary phosphate excretion (Noori et al., 2010; Nouri et al., 2010; Roy et al., 2002; Vielma and Lall, 1998). Fish kidneys are evolutionarily primitive (Vize, 2004). We suspect them to be more sensitive to high PPR diets (Marshall Jr and Smith, 1930), e.g., when feeding predominantly on natural food, they might end up with higher urinary losses of already digested P. Cereals themselves have low PPR (D'Alessandro et al., 2015), which can be attributed to their poor P digestibility relative to good N digestibility (Roy et al., 2020a). Cereals can neutralize the high PPR effect of natural food on carps if added early enough in the beginning season. Lowering the PPR would also involve a slight reduction in protein intake (Noori et al., 2010), as cereals (carbohydrate) would satiate and replace some natural food (protein). The lowered protein intake could be compensated by protein sparing (Stone, 2003). Protein sparing is essential for realizing efficient growth and N retention (Bureau et al., 2003; Kaushik, 1995), especially from a high-quality protein and lipid source like natural food. In the absence of an adequate NPE source (carbohydrate), valuable PUFAs, EAAs, and functional NEAAs in natural food are catabolized for meeting energy demand (Bureau et al., 2003; Stone, 2003). Natural food has a low protein-sparing potential (present study). As a result, maximum resource use efficiency (RUE) of ponds (by fish) may not be achieved (Hodapp et al., 2019); when fed on zooplankton-zoobenthos alone. Here the prominent role of cereals or plant-based food as a rich source of digestible carbohydrates needs to be recognized. As the efficacy of N retention is maximized, an improvement in P retention also follows (Sugiura et al., 2000). It might be because when a fish grow musculature, the skeletal (e.g., vertebrae storing Ca, P; (Roy et al., 2002; Vielma and Lall, 1998)) and connective tissue (e.g. membrane lipids integrating P (Berg et al., 2002)) also grow in tandem.

The central problem with plant feedstuffs (e.g., cereals) lies with protein profile and P loading into fishpond environments. Up to 80% of P in cereals can be locked in phytate forms (Vashishth et al., 2017), 'agastric' carps are unable to absorb (digest) them (reviewed in (Roy et al., 2020a)). For example, wheat-P is only 36% digested while wheat-N is far better digested (~76%) (Roy et al., 2020b). Therefore, most of the P in cereal grains can be 'selectively' loaded back to the fishpond. Additionally, the difficult-to-break complex fibers (non-starch polysaccharides, NSP) of plant feedstuffs trap most minerals, rendering them biologically unavailable (Goff, 2018). To make such minerals bioavailable (and not loaded to ponds), breaking down of those NSPs would be required (Goff, 2018), which carps are primarily incapable of doing (Polakof et al., 2012; Stone, 2003). This line of thought negatively affects the practice of supplementary feeding in regional ponds for water pollution and eutrophication concerns (Roy et al., 2020b). However, in ponds, this line of thought might not be straightforward applicable. Natural food has high enzymatic activities and can contribute them to fish gut, e.g., phosphatase (breaking down phytates; (Wynne and Gophen, 1981)), cellulase, or chitobiose (breaking down NSPs; (Avila et al., 2011; Gangadhar et al., 2018)). A sufficient share of natural food in diet can help digest the originally indigestible fractions in carp diet (e.g. wheat P, fibre, and inorganic minerals); probably in the same way they help digest algal cell walls. Such insights were largely non-validated till now.

Unfortunately, these better-digested P (from natural food) could still be released back to the environment through urine, depending on diet scenarios. For example, when natural food predominates in beginning-season diets, there is a high supply of NEAA proline (present study). Proline is a precursor for the biosynthesis of EAA arginine (Tomlinson et al., 2011a,b. *De-novo* biosynthesis of arginine has often been observed in animals along the intestinal-renal axis (Hou et al., 2016), with renal arginine biosynthesis being the final pathway (Brosnan and Brosnan, 2004). The present study indicated *de novo* biosynthesis of arginine, double confirmed from carp body storage pattern of proline and arginine under 'high diet' (presented above). During arginine biosynthesis, renal phosphate flux increases due to enzymes and phosphate donors involved in the chain reaction (Bolte and Whitesides, 1984; Brosnan and Brosnan, 2004). Under a high PPR beginning-season diet, this excess phosphate flux might be freely wasted through urine as part of P homeostasis (Roy et al., 2002; Vielma and Lall, 1998). Looking at exceptionally high urine production by freshwater fish kidneys for osmoregulatory reasons and evolutionary primitive glomeruli (Marshall Jr and Smith, 1930), phosphate flushing under those circumstances mentioned above seems plausible. Urinary P of carps, mostly in orthophosphate forms, is readily assimilable by plankton (Lamarra Jr, 1975; Vanni, 2002) and can trigger eutrophication (Chumchal and Drenner, 2004). Therefore, under high natural food availability, carps can simply act as 'pumps' of reactive P to the environment (converting organic P from natural food) if not sufficiently balanced with cereals. Balancing with cereals makes N-retention efficient and P-retention efficient too (present study), thereby maximizing the RUE potential by fish in ponds (Hodapp et al., 2019).

Indirect connection of impaired energy metabolism and nutrient loading

Despite offering high protein-sparing potential (efficient N retention followed by P retention; discussed) and low PPR (reduced risk of releasing digested P; discussed), the supplementation of plant feedstuffs beyond a certain limit creates physiological imbalances. Almost all EAAs in carp body, except sulphur (S) containing AAs, decrease with decreasing natural food and increasing cereals in fishponds. S-containing AAs, methionine+ cysteine, are stored optimally only under a balanced diet because their storage in body at higher digestible intake increases risk of several toxic end-products (Baker, 2006; Brosnan and Brosnan, 2006). However, there are few EAAs apart from lysine that have a large share in carp body protein. One of them is isoleucine, a branched-chain EAA (Zhang et al., 2017). Isoleucine quality of intake protein can decrease sharply through the vegetative season; difference in DIAAS of isoleucine between zooplankton-zoobenthos and plant feedstuff like wheat average 3.4 folds (present study). Recent evidences suggest that isoleucine play a major role in glucose (carbohydrate energy) metabolism via glucose transportation and consumption in body (Yoshizawa, 2015; Zhang et al., 2017); also keeps in check muscle triglyceride deposition (Nishimura et al., 2010). Cereals despite being the richest source of digestible carbohydrates, does not provide the crucial EAA to help in carbohydrate metabolism. It is relatable to the disturbed energy metabolism and *de-novo* lipogenesis (DNL) observed under excess digestible carbohydrates, and poor protein profile of end-season diets (present study). Although carps can tolerate high dietary carbohydrate levels, they cannot sustain high glucose levels (=labile energy) for a long time. Excess energy is either stored as glycogen (glycogenesis) or new lipids (DNL) (Polakof et al., 2012). DNL is inhibited by high PUFA intake and secretions of growth hormones (Kersten, 2001). In end-season, PUFA intake drops significantly (present study) and growth hormone is probably supressed too under poor protein regime, protein metabolism, and growth (Li et al., 1996). So, basically at the end of season DNL gets a free hand, and it coincides with stagnated growth, highest N, P loading by fish.

Concept of balanced fish nutrition to tackle eutrophication: future applications

The applied side of our proposed concept is in line with the state-of-the-art understanding of fish (animal) nutrition and in line with the PEG principles to tackle eutrophication in shallow lake ecosystems, e.g., clear water phase or zooplankton dominance (Lepori, 2019; Scheffer and van Nes, 2007; Sommer et al., 2012). Cereals in addition to offering lowered PPR (D'Alessandro et al., 2015) and protein sparing potential (Bureau et al., 2003; Kaushik, 1995), might contribute to carp's feeling of satiety too (Holt, 1995). Energy and carbohydrate dense food items are known to have quite high satiety index *i.e.,* feeling of fullness (Holt, 1995). Carp stocks mostly start hungry from the feed-deprived, ~3 months long overwintering phase (Bauer and Schlott, 2004), severely depleting their body energy reserves (Zhao et al., 2021). Whether consuming a few grains of cereals at the beginning of vegetative season could spare sizeable amounts of zooplankton from being grazed upon, need further investigations to look upon. Observations in Mehner et al. (Mehner et al., 2019) hint one such possibility. Carps reduced their homing range (≈grazing ground), reliance on natural food, and 'opportunistically' spent more time around artificial feeding sites ((Mehner et al., 2019)). If manipulating carp's satiety is successful right from the beginning of vegetative season, the clear-water phase in these shallow lake systems might be stretched longer (Scheffer and van Nes, 2007; Sommer et al., 2012), and present bottleneck of low natural food availability beyond mid-season could be avoided.

However, at the end of vegetative season, the cereals alone are not effective. They must be replaced (completely or majorly) with 'treated' (e.g. soaking, fermentation; (Samtiya et al., 2020)), low anti-nutritional factor varieties (Francis et al., 2001) of plant-protein (e.g. lupines, peas, rapeseed, sunflower (Kaushik and Seiliez, 2010)), in certain combination(s) to ameliorate the deficient EAAs and NEAAs (Li and Guoyao, 2020). Simultaneously, non-manuring interventions to boost zooplankton production should also be explored for mobilizing ecosystem P to fish body (Scheffer and van Nes, 2007; Sommer et al., 2012), e.g. elimination of weed fishes (Musil et al., 2014), creation of plankton refugia (Sarkar et al., 2018), and artificial floating islands (Park et al., 2018). Maintenance of larger-bodied zooplankton keeps dissolved reactive P concentration and eutrophication in check (Lepori, 2019), but if combined with balanced fish nutrition, least excretory footprint from fishes (Roy et al., 2020b) and maximum RUE by fish in fishponds can be ensured too (Hodapp et al., 2019). These nutritional intricacies must be understood by ecologists who are tracking eutrophication in large European fishponds (IR: 4.4–64 ha area, 1.3–3 m depth; Kajgrova et al., unpublished); resembling temperate, shallow lake ecosystems. The current approach alone will be of limited efficacy in mitigating eutrophication if other good management practices are not followed (Jeppesen et al., 2012); for example, unchecked agricultural and municipal nutrient loading (Moss et al., 2004) or destruction of aquatic macrophytes (Francová et al., 2019).

Conclusion

The present study is the first of its kind which provides a deep understanding how fish nutrition shapes excretion and eventually eutrophication in temperate shallow lake ecosystems like most large-sized European ponds. We point out that improved ecosystem resource utilization efficiency (RUE) and tackling eutrophication may be achieved by 'bio-manipulating' temperate shallow lake ecosystems like large European ponds towards a balanced fish nutrition (≈proposed approach). Besides extrinsic factors, the highest ecosystem RUE, highest ecosystem services and least internal N, P loading depends much on nutrition availability for fish in ponds.

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CHAPTER 7

RECYCLE AND RE-USE OF NUTRIENTS: FEED FORMULATION DATABASE FOR AQUAPONICS

Roy, K., Kajgrova, L., Mraz, J., 2021/2022. TILAFeed: a bio-based inventory for circular nutrients management and achieving bioeconomy in future aquaponics. Manuscript.

My share on this work was about 60%

TILAFeed: a bio-based inventory for circular nutrients management and achieving bioeconomy in future aquaponics

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Abstract

Future food systems aim to achieve improved resource use efficiency and minimized environmental footprint through a circular bioeconomy-based approach. Aquaponics is a hallmark of such circular food production. The image of a circular nutrient utilization efficiency in aquaponics is often weakened due to the daily use of additional inorganic fertilizers in such systems. As circular bioeconomy greatly emphasizes developing bio-based solutions, the presented novel inventory called 'TilaFeed' and its associated utility tools is a step towards achieving more circular nutrient utilization and bioeconomy in future aquaponics. Through the formulation of tailored fish feed that is compatible with aquaponic systems' needs (e.g., plant nutrient requirement, mineralization efficiency of microbial sludge digesters), the objectives of 'TilaFeed' are to (i) solve nutrient constraints in aquaponic systems, both for fish and plants; (ii) avoid or strongly limit artificial fertilizer use in aquaponics by smartly tailored aquafeeds; (iii) equip system managers with decision-making tools for improved nutrient planning of their aquaponic systems. TilaFeed is a bio-based inventory. It integrates material (nutrient) flow information from feed to fish (*in-vivo* nutrient partitioning, forms of excretion) to environment (*in-situ* nutrient loading, nutrient forms) and primary producers (mineralization by microbes, available nutrients to plants). Based on TilaFeed-Model, feed for future aquaponics may be more precisely formulated with the principle that nutrients are not only a resource for fish, but excreted nutrients from fish (feed) also fertilize the microbes and plants.

Keywords*: feed formulation; plant fertilization; feedstuffs; minerals; nutrition; excretion*

Highlights

- Present database may aid in centralized and 'one formula' solutions for aquaponics.
- Avoid or limit artificial fertilizer use in aquaponics by smartly tailored aquafeeds.
- Equip system managers with improved nutrient management of their aquaponic systems.
- Compatible with nutrient formulation software using linear or stochastic programming.

Introduction

As part of its green deal, the European commission vows to promote a future that would embrace a 'circular bioeconomy framework' for its food systems (Commission, 2019, 2020). The circularity aims to 'maintain the value' of land, products, materials, and resources for as long as possible. Its core principle is to minimize or avoid losses by design, reuse, remanufacturing, integrating, and recycling materials (Colombo and Turchini, 2021; Commission, 2019, 2020; de Boer and van Ittersum, 2018). Waste is not considered a waste per se but a resource in a totalitarian circular framework (Roy et al., 2021). Circularity demands a paradigm shift in thinking, changing focus from increasing productivity (present) to increased resource use efficiency (future), from animal to the farm level. Aquaponics is a hallmark of circular food production (Lunda et al., 2019; Roy et al., 2021). It combines intensive aquaculture by recirculation aquaculture systems (RAS) and hydroponic plant production in a closed-loop system. Waste from one system (fish) serves as a resource to the other system (plant) (Lunda et al., 2019). Although most nutrients for sustaining the plant's optimal growth are expected to be derived from RAS effluents (wastewater and sludge from fish excreta, uneaten food), plant nutrient deficiencies often jeopardize the closed-loop operation of such systems (Lunda et al., 2019). Approximately 30–40% of plant nutrients may still be missing in aquaculture effluents (Monsees et al., 2019). It is partly due to limited efficacy in the valorization of sludge, a nutrient reservoir (Lunda et al., 2019). Moreover, partly due to initially low, non-prioritized contents of some plant essential nutrients in the fish feed that would be left in ample amounts (for microbial sludge digestors or plants) after fish absorption. Like agriculture or hydroponic, plant nutrient fertilization is often carried out in aquaponics using artificial fertilizers (Baganz et al., 2021). Inorganic or synthetic fertilizers have an environmental footprint (Chen et al., 2020) and weaken aquaponics' circular, sustainable hallmark. The future bioeconomy targets all renewable biological resources to produce food, materials (e.g., nutrients), and energy; simultaneously, lesser dependency on synthetic alternatives.

Several models have been developed in the academic sphere of aquaculture to improve nutrient utilization by fish or minimize the environmental impacts of fed aquaculture (Conceicao et al., 2018). Existing fish growth models in intensive aquaculture (e.g., specific growth rate, daily growth rate, thermal-unit growth coefficient) combined with waste throughput models (e.g., Fish-PrFEQ Model, WASTEst tool, FEEDNETICS dynamic simulation model, Wittaya AquaOp farm) have significantly advanced the understanding of material flow in aquaculture, like the fate of nutrients in feed, from *in-vivo* (fish) to *in-situ* (water) (Bureau and Hua, 2008; Bureau et al., 2000; Cho, 2004). Most waste throughput models in aquaculture are fish bioenergetics and mass balance-based models. They have varying complexity, which either limits or broadens their application. Not all but some of these models have gone beyond the academic level or put into a day-to-day use in the aquaculture industry (Conceicao et al., 2018). Perhaps because most publicly available tools are currently limited in scope and do not include all published information or accruing knowledge regarding the nutrition of fish species. Moreover, these tools are based on statistical regression analysis. Each time a new species or a new concept of aquafeed formulation or feeding management is introduced into culture, it is necessary to start from scratch (Bureau and Hua, 2008; Conceicao et al., 2018). Contrary to just mathematical or single click models, a systematic collection of data packaged into a database or inventory allows transparency on the foundations used, enables flexibility in information flow, and simplifies refurbishing existing models and calculations whenever necessary.

Development of this novel inventory entitled 'TilaFeed' and its associated utility tools are aimed towards improved nutrient circularity and bio-based solutions to minimize inorganic fertilizer use in future aquaponics; also better nutrient planning of aquaponic systems. The objectives of 'TilaFeed' are: (i) to solve nutrient constraints in aquaponic systems, both for fish and plants; (ii) avoid or strongly limit artificial fertilizer use in aquaponics by smartly tailored aquafeeds; (iii) equip system managers with decision-making tools for nutrient planning of their aquaponic systems. Future circular bioeconomy greatly emphasizes the development of such bio-based technologies or solutions (de Boer and van Ittersum, 2018).

Materials and methods

Core working principle

We recognize that perhaps nutrition (and resultant excretion) is the most dynamic and regular process in living organisms, which involves the exchange of nutrients to and/or from the environment (Bureau et al., 2003; Cho and Bureau, 1998; Guillaume et al., 2001; Kaushik, 1995; Lunda et al., 2019; NRC, 2011; Roy et al., 2020a,b). In an aquaponic system, fish feed is supposed to be the only nutrient input daily followed by pH adjustment buffers or carbon sources, not artificial fertilizers. Fish feed is hereinafter referred to as aquafeed. Fishes through their particulate fecal (digestible) and dissolved non-fecal (metabolic) excretion may be regarded as pumps of nutrients for the plants (Roy et al., 2020a,b); via microbial digesters (Goddek et al., 2018; Lunda et al., 2019). Feeding and excretion processes may be increasingly targeted for bio-manipulation to address improved nutrient loop or circularity from animal (in-vivo) to farm (in-situ) level. Here an aquaponic fish waste throughput model is stepwise assembled. A set of data and their synthesis is compiled into sub-datasets for each step. These sub-datasets covered information (data) on all the necessary aspects like feed formulation, fish excretion, and aquaponic system fertilization. Lastly, a collection of sub-datasets is integrated into a data-based model (called TilaFeed-Model). TilaFeed-Model focuses on tailoring future aquafeeds and bio-manipulating fish excretion in a way that fulfills aquaponics plant needs. 'TilaFeed' is a systematically organized inventory, presented in the form of an Excel workbook.

Much work has gone behind to improve the mineralization performance of microbial sludge digestion processes in RAS and aquaponics (Goddek et al., 2018; Martins et al., 2010). However, not much has been done on tailoring aquafeed formulation that would tweak fish excretion (digestible losses, metabolic losses, and total losses) to fulfill aquaponics needs. It would require a thorough, representative, and quantified knowledge of fish nutritional requirements and nutrient partitioning schemes in fish bodies. To the best of our knowledge, TilaFeed is the first-of-its-kind inventory. It can be easily imported with any basic 'feed formulation software' in the market (e.g., WinFeed™). Tailored aquafeed for aquaponics can be formulated based on: (a) digestible nutrient content of different feed ingredients that would fulfill fish species' optimum nutritional requirements and; (b) excretion of plant-essential nutrients from fishes or nutrients available to plants from a given aquafeed. The fish nutrients considered were as follows: protein, lipid, ash, fiber, carbohydrates, energy, phosphorus, essential amino acids (n= 10), and some essential fatty acids. The plant-essential nutrients (n=14) considered were as follows: N, P, K, Mg, Ca, S, Na, Cl, Fe, Zn, Cu, Mn, Se, and Co. A justification of their selection is provided below. The inventory also includes values on mean and standard deviation separately so that stochastic formulas (with some degrees of certainty), instead of just linear formulas, can be computed by advanced software(s).

Global digestibility data on model fish species – Tilapia

In Europe, the most common fish species in aquaponics is tilapia (*Oreochromis* sp.), followed by other species like rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), and African catfish (*Clarias gariepinus*) (Baganz et al., 2021; Lunda et al., 2019; Villarroel et al., 2016). A global compilation on tilapia's apparent digestibility coefficient (ADC) data of different nutrients and different ingredients (plant- or animal origin) and/or mixed compound diets were collected from 109 peer-reviewed scientific articles in the English language. The articles were searched on 'Google Scholar' and 'Scopus' using the standard set of keywords used in Roy et al. (Roy et al., 2020a). The articles are listed in supplementary appendix.

The ADCs data were available in plenty for major proximate nutrient fractions (protein or N, lipid, ash, fiber, carbohydrates, essential amino acids) and up to macro-minerals like P or Ca. However, tilapia has limited to none of the ADC data for other plant-essential nutrients (except N, P, and Ca). In almost all the cases (Fig. 1–2), it can be noticed that the central representative value (mean) of a pooled dataset of ADCs irrespective of plant-, animal- origin ingredients or mixed diets corresponds well with the mean of either of those categories. The standard error belt of fitted generalized linear model (GLM) trendline was also narrow over the pooled dataset, implying high confidence in adopting it as a representative of others (Fig. 1–2). Thus, pooled mean and standard deviation values of the overall tilapia ADC dataset (on a diverse range of feedstuffs) were assumed as a good representative for further calculations (Fig. 1–2). For the data-deficient nutrients (e.g., K, Mn, Na, and others), the ADC value of ash was assumed as a representative proxy for further calculations as it fell within the range and was reasonably comparable (Fig. 3).

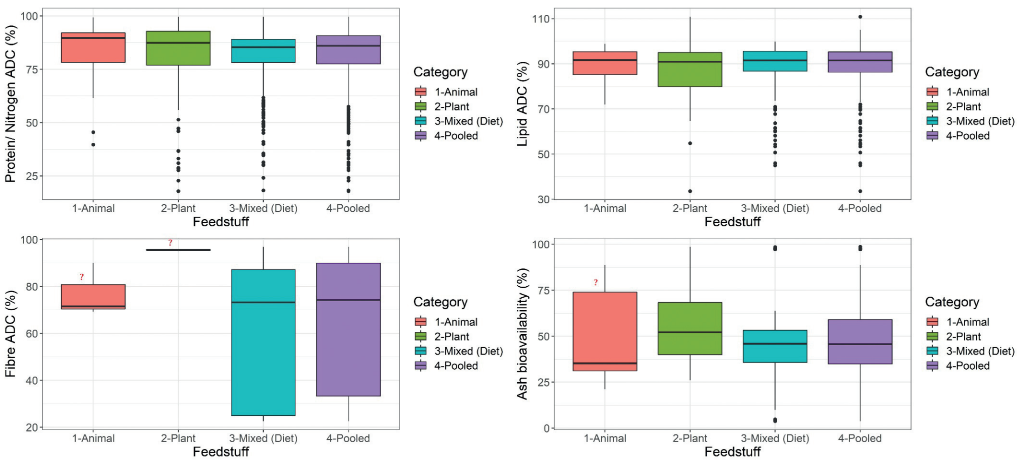

Figure 1. *Data on the feedstuff specific digestibility distribution of selected nutrients (protein or nitrogen, lipid, fibre, ash) by tilapia. ADC=apparent digestibility coefficient. Red question marks indicate limited data availability in the category. 'Pooled' data show the most representative values of digestibility which is well harmonized with other feedstuff specific digestibility distribution. Black dots indicate outliers.*

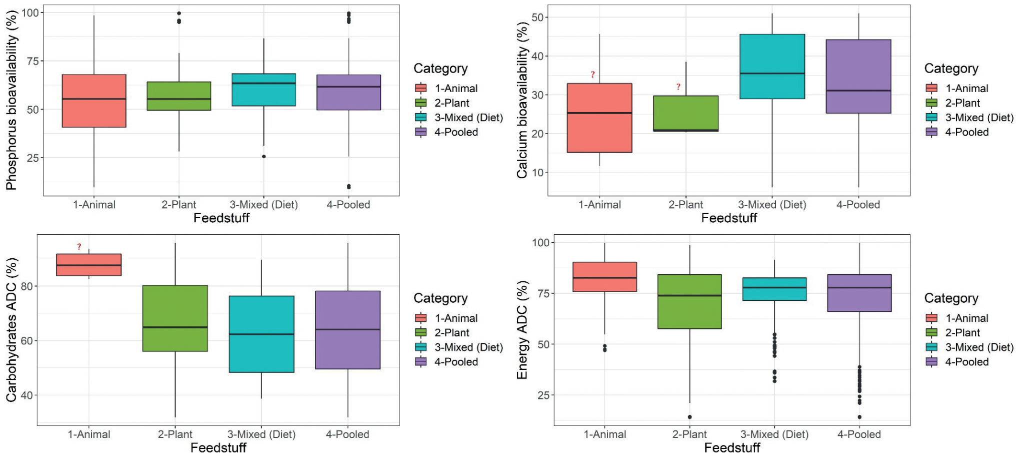

Figure 2. *Data on the feedstuff specific digestibility distribution of selected nutrients (phosphorus, calcium, carbohydrates, energy) by tilapia. ADC=apparent digestibility coefficient. Red question marks indicate limited data availability in the category. 'Pooled' data show the most representative values of digestibility which is well harmonized with other feedstuff specific digestibility distribution. Black dots indicate outliers.*

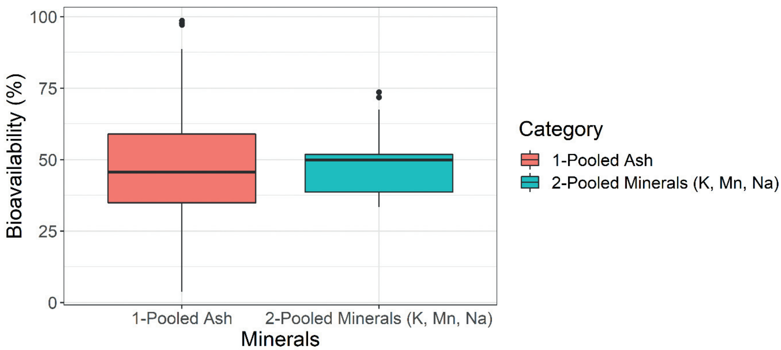

Figure 3. *Data on bioavailability of some minerals (K, Mn, Na; with limited data) in tilapia and bioavailability of ash being a potential proxy for such data-deficient minerals. Black dots indicate outliers.*

Global retention and losses data on model fish species – Tilapia

Apart from the digestible losses (=100–ADC% of crude intake) from fish, the metabolizable losses (=100-retention% – digestible losses % of crude intake) also contribute significant environmental loading of nutrients from the fish (Bureau et al., 2003; Hardy and Barrows, 2003; Kaushik, 1995; NRC, 2011; Roy et al., 2020a,b). The digestible losses, also called fecal losses, are particulate matter. They need microbes to mineralize the nutrients into reactive forms that the plants can readily assimilate. The metabolizable losses are also known as non-fecal losses (includes losses through urine and gills), and they are of dissolved nature (readily available forms of nutrients for plants). Altogether, they comprise total losses from fish, and it can be easily calculated from the retention as 100-retention% of crude intake. However, to demarcate the metabolic losses from digestible losses, one must also know the ADC values. For mapping the complete scheme of nutrient partitioning in fish, all four components are needed, *viz.* crude intake, retention, digestible losses, metabolizable losses. We, therefore, had to develop a representative nutrient partitioning scheme for tilapia, based on which the aquaponic nutrients flow can be mapped from fish feed over fish metabolism to plants.

To develop such a scheme of nutrient partitioning in tilapia, we first had to review and meta-analyze data from 46 globally published feeding and growth trials on tilapia, reporting the body nutrient composition. The articles were searched on 'Google Scholar' and 'Scopus' using the standard set of keywords used in Lunda et al. (Lunda et al., 2020), but for tilapia. The articles are listed in supplementary appendix.

Thermal growth coefficient (TGC), a marker of growth (Dumas et al., 2007; Iwama and Tautz, 1981), and retention of different nutrients were re-calculated according to Lunda et al. (Lunda et al., 2020). In terms of nutrients, about 14 nutrients (or minerals) were selected from the feed ingredient composition database (FICD) of the international aquaculture feed formulation database (IAFFD) that shared common importance both for fish (NRC, 2011) and plants (Lunda et al., 2019). The common priority nutrients (n=14) included in our inventory were (in ascending order of atomic mass): nitrogen (N atomic mass, 14.00 u), sodium (Na, 22.99 u), magnesium (Mg, 24.30 u), phosphorus (P, 30.97 u), sulfur (S, 32.06 u), chlorine (Cl, 35.45 u), potassium (K, 39.09 u), calcium (Ca, 40.07 u), manganese (Mn, 54.93 u), iron (Fe, 55.84 u), cobalt (Co, 58.93 u), copper (Cu, 63.54 u), zinc (Zn, 65.38 u), and selenium (Se, 78.96 u). From the reviewed literature on tilapia, retention data on 9 out of 14 nutrients, *viz.* N, Mg, P, K, Ca, Mn, Fe, Cu, Zn could be extracted. Retention values (Fig. 4) were used to estimate total losses, hereinafter termed as 'nutrient efflux' in the inventory.

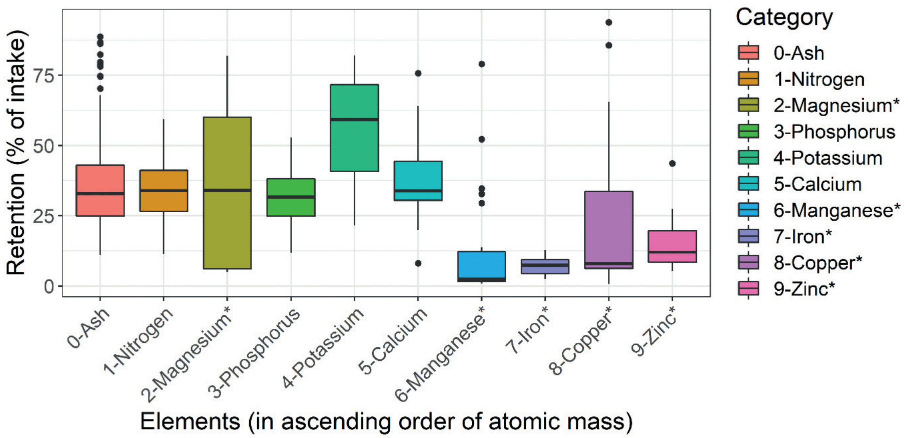

Figure 4. *Data on the retention of some common fish and plant essential minerals by tilapia. Arranged in the ascending order of atomic mass (see text). Note the decreased retention for heavier minerals. Asterix indicate recognized microminerals for fish (tilapia) nutrition. Black dots indicate outliers.*

Preparation and visualizations of TilaFeed inventory

For all the major feedstuffs used in the aquafeed industry worldwide, their crude nutrient content was acquired from IAFFD's open-access FICD database (iaffd_ingredients-v4.3.csv; provided in the supplementary). The TilaFeed has two main parts: (a) nutrition for fish (tilapia) and (b) nutrients for aquaponics (plants). The first part is covered within two sheets of the (Excel) workbook or TilaFeed inventory, namely- 'NOTES – FEED' and 'FEED FORMULATION.' The second part is covered within two other sheets of the inventory, namely- 'NOTES – AQUAPONIC' and 'AQUAPONIC MINERALS.'

For the first part, the crude nutrient contents were multiplied with average and standard deviation ADC values of tilapia (but percentages were converted to coefficients; 100%=1) to obtain average digestible and standard deviation of digestible nutrients, respectively, of a given ingredient. The information was then visually coded as follows: (a) relative strengths between feedstuffs in supplying a given nutrient (digestible) was visualized with in-cell bar-type conditional formatting of the datasheet, and (b) individual values of digestible nutrients were compared at par with tilapia's recommended nutrient specifications ((Chen et al., 2013; do Nascimento et al., 2020; Lim et al., 2011; NRC, 2011); also provided in 'NOTES – FEED') and applied in-cell value limit-type conditional formatting for visualization. Feedstuffs that were either deficient, rich, or balanced in nutrient supply (as per tilapia standards) were flagged for facilitating quick look and first-hand decisions by the users. Detailed instructions are provided in the inventory's 'NOTES – FEED' sheet.

For the second part, the crude nutrient contents of ingredients were multiplied similarly, but with nutrient efflux (total losses, including digestible and metabolizable losses) coefficients for tilapia. The average excretable and standard deviation of excretable nutrients of a given ingredient was obtained. The information was also visually coded as follows: (a) demarcating nutrients for plants that are essential, non-essential but beneficial for plants or risky in an aquaponic setup due to daily use of some pH adjustment buffers like sodium bicarbonate (Lunda et al., 2019), (b) relative richness or deficiency of nutrients (to be later available for plants) among feedstuffs were visualized by in-cell color-scale type conditional formatting of the values nutrient-wise. Detailed instructions are provided in the inventory's 'NOTES – AQUAPONIC' sheet.

Technical validation

To understand if the needs of fishes and plants can be addressed simultaneously in an aquaponic setup from a lens of tailored aquafeed, the nutritional dependencies for growth (Lunda et al., 2019) in tilapia were mapped from the growth trial data collected above. Since the inventory is mainly concerned with essential plant nutrients present in aquafeed, and ash is an overall crude representative of such group (clarified above), we focused on meta-analyzing the growth (*i.e.,* TGC) dependencies surrounding dietary ash. Generalized additive model (GAM), a non-parametric class of regression, was chosen because the model framework is based on the assumption that relationships between the predictors and the dependent variable follow smooth patterns that can be linear or nonlinear (Hastie and Tibshirani, 1986). Following Lunda et al. (2020) and Roy et al. (Roy et al., 2020a), generalized additive models (GAM) were generated individually with TGC as a response variable and dietary crude ash levels or ash retention % by tilapia as predictor variables. Whether the pattern of retention of ash (vis-à-vis pattern of excretion of ash) itself is a function of growth stages of tilapia was also assessed through a GAM having ash retention as response variable and body weight as predictor variable.

Data records

The open-access inventory (license type: attribution 4.0 International; CC BY 4.0) entitled 'TILAFeed' can be accessed as a .xlsx format spreadsheet; the main file is named 'Stochastic TILAFeed Inventory.xslx'. There are linked dependencies or associated sub-files to the inventory (e.g., WinFeed™ compatible files for feed store inventory and nutritional specifications of the animal concerned; also a directly importable template-specific spreadsheet for WinFeed™, a basic feed formulation software). Caution must be borne in mind to keep the dependencies within the same folder as the main file. The complete bundle of files as a zipped archive can be downloaded from the URL < https:// link provided by the publisher >.

Basic TilaFeed use case simulation – overall nutrient dosing from tailored feed to aquaponic system

A detailed step-by-step method of feed formulation in WinFeed™ using importable feedstuff inventory (like TilaFeed inventory) and set species requirements (like tilapia) is provided in a methodology on feed formulation (Roy and Mráz, 2021a). A model feed formulation for tilapia in an aquaponic system derived from TILAFeed and generated in WinFeed™ is used as a case example (see supplementary appendix, Fig. S1). We theoretically tested how the model feed would fertilize a standard aquaponic system. We simulated TilaFeed derived 'model feed' in the University of Virginia island (UVI) aquaponic system. UVI aquaponics has an aquaculture unit consisting of four fish rearing tanks (7800 L or 7.8 m^3 fish culture volume per tank) with a total aquaculture volume of 31,200 L (31.2 m^3). We considered the scenario that the UVI aquaponic system could support an average fish (red tilapia) biomass of ~44 kg m^{-3} and hold red tilapia with a median individual weight of 285.6 g (154 fish m^{-3}) (Rakocy et al., 2004). The median individual body weight was calculated from the average initial body weight (stocking material, 58.8 g) and average final body weight (harvest material, 512.5 g). To maintain such fish biomass (44 kg m^{-3}) and feed them (250 to 300 g fish) daily to apparent satiation (~3% of body weight), about 41.17 kg of model feed would be necessary every day in the overall aquaculture unit (31.2 m^3). Since the feed formulation using TilaFeed inventories in WinFeed™ also enable prediction of plant nutrient efflux per kg of feed (after passing through fish), we used the predicted values (per kg) of model feed. We estimated approximate nutrient dosing in the UVI aquaponic process water (reactive and particulate forms combined) 'weekly' when nutrient effluxes from feed use in the aquaculture compartment (41.17 kg per day) get diluted in the total system water volume of 111,196 L (Rakocy et al., 2004).

In aquaponic systems, it is recommended that hydroponic nutrient solutions, like Hoagland's solution (Hoagiand, 1933; Hoagland and Arnon, 1950; Trejo-Téllez and Gómez-Merino, 2012), be analyzed and replenished weekly. Without weekly analysis and replenishment, hydroponic nutrient solutions must be discarded after 2 to 3 weeks (Rakocy et al., 2004; Resh, 2012). We have estimated weekly (7 days) dosing of nutrients (irrespective of particulate or reactive forms) in the same logic. Because varying stages of growing plants (e.g., batches of basil, in a plant growing area of 214 m^2) in the UVI system seldom need all the nutrients daily or absorb them in equal proportions daily. As a result, the build-up or deficiency of specific nutrients in aquaponic process water is realized only after some time; ranging from 1 to 3 week(s) (Rakocy et al., 2004; Resh, 2012). Since there is a time lag between nutrient loading and utilization (Eck et al., 2019), at least weekly accumulation of nutrients in UVI aquaponic process water was estimated (as a benchmark). For this purpose, per day dosing of nutrients from feed (after dilution in total system water volume) was multiplied by seven. However, there is a provision of some daily water exchange in the UVI system (average daily from 0.26 to 0.46% of system volume) to compensate the water lost through sludge removal from filter tanks and clarifiers (cleaned once or twice a week); this also means loss of nutrients. Our present calculations do not cover this aspect and assume the scenario that sludge and associated wastewater are reused (a potent fertilizer, (Lunda et al., 2019)).

The weekly nutrient accumulation in UVI process water and its final concentration (in mg L^{-1}) was compared with the nutrient composition of Hoagland's solution. Hoagland's solution is a standard and popular hydroponic nutrient solution that provides all the necessary nutrients for plant growth and is appropriate for many plant species (Hoagiand, 1933; Hoagland and

Arnon, 1950; Trejo-Téllez and Gómez-Merino, 2012). Among the complete set of essential plant nutrients considered in TilaFeed, the nutrient composition of Hoagland's solution is as follows: N (210 mg L^{-1}), P (31 mg L^{-1}), K (234 mg L^{-1}), Mg (34 mg L^{-1}), Ca (160 mg L^{-1}), S (64 mg L^{-1}), Na (1.20 mg L^{-1}), Cl (0.65 mg L^{-1}), Fe (2.50 mg L^{-1}), Zn (0.05 mg L^{-1}), Cu (0.02 mg L^{-1}), Mn (0.50 mg L^{-1}), Se (not defined), and Co (not defined). Compared with typical nutrient ranges in hydroponic nutrient solutions suggested for different crops (e.g., tomato, pepper, lettuce, herbs, watercress, cucumber, strawberry, melon, rose; (Resh, 2012), https://www.smart-fertilizer.com/articles/hydroponic-nutrient-solutions/), Hoagland's solution seems to be a standard reference that fulfills most plants' needs. UVI aquaponic system had a maximum basil yield of 25 kg m^{-2} by batch culture method when optimum plant nutrient requirements were continuously met (Rakocy et al., 2004). Therefore, the differences of nutrient concentrations between Hoagland solution and nutrient dosed in UVI system process water (following use of model feed) were expressed in percentage to assess additional fertilization requirements to fulfill plant needs. Besides nutrient concentration, we considered nutrient balances or stoichiometry of nutrients, which is also vital for plant growth (discussed in (Lunda et al., 2019)). Since N is the most abundant nutrient in aquaponic process water (Eck et al., 2019; Lunda et al., 2019), N was taken as a reference (100%). The concentration of other nutrients relative to N was calculated to estimate 'nutrient balance.' The nutrient balance of Hoagland's solution was calculated and assumed as an ideal balance for plant growth (clarified above). Nutrient balance of UVI system process water (caused by nutrient dosing from model feed alone) was also estimated and compared with that of Hoagland's solution.

Advanced TilaFeed use case example – mapping plant available nutrient forms per kg of model feed

Standard aquaponic systems have mineralization units or microbial sludge digesters. For example, in the UVI system, a combination of a sump, filter, and degassing tanks having an altogether volume of 1306 L (1.31 m^3) connects the fish compartment to the plant compartment. The mineralization units convert the particulate form of plant essential nutrients (elements) to their dissolved, reactive forms, readily assimilated by the plants. Depending on the design and operating conditions, these mineralization units may have varying levels of efficiency (reviewed in (Goddek et al., 2018)). Based on the mineralization efficiency of some common microbial sludge digesters in aquaponics, data on mineralization of primary plant nutrients (N, P, K, Ca, Mg; available in the literature) was taken from Goddek et al. (Goddek et al., 2018). The best-reported mineralization efficiencies of these nutrients were as follows: N (53% of 'particulate' influx), P (26%), K (26%), Ca (26%), and Mg (71%). These values were divided by 100 and converted to 'mineralization coefficient.'

Based on the TilaFeed-Model scheme of the breakdown of tilapia's excretion (see Fig. S2 in supplementary appendix), the model feed's N, P, K, Ca, and Mg effluxes were divided into dissolved losses and particulate losses. The dissolved loss was calculated by multiplying the nutrient efflux of model feed (per kg) with the coefficient of dissolved loss of a particular nutrient from tilapia (*i.e.,* percentage share of dissolved losses on total losses). Coefficients of dissolved losses were as follows: N=0.73, P=0.39, K=0, Mg=0.22, and Ca=0 (re-calculated from TilaFeed-Model excretion breakup). Likewise, the particulate loss was calculated by multiplying the nutrient efflux of the model feed with a coefficient of particulate loss of the concerned nutrient. Coefficients of dissolved losses were as follows: N=0.27, P=0.61, K=1.0, Mg=0.78, and Ca=1.0 (re-calculated). The dissolved losses, *i.e.,* in 'originally reactive forms' (ORF), do not require mineralization; so, the values were kept as it is. The particulate losses were multiplied by their respective mineralization coefficients to convert them to equivalent 'mineralized reactive form' (MRF). Lastly, the ORF and MRF were summed to determine plant available nutrient forms per kg of model feed.

Results

Origin and uses of TilaFeed

TilaFeed is a centralized inventory for fish (tilapia) nutrition and aquaponic system (plant) fertilization. It is workable with most feed formulation software, through importable feed ingredient inventory and inventory for predicted nutrient (mineral) efflux from the feed ingredients or any feedstuff combination fed to Tilapia. The former, i.e., feed ingredient inventory, covers all major aquafeed ingredient categories with data spanning from proximate composition to energy and micronutrient level (amino acids, fatty acids). The latter, *i.e.,* inventory for predicted nutrient efflux from ingredients, covers 14 essential plant elements (nutrients) for planning fertilization of aquaponic systems. The data in TilaFeed Excel workbook can also be imported and worked with nutrient or feed formulation software(s) that support stochastic (probabilistic) formulation approach and linear programming approach. In a nutshell, TilaFeed can generate stochastic results using mean and standard deviation data included in the worksheet separately. Whether linear or stochastic, the formulations can also be optionally combined with the least-cost approach (if the software supports) because TilaFeed has a provision of prices data for each feedstuff. TilaFeed also enables tracking of strength or weaknesses of each feed ingredient in terms of either optimum digestible nutrient supply for fish or plant fertilization strength through their predicted mineral efflux. A mind map of TilaFeed and its logic is given in Fig. 5.

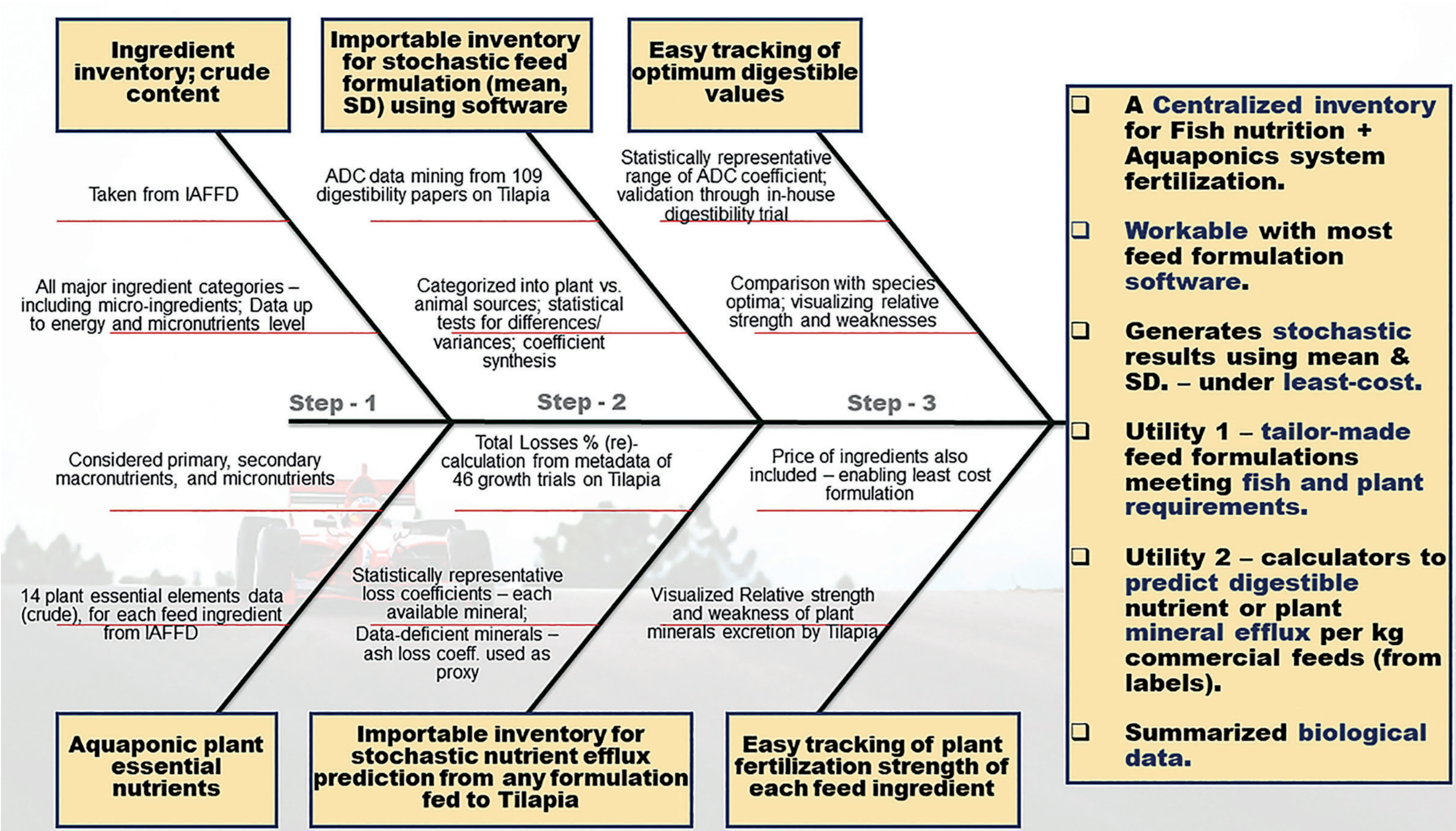

***Figure 5**. Mind-map of the origin and uses of TilaFeed inventory. Overlayed on a 'formula-one' background to accent its intended 'one formula' solution for aquaponics (visible in electronic form only).*

Composition of 'TilaFeed'

Main inventory (TilaFeed inventories)

The central inventories include inventory for digestible nutrients for fish (tilapia); inventory for available nutrients for re-valorization in the aquaponic system (plants) from a formulated feed (combination of feedstuffs). The central inventories are included within the "FEED FORMULATION" and "AQUAPONIC MINERALS" sheets of TilaFeed. Indicative prices of feedstuffs (from alibaba.com and tridge.com; as of August–September 2020) are also included in TilaFeed to enable least-cost formulations. Tailor-made feed formulations meeting fish and plant requirements can be made using these inventories. However, due to the dynamic nature of prices, the users are advised to reconsider the prices of the ingredients according to their source. Also, if users have precise knowledge of the crude nutrient composition of their exact feedstuffs (under consideration), either the values nearest to them should be relied upon from central inventories, or the users may re-calculate based on the crude data.

Calculators or converters (TilaFeed-Model)

As part of some utility tools, some calculators or converters are included. For example, the crude nutrient to digestible nutrient in feed, or digestible nutrient to crude nutrient in feed; crude nutrient in feed to aquaponic system fertilizable nutrient content. They are included within the "NOTES – FEED" and "UTILITIES & FORMULATION" sheets of TilaFeed. Prediction of digestible nutrients for fish or plant mineral availability (efflux) per kg of any feed can be made through these calculators or converters. Only information on crude nutrient contents of such feed (either from the label of commercial feed or proximate composition of farm-made feed) is necessary. These utility tools are based on TilaFeed-Model (see methods).

Approximate breakdown of tilapia's excretion (TilaFeed-Model)

A breakdown of tilapia's excreta between dissolved and particulate losses is given for aquaponic system planners. Plants readily assimilate dissolved losses, while particulate losses need to be mineralized first and then assimilated by the plants. The mineralization efficiency of some common microbial sludge digesters in aquaponics (Goddek et al., 2018) is summarized for precise calculations by the users. It is included within the "UTILITIES & FORMULATION" sheet of TilaFeed. This utility tool is also based on TilaFeed-Model.

WinFeed™ (feed formulation software) compatible files and examples of output

One-click downloadable files for the inventory of feed ingredients (feedstuffs) and animal (tilapia) requirements are included. Two sets of files are given: (a) for direct launch in WinFeed™ program (file formats: .fst and .wff); (b) for import to WinFeed™ program from a pre-programmed (template) excel file provided by the software developer. Links with download icons are included within the "UTILITIES & FORMULATION" sheet of TilaFeed.

Basic TilaFeed use case example – overall nutrient dosing from model feed to aquaponic system

A model feed formulation derived from TILAFeed and generated in WinFeed™ is given as a case example. It is referred to as 'model feed.' Detailed information on the model feed can be found in the "UTILITIES & FORMULATION" sheet of TilaFeed. As a good example of circularity, the model feed was deliberately formulated using by-product derived or alternative feed ingredients. It fulfills tilapia's optimum 'digestible' protein, amino acids, lipid, fatty acids, phospholipids, Calcium (Ca), Phosphorus (P), and dietary energy requirements, including proper protein: energy balance. The model feed's nutrient efflux or plant fertilization potential is also estimated. The cost of the model feed formulation can be better estimated if the current prices of the ingredients are known. Since the cost of ingredients may vary quite dramatically and often unpredictable at times, the estimated cost of the model feed formula is not discussed. A screenshot from the "UTILITIES & FORMULATION" sheet of TilaFeed is provided and explained at the end of the supplementary appendix (Fig. S1).

The model feed was simulated in UVI aquaponic system (see methods). A detailed breakup of model feed's plant fertilization potential is provided in Table 1. Results suggest that the model feed fulfills plant needs for nutrients differently, from fulfilling only 5.6% of plant requirement for S to complete fulfillment of Cu. In terms of nutrient contribution and nutrient imbalances in UVI process water (caused by model feed), three elements were identified as deficit or imbalance 'of concern,' *viz.* K, Ca, and S (Tab. 1). For some elements like Na, Cl, and Zn, the model feed can fulfill more than plant requirements; excessively for Na (alarmingly) and Cl. Considering the nutrients that are supplied in moderately deficient (N, P, Fe, Mn) to highly deficient (K, Mg, Ca, S) amounts, it seems that the model feed may contribute on an average ~31% of plants' needs. In other words, up to 1/3rd of the maximum possible basil yield in the UVI system (*i.e.,* ~8 kg m^{-2} out of 24 kg m^{-2}) may be realized if the model feed is applied in the UVI system carrying specified biomass of red tilapia and fed to apparent satiation (see methods).

Rest of the plant growth is necessary to be contributed as follows: (a) either by supplementary fertilization on an average +3.4 times for all the deficient nutrients (except S) and +18 times for S, than the feed could provide; (b) or by downsizing the plant cultivation area by at least half, to double (concentrate) the nutrients in order to contribute ~62% of plant growth or achieve up to 2/3rd of maximum basil yield in UVI aquaponic system (*i.e.,* ~16 kg m^{-2} out of 24 kg m^{-2}), through use of feed alone. However, the downsizing approach needs probing since the risk of higher Na and Cl concentrations may or may not stress the plants (discussed later). Concerning plant needs, elements K, Mg, Ca, S need simultaneous optimization in model feed formula and may also require fertilization (even smart choices of daily pH buffer, e.g., KOH or $CaMg(CO_3)_2$) simultaneously (Tab. 1). Besides, the model feed needs to be optimized by selecting ingredients lower in Na and Cl efflux (Tab. 1). These examples demonstrate how TilaFeed could contribute to aquaponic system fertilization planning.

Table 1. *Nutrient dosing and plant fertilization potential of TilaFeed model feed when used in UVI aquaponic system raising red tilapia and basil (see, methods). Detailed composition of model feed can be found in supplementary appendix (Fig. S1).*

Nutrient	**Impact of model feed in process water**			**Standard reference for plants**		**Strength/ weakness of model feed**	
	Nutrient efflux per kg of model feed (mg)*	**Nutrient accumulation in process water – weekly (mg L-1)****	**Nutrient balance in process water (% of N)****	**Reference conc. – Hoagland hydroponic solution (mg L-1)#**	**Nutrient balance in Hoagland solution (% of N)#**	**Nutrient contribution by model feed (% of reference conc.)##**	**Nutrient imbalances in process water (due to model feed)†**
Nitrogen	4,1310	107.06	100 (ref.)	210	100 (ref.)	51.0	Not computed
Phosphorus	4,640	12.02	11.23	31	14.76	38.8	-3.53
Potassium	17,340	44.94	41.97	234	111.43	19.2	-69.45
Magnesium	2,780	7.20	6.73	34	16.19	21.2	-9.46
Calcium	13,170	34.13	31.88	160	76.19	21.3	-44.31
Sulfur	1,380	3.58	3.34	64	30.48	5.6	-27.14
Sodium	39,670	102.81	96.03	1.20	0.57	8,567.31	95.46
Chlorine	2,880	7.46	6.97	0.65	0.31	1,148.27	6.66
Iron	326.41	0.85	0.79	2.50	1.19	33.8	-0.40
Zinc	55.83	0.14	0.14	0.05	0.02	289.39	0.11
Copper	8.20	0.02	0.02	0.02	0.01	106.3	0.01
Manganese	103.58	0.27	0.25	0.50	0.24	53.7	0.01
Selenium	1.29	0.003	0.0031	Undefined	–	–	–
Cobalt	0.01	<0.001	<0.001	Undefined	–	–	–

*After passing through fish. See Fig. S1.
**After dilution in UVI system process water, 24,247 L. See methods.
#Standard requirement of most hydroponic plants (see methods). Nutrient balances calculated on percentage of nitrogen basis (N content as 100%).
##Nutrients <100%= deficit, <25%= deficit of concern (K, Mg, Ca, S – **require fertilization**), >100%=excess, >200%=concerningly excess (Na, Cl, Zn).
†Value ≈ 0 is balanced. Values below -10 or above +10=imbalanced (K, Ca, S, Na – **need feed re-formulation**).

Advanced TilaFeed use case example – mapping plant available nutrient forms per kg of model feed

Besides the abovementioned use case example, which is somewhat basic, a more advanced use case example can also be facilitated by TilaFeed. For instance, tracking nutrient efflux from the model feed in higher definitions, such as particulate fractions (later mineralized to reactive forms) and originally reactive forms (ORF) of excreted nutrients. In the end, prediction of plant-available nutrients after considering ORF and mineralization efficiency (of particulate nutrients) in microbial sludge digestors of aquaponic systems can be made. A model simulation of plant available nutrient forms that can be expected from 1 kg of model feed is demonstrated in Tab. 2. One good outcome of such high-definition simulation may be an estimation of 'dilution water volume' in which reactive (dissolved) plant-available nutrients per kg of model feed may be dissolved 'responsibly.' Here, the term 'responsible' imply achieving two contexts simultaneously: (a) avoiding excess concentrations of some nutrients in process water (e.g., N, P; Tab. 2) and, (b) ensuring minimum use of fertilizers to supply largely missing nutrients (e.g., K, Mg; Tab. 2).

Table 2. *Breakdown of nutrient forms and plant available nutrient supplied per kg of TilaFeed model feed. Nutrient selection in table is limited to those nutrients whose mineralization efficiency data in aquaponics is available (see methods). Detailed composition and nutrient efflux of model feed can be found in Fig. S1. Breakup of tilapia's forms of excreted nutrients can be found in Fig. S2.*

Nutrient	Nutrient efflux of model feed (g)	Excreted– originally reactive form (g)	Excreted-particulate form (g)	Mineralized reactive form (g)	Plant available nutrient (g)	Dilution to Hoagland's solution concentration*
Nitrogen	41.31	30.04	11.27	5.97	36.01	170 L
Phosphorus	4.64	1.80	2.84	0.74	2.54	80 L
Potassium	17.34	0.00	17.34	4.51	4.51	19 L
Magnesium	2.78	0.61	2.17	0.56	1.18	34 L
Calcium	13.17	0.00	13.17	9.35	9.35	58 L
Average**						72.2 L

*Amount of water required for dissolving the plant available nutrients to achieve concentrations equivalent to that of Hoagland's solution – an ideal hydroponic solution (see, Tab. 1).
** 'Responsible dilution volume' that has trade-offs of avoiding too high N, P concentrations and ensuring minimum fertilizer requirement for K, Mg and Ca.

Of course, advanced simulations using TilaFeed have some inherent limitations. Mineralization efficiency (performance) of mineralization units may greatly vary depending on the nutrient load (influx, efflux), operating conditions (biological, chemical parameters, turnover of sludge), or even day-to-day fluctuations. Even the ratio of particulate and dissolved forms of nutrient excretion by fish may have daily variations. Therefore, such simulations should only be deemed representative, first-hand information that needs to be practically validated depending on the type of system (beyond the scope).

Discussions

Global digestibility data on model fish species – Tilapia

A summary of tilapia's ADC data for animal-origin feed ingredients, plant-origin feed ingredients, mixed compound diets, and overall pooled data can be found in Fig. 1, 2, and 3. The summarized values of ADC used for the calculation are given in TilaFeed's "NOTES – FEED" sheet. The justification of using such central values is because of the statistically insignificant differences that were seen in the end in terms of the measures of central tendency between ingredient levels (plant- or animal-), compound diets (with ingredients of different origins mixed), and pooled data (mentioned above). One of the possible reasons could be that no feedstuff is fed alone; they are always mixed in different proportions into a diet (individual ingredients seldom exceed ~40–50% of a diet in commercial formulations; mostly around ≤30%) fed by the fish. So, the ingredient-specific digestibility differences tend to dampen, average out, and reach a central tendency that we used in the present calculations.

To determine if the literature's ADC values are reliable enough, we also conducted in-house digestibility trials with tilapia on six locally important protein sources in tilapia feed (Roy and Mráz, 2021b). We found the ADC values to be mostly reliable. The processes and results of ADC validation can be found in detail (Roy and Mráz, 2021b). Nonetheless, a comprehensive list of ingredient-specific digestibility is given in the "NOTES – FEED" sheet of TilaFeed if the users customize the values to suit their needs better. A snapshot of validation results is also provided at the end of the digestibility data table in the "NOTES – FEED" sheet of TilaFeed.

Global retention and losses data on model fish species – Tilapia

The retention range of these nutrients is depicted in Fig. 4. The retention of ash is somewhat comparable to the retention of nutrients having an atomic mass below 40 u (*i.e.,* below calcium; Fig. 4). It is perhaps because ash is a crude mixture of all these generally incombustible inorganic minerals present in higher proportions (ISO 1575:1987). However, heavier nutrients having atomic mass >54 u like Mn, Fe, Cu, and Zn seem to be retained at much lower levels (Fig. 4). However, to the best of our effort, there was no possibility to extract retention data on the rest 5 out of 14 nutrients *viz.* S, Na, Cl, Se, Co. The summarized coefficient of nutrient efflux or non-utilization of different minerals in tilapia is given in the "NOTES – AQUAPONIC" sheet of TilaFeed. Since there were no available data on S, Na, or Cl, and their atomic mass fell below 40 u (or that of calcium), the retention average and standard deviation of ash were assumed for these nutrients (clarified above). Besides, data were also unavailable for Se and Co, which are much heavier. Considering the lower retention levels of heavier nutrients (>54 u atomic mass, or greater than Mn; see above), a representative average and standard deviation of retentions of Mn, Fe, Cu, Zn by tilapia were assumed for Se and Co. The lower retention of heavier minerals in aquafeed by fish is perhaps linked to their inherent toxicity effects at higher levels (Lall and Kaushik, 2021). Hence, caution must be exercised while tailoring them to suit aquaponic plant needs so that the aquafeed itself does not become toxic to fish.

On the other hand, plants have low requirements of Na, and it is generally considered toxic to hydroponic crops at higher concentrations (Kronzucker et al., 2013). The maximum acceptable Na level in the root zone of most greenhouse crops can range from 23 to 184 mg L^{-1} (AkzoNobel et al., 2020); as a thumb rule, less than 50 mg L^{-1} Na content is considered safe for hydroponic nutrient solutions (https://www.smart-fertilizer.com/articles/hydroponic-nutrient-solutions/). Unlike other elements, Na and its associated element Cl are not readily absorbed by plants. Sodium can easily accumulate in aquaponic systems where sodium bicarbonate is used daily. Daily application of fish feed containing fishmeal and animal meal also brings high salt content (Eck et al., 2019; Robaina et al., 2019). There are also instances where high Na levels did not retard the growth of aquaponic crops (Goddek and Vermeulen, 2018), or the upper limit of Na toxicity in aquaponic systems could be higher (Lunda et al., 2019; Resh, 2012). Future tailor-made feed formulations for aquaponics may not solve such issues alone. Instead, feed should complement aquaponic system management itself. An example is given as follows: (a) formulating feed with lower Na, Cl efflux, (b) minimize the use of sodium bicarbonate as pH buffer, replaced with alternatives tailored to system needs (KOH or $CaMg(CO_3)_2$), (c) probing tolerance of various greenhouse crops in aquaponic conditions and perhaps growing without any obvious salt stress.

Technical validation

Results of the GAMs indicated few thresholds and patterns. TGC in tilapia attenuates a plateau when dietary crude ash reaches around 5% of the diet and then remains unfazed (TGC vs. dietary ash GAM statistic: deviance explained 6.13%, $p<0.01$) (Fig. 6). TGC vs. ash retention GAM suggests that after a required level of ash is retained (~30% of crude intake), no further reinforcement in growth is provided (GAM statistic: deviance explained 18%, $p<0.01$) (Fig. 6). Besides, as the tilapias become older and bigger (≥75 g body weight), their retention efficiency of dietary ash also declines (ash retention vs. body weight GAM statistic: deviance explained 14.4%, $p<0.01$) (Fig. 6). Perhaps because of decreasing requirements compared to juvenile stages (≤50 g body weight). Looking at such low but highly significant association between growth and dietary ash or ash retention in GAMs statistics (*i.e.,* deviance explained

6.13% to 18%, but *$p<0.01$*), it is indicative that ash (or minerals) are necessary for fishes (quite obvious; (Lall and Kaushik, 2021)), but they do not contribute very strongly to growth. Also, beyond some required levels, the fishes most likely eliminate the excess dietary minerals; available for re-valorization in aquaponics (plants).

These findings validate the opportunity to tailor aquafeed to suit aquaponics needs or aid nutrient planning of aquaponic systems in general. Also, caution must be exercised while tailoring them to suit aquaponic plant needs so that the aquafeed itself does not become toxic to fish (clarified above; (Lall and Kaushik, 2021)). We also qualitatively determined the effects of feeding ration or daily feed dose (in % of body weight fed) on the ash retention efficiency (or total losses of ash) by tilapia. The ash retention efficiency remains almost unfazed irrespective of the daily feeding dose (Fig. 6). Therefore, feeding tilapias in aquaponics near apparent satiation on a tailored aquafeed most likely may secure better dosing of nutrients, without any significant compromises caused by feeding management decisions itself, for the plant compartment.

Conclusion

The present study delivers an important contribution to improve the nutrients efficiency of aquaponics. It introduces an Excel workbook concerning nutrient components (both for fish and plants) which is compatible with nutrient formulation software.

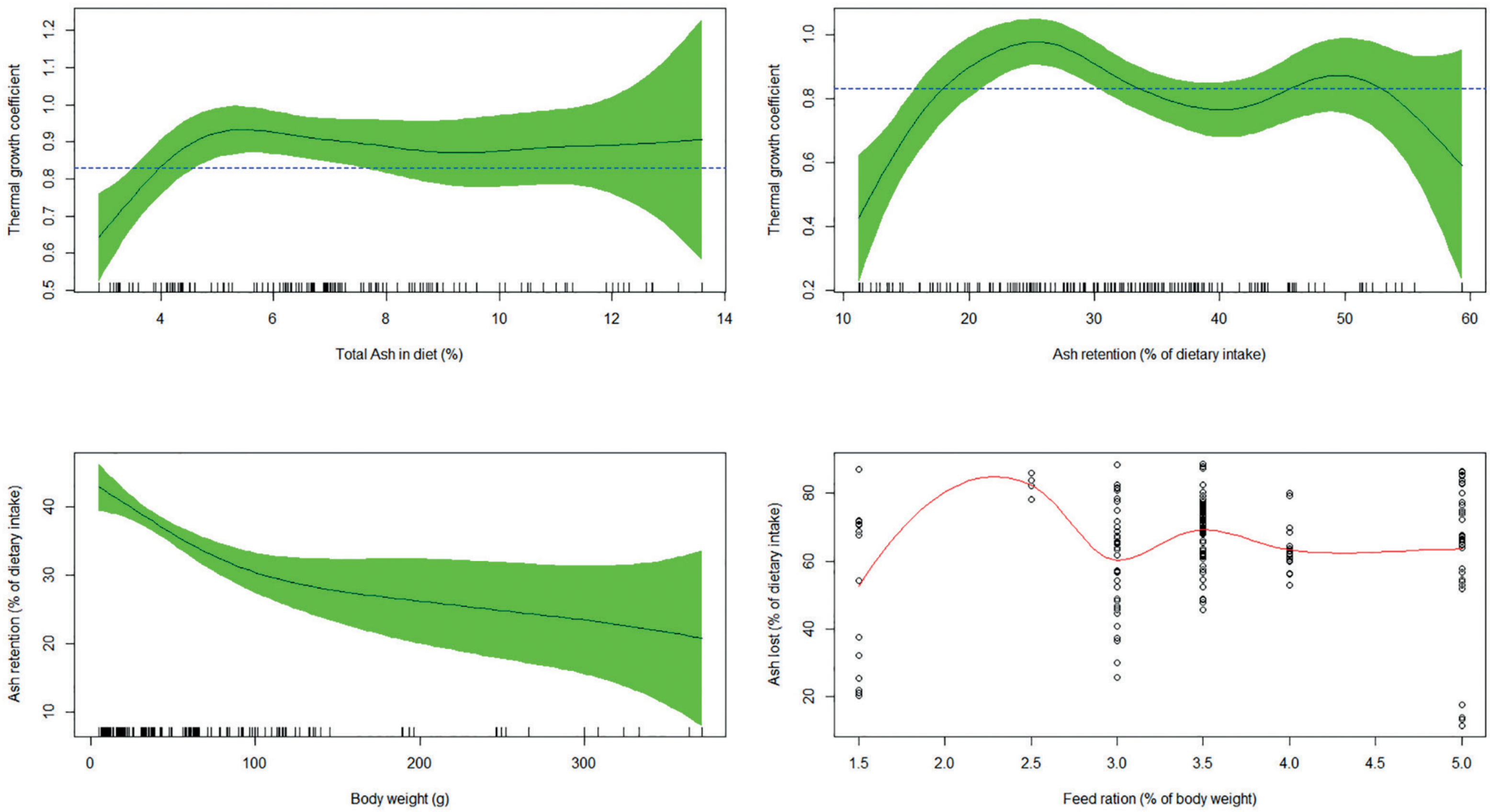

Figure 6. *Utilization and excretion of minerals (represented by ash here) by tilapia under different conditions. The horizontal blue dashed line on thermal growth coefficient (~0.83 units) indicate reasonably good growth levels in tilapia. Detailed explanations individually can be found in text.*

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CHAPTER 8

ALTERNATIVES TO FINITE RESOURCES: FISHMEAL FISH OIL REPLACEMENT IN CARP FEED

Roy, K., Mraz, J., 2021. Alternative feed components to replace fishmeal and fish oil in carp feed. Edition of Methodics. University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic, No. 184, 30 pp.

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My share on this work was about 60%.

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Edition of Methodics 184

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CONTENT

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL AND FISH OIL IN CARP FEED

1. BACKGROUND

Cyprinids comprise about 38% of all aquaculture (by weight) and represent a crucial edible protein source produced through aquaculture. Carps, feeding lower on the food chain, need a relatively large amount of land per unit of protein produced (Waite et al., 2014). The common carp (*Cyprinus carpio* L.) is the oldest domesticated aquaculture species in the world and the most popular representative of cyprinids in aquaculture (Balon, 1995). It is the main farmed species in European freshwater aquaculture with production localized mainly in central and eastern Europe. The Russian federation (0.06 Mt) followed by Poland (0.02 Mt), the Czech Republic (0.02 Mt), Hungary (0.01 Mt) and Ukraine (0.01 Mt) represented about 70% of carp production in Europe in 2016 (FAO FishStat, 2017). The land-locked central European countries rely heavily on common carp aquaculture. For example, in the Czech Republic with 41,080 ha of fishponds (70% of which are of 0.5–3 ha), common carp has consistently comprised >85% of total aquaculture production (CZ-Ryby, 2019). The average productivity of carp culture systems in central Europe ranges between 0.3–1 ton ha^{-1} (Sterniša et al., 2017). Like other aquaculture practices worldwide, the common carp aquaculture has considerably intensified over the years. This has led to an increase in both stocking density and provision of supplementary feeding to enhance the yield (Potužák et al., 2007; Hlaváč et al., 2014). The European common carp production, in terms of volume, reached its peak (0.18 Mt) during 2009–2010 and has been declining since. In terms of value, the decline appeared later – the production peaked during 2011–2012 (0.45 million USD) and started to decline afterwards (0.38 million USD in 2016) (FAO FishStat, 2017).

On a global scale, the estimated commercial aquafeed production is approximately 40 million tons, and it is predicted to increase to more than 85 million tons by 2025 (Kim et al., 2019). Globally, carp aquaculture was estimated to consume about 13.5 Mt of aquafeed, i.e. 27% of the global aquafeed produced in 2015 (Tacon and Metian, 2015). Common carp alone consumed ~37.5% (~5.1 Mt) of aquafeed globally produced for carp aquaculture in 2015; only ~0.2 Mt of aquafeed was used in Europe (Roy et al., 2019). Fishmeal and fish oil from capture fisheries have been the main protein and lipid sources in aquafeed, especially those designed for intensively reared high-trophic-level species of fish. The production of fishmeal and fish oil is anticipated to be exhausted in near future, meaning it will not be able to cover the increasing demand of these ingredients for animal feed manufacturing industries. Thus, continued dependency on fishmeal and fish oil is ultimately unsustainable for the aquaculture sector (Kim et al., 2019). Moreover, the increased environmental footprint associated with the use of fishmeal and fish oil demands cheaper, readily available, highly digestible and eco-friendly feedstuffs of plant and microbial origin to be used (Papatryphon et al., 2004; Aubin et al., 2009).

2. THE AIM OF THE METHODOLOGY

Compared to the 1990–2000s, the proportion of fishmeal in carp feeds has decreased in recent years (Searchinger et al., 2013; Waite et al., 2014; Tacon and Metian, 2015). It is presumed that common carp is much easier to be produced without the use of fishmeal or oil than predatory fish species, such as trout or salmon (Biermann and Geist, 2019). In this light, the aims of the methodology are the following:

- To informed on the range of feed ingredients and compositions of present-day commercial carp feeds.
- To informed on the general range of carp's nutrient utilization capacity and crude nutrient-energy levels in artificial feedstuffs.
- To demonstrate a methodology (i.e. fishmeal, oil substitute) using a database of feed ingredients (containing information on digestible nutrients and energy).
- To provide a database of the optimum nutritional requirements of carp at macro- and micronutrient levels.
- To understand inclusion levels of different feedstuff groups for achieving nutritional balance in the carp.
- To identify bottlenecks of the production and formulate carp diets with no or minimal use of fishmeal and fish oil.
- To discuss problems of fishmeal and fish oil replacement and identify potential alternatives.

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL AND FISH OIL IN CARP FEED

3. NOVELTY OF THE METHODOLOGY

Many alternative ingredients (vegetable, microbial, animal and insect origins, etc.) may have lower digestibility than highly digestible fish meal and fish oil (>90% digestibility) due to presence of anti-nutritional factors such as ash, fibers, chitin, phytate and bone-phosphorus, hence it is not advisable to formulate 'replacement (fish meal, fish oil) diets' using crude nutrient values or assuming equally high digestibility (>90% of crude content) of alternative ingredients. In this case, it is wiser and safer to base the formulation on 'digestible nutrient basis' rather than simply use crude values of alternative ingredients replacing fish meal and fish oil.

Detailed nutritional information on the ingredients (i.e. alternative protein or lipid sources for carp) are usually compiled and made available through databases. Some databases are open access while others are proprietary. While most databases list data on 'crude content basis' only, there are few that list 'digestible values' of ingredients besides crude content. A list of such databases is provided below. The novelty of this methodology does not consist in the database itself, but in the approach to formulation and utilization of such databases (inventory) for successful fishmeal and fish oil replacement in carp feed.

By following this methodology, the R&D (research and development) section of any local feed manufacturer can create their own tailor-made database(s) based on the ingredients they plan to use or have in stock. The methodology is original in the following aspects: (a) formulating the feed in a more rational way rather than just using crude contents of feedstuffs; (b) using digestible nutrient values in feed formulation; (c) utilizing such feedstuff inventory; (c) creating least cost formulation considering the optimum 'digestible' nutrient (from macro- to micro-) requirement. For demonstration purposes, we worked with an internally-developed in-house database tailor-made for common carp known as "ZeroFish CarpFeed" (fishmeal and fish oil-free carp feed ingredients database) which is be available from the author on request (Assoc. Prof., Jan Mráz, Jihočeská univerzita v Českých Budějovicích, Fakulta rybářství a ochrany vod, Na Sádkách 1780, 370 05 České Budějovice jmraz@frov.jcu.cz). Since any database needs to be continuously updated (otherwise it becomes obsolete), there is no permalink to the database.

4. AVAILABILITY AND DEVELOPMENT OF THE DATABASE

Few online free aquafeed ingredient databases, which can be used by software for feed formulation, are listed below. The readers are advised to check whether 'digestible values' are provided and whether these digestible values are derived from (or apply to) carp.

- International Aquaculture Feed Formulation Database (IAFFD) – https://www.iaffd.com/
- Digestibility Database (Trout-Grains Project, USDA) – https://www.ars.usda.gov/pacific-west-area/aberdeen-id/small-grains-and-potato-germplasm-research/docs/fish-ingredient-database/
- INRAE-CIRAD-AFZ Feed Tables – https://www.feedtables.com/
- AMINODAT®/AMINOCARP® (Evonik Industries; premium only) – https://animal-nutrition.evonik.com/en/services/animal-nutrition/aminodat
- ZeroFish CarpFeed (digestible database tailor-made for carp) – Internally developed. Available on request at jmraz@frov.jcu.cz

While creating such database at company level, please follow the following instructions: (a) review common carp's **digestibility data** (calculate interquartile range IR; see, Roy et al., 2019), or, directly use the values in Tab. 3; (b) search **crude nutrient content** of ingredients (dry matter basis) in databases like IAFFD; (c) multiply it with the IR of digestibility to calculate the IR of '**digestible nutrient**'; (d) cross-match digestible values of ingredients with **optimum requirements** of carp (NRC, 2011) and make the datasheet self-explanatory using **conditional formatting** (to find 'strengths and weaknesses' of each ingredient); (e) include **prices** of ingredients (tax included) in the database (from local suppliers or Alibaba.com®) to enable the software to calculate the **least cost formulation**; (f) use the compiled datasheet including digestible values + prices of ingredients as 'feedstuff inventory' and optimum species (carp) requirement as a separate 'standards sheet' for the feed formulation software to compute the **optimum feed formula with the minimum cost**.

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL
AND FISH OIL IN CARP FEED

5. GENERAL FEED PROFILE AND NUTRIENT UTILIZATION IN CARP

In terms of crude nutrient and energy level, artificial carp diets contain protein (310–500 $g.kg^{-1}$ feed), lipid (37–118 $g.kg^{-1}$), carbohydrate (210–585 $g.kg^{-1}$), phosphorus (6.8–11.7 $g.kg^{-1}$) and energy (2,413–5,402 $kcal.kg^{-1}$), depending on the growth stage. A list of ingredients (excluding micronutrient premixes) presently used in premium carp feeds by the top aquafeed producers in Europe is included below (Tab. 1). The general range of artificial feedstuffs digestibility for carp is summarized in Tab. 2.

Tab. 1. *Checklist of macro-ingredients (excluding micronutrients) used in premium carp feeds in the EU.*

Category	Ingredients
Starter/ Fry feed	Corn Gluten, **Fishmeal, Fish Oil,** Hemoglobin, Krill Meal, Rapeseed Oil, Soya, Soya Protein Concentrate, Wheat, Wheat Gluten
	Corn Gluten, DDGS, Feather Meal, **Fishmeal,** Hemoglobin, Poultry Meal, Rapeseed, Rapeseed Oil, Soya, Soya Protein Concentrate, Sunflower Protein Concentrate, Triticale, Wheat
Grower feed	Corn Gluten, DDGS, Feather Meal, **Fishmeal,** Hemoglobin, Poultry Meal, Rapeseed, Rapeseed Oil, Soya, Soya Protein Concentrate, Sunflower Protein Concentrate, Triticale, Wheat
	Corn Gluten, Soya (GMO free), Wheat Whole, **Fishmeal**, Faba Beans, Rapeseed Oil
	Soy Meal (GMO), Wheat Flour, Toasted Soybeans (GMO), **Fishmeal**, Peas, Guar, Haemoglobin Powder, **Fish Oil**

Tab. 2. *Normal range of digestibility of different dietary components (from artificial feedstuffs) for carp.*

Dietary component	Range of digestibility (% of crude level)
Protein	79–99%
Lipid	80–93%
Carbohydrate	52–89%
Phosphorus	27–47%
Energy	77–99%

6. DIGESTIBLE NUTRIENT SUPPLY FROM DIFFERENT FEED INGREDIENT CATEGORIES

Digestibility and digestible supply (bioavailability) of protein, phosphorus, lipid and carbohydrate from some common aquafeed ingredient categories for common carp are summarized in Tab. 3.

Tab. 3. *Digestibility, digestible nutrient and energy supply of different feed ingredient categories for carp.*

Ingredient category (included variants)	Nutrition parameters	Digestibility (%)	Bioavailability ($g.kg^{-1}$)
Cereals (whole, middling, bran, flour, germ meal, gluten meal)	Protein	70.9–93	92.2–290
	Lipid	77.7–84.7	21.9–45.6
	Carbohydrate	44.8–90.1	252.7–743.3
	Phosphorus	25–57	0.65–3.93
	Digestible Energy	1,576.7–4,543.6 $kcal.kg^{-1}$	
Oilseeds (pressed, defatted and extruded meals, protein isolates)	Protein	82.4–91.3	314.1–430.8
	Lipid	91.6–95	17.7–104.5
	Carbohydrate	41.6–54.1	90.7–186.6
	Phosphorus	16.4–26.7	1.24–3.92
	Digestible Energy	1,778.5–3,419.1 $kcal.kg^{-1}$	
Fish derivatives (fishmeal, silage, protein hydrolysates and oil)	Protein	85.6–93	351–639.4
	Lipid	69.2–91.2	43.9–107.6
	Carbohydrate	83.1–87.9	19.1–187.2
	Phosphorus	22.8–34.4	2.05–8.08
	Digestible Energy	1,875.5–4,274.8 $kcal.kg^{-1}$	
Animal proteins (terrestrial) (meals, hydrolysates)	Protein	52.8–86.2	165–594.8
	Lipid	83.5–91.6	81–128.8
	Carbohydrate	–	N.A.
	Phosphorus	–	N.A.
	Digestible Energy	–	
Alternative ingredients (hydro-thermally treated legumes-pulses, brewery wastes, malt protein flour, brewers' and petroleum yeast)	Protein	73.7–85.4	276.4–427
	Lipid	75.6–81.3	16.6–96.7
	Carbohydrate	37–85.7	68.6–474.8
	Phosphorus	47.1–80	4.38–8.72
	Digestible Energy	1,529.4–4,477.5 $kcal.kg^{-1}$	

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL
AND FISH OIL IN CARP FEED

7. FULFILLING OPTIMUM NUTRITION FOR CARPS

The recommended nutritional and energy levels for various growth stages of carp are summarized in Tab. 4 (macronutrients) and Tab. 5 (micronutrients). The ingredients should be combined in proportion based on digestible nutrient supply (Tab. 3) in order to reach the 'macronutrient' targets outlined in Tab. 4. It is advisable to increase the diversity of plant-based proteins in feed formulation to ensure minimal use of fishmeal (e.g. maximum 15% by weight) and fish oil (e.g. none to maximum 0.5% by weight). Using plant-based protein also keeps the cost of formulated feed low. For example, see the commercial formulations listed in Tab. 1. When replacing fishmeal with plant/microbial origin feedstuffs, additional factors such as crude fiber and total ash content of those ingredients need to be taken into consideration. The formulation must respect their upper limits (in the final feed) specified in Tab. 4.

Despite fulfilling the macronutrient requirements, deficiency in essential amino acid(s) (EAAs) and/or essential fatty acid(s) (EFAs) can occur in the formulation. To ensure that replacement of fish derivatives does not cause omission of specific EAAs or EFAs, novel formulations must pass additional quality check(s). The crude amino acid and fatty acid content of ingredients is multiplied by protein and lipid digestibility coefficients respectively (digestibility coefficient = digestibility in % divided by 100). This calculation is made for each ingredient category (given in Tab. 3), which provides an estimate of digestible micronutrient supply of the ingredient(s) in the formulation. Tab. 5 summarizes the achievable 'micronutrient' targets in the diet. Ideally, the combination of ingredients achieves optimum nutrition.

Tab. 4. *Macronutrient and energy recommendations for artificial carp diets.*

Parameter	Body weight (g)	Recommended ($g.kg^{-1}$ feed)*
Protein *(Digestible)*	<20	450
	20–110	380
	200–600	**320**
	>600	280
Lipid *(Crude)*	<20	150
	20–110	100
	200–1,000	**70–75**
	>1,000	50
Carbohydrate/ Nitrogen-Free Extract *(Crude)*	<100	300
	>100	**400**
Fiber *(Crude)*	–	**Below 100**
Total Ash *(Crude)*	–	**Below 100**
Dietary energy *(Digestible)*	–	**~3,200 $kcal.kg^{-1}$ diet**

*The highlighted values are generally recommended nutritional and energy levels for carp over 110–200 g.

Tab. 5. *Essential micronutrient recommendations for artificial carp diets.*

Parameter	Dietary level
Essential amino acids ($g.kg^{-1}$ feed) – Digestible content	
Arginine	17
Histidine	5
Isoleucine	10
Leucine	14
Lysine	22
Methionine	07
Phenylalanine	13
Threonine	15
Tryptophan	3
Valine	14
Essential fatty acids ($g.kg^{-1}$ feed) – Digestible content	
18:3n-3	5–10
20:5n-3 and/or 22:6n-3	Required, not quantified
18:2n-6	10
Essential minerals ($g.kg^{-1}$ feed) – Crude content*	
Calcium	3.4–6.8
Magnesium	0.5–1
Phosphorus	7–12

*Lower limits are applicable for ingredients containing highly bioavailable form of minerals with digestibility above 70%. For carp, values near upper limits are recommended.

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL AND FISH OIL IN CARP FEED

8. PROBLEMS AND PROSPECTS OF FISHMEAL-FISH OIL REPLACEMENT

Growth-retarding antinutritional factors in plant origin feedstuffs

Most of the plant-derived feed ingredients contain several antinutritional factors. Carp feeds with high ratio of plant protein source (~50–75% of total protein) or large amount (by weight, >40%) of antinutritional factor rich plant origin feedstuffs are poorly utilized. Carps exhibit decreased nutrient utilization and retarded growth when fed a plant-based diet with excessive with antinutritional factors (e.g. 5–6 $g.kg^{-1}$ of phytates, 20 $g.kg^{-1}$ tannin; reviewed in Kokou and Fountoulaki, 2018; Roy et al., 2019).

Nutritional bottlenecks in plant origin feedstuff compared to fishmeal

Plant origin feedstuffs can contain several antinutritional factors such as anti-tryptic factors or phytate (P) affecting availability of nitrogen and phosphorus (reviewed in Francis et al., 2001). Carps lack intestinal phytase activity and are unable to digest phytate from plant ingredients, since phytases perform optimally at the low pH (3–6 units), which carps lack (gut pH above 6). Mere inclusion of phytases in the plant-based feed formulation often reduces P bioavailability (reviewed in Hua and Bureau, 2010; Roy et al., 2019). Adding protein concentrates from grains and oilseeds can add phytate, thus further lowering levels of available phosphorus in fish feeds. High fiber content (above 9.5%) and high level of complex carbohydrates in plant origin feedstuff negatively interferes with the nutrient utilization. In most plant-based protein sources, the essential amino acids are inadequate compared to the requirements of carp. Such imbalanced plant protein aggravates metabolic N losses (dissolved N losses through branchial and urinary system). Supplementation of crystalline amino acids (AAs), and particularly the essential AAs like methionine and lysine in formulated feeds (at 0.4% inclusion, dry matter basis) is known to improve protein utilization (reviewed in Kaushik 1995; Roy et al., 2019).

Despite sustainability concerns, the nutritional profile of fishmeal is ideal for the majority of aquafeeds. Available data on the essential amino acid requirements of fish and shrimp show that fishmeal is ideal in terms of protein quality and amino acid profile (Kim et al., 2019). Reducing fishmeal levels in fish feeds also compromises the source of critical trace minerals and essential vitamins which need to be supplemented by using 1–2% vitamin and mineral premix in the feed formulation. Recent researches also suggest that taurine, a semi-essential nutrient present abundantly in fishmeal but insufficient in plant origin feedstuff (NRC, 2011), needs to be supplemented in plant-based feeds, especially in feeds designed for juvenile stages (Gunathilaka et al., 2019; Kotzamanis et al., 2020).

Contemporary fishmeal replacements

The proportion of fishmeal in present-day commercial carp feeds is usually ≤15% (≤150 $g.kg^{-1}$). In recent years, soy protein concentrate, pea protein, faba beans, horse beans, sunflower expeller, wheat gluten and maize gluten have been included among the vegetable protein components in commercial fish feeds. Plant protein isolates (like **corn gluten meal, wheat germ meal, soy protein and jatropha protein concentrates**) can also fully replace fishmeal in practical carp diets if some essential amino acids (**lysine, methionine**) are supplemented. **Earthworm meal** can fully replace fishmeal even without supplementing inorganic P salts or essential amino acids. The European Commission recently approved **insect meal** for use in fish feeds. Although single-cell products like bacterial meals were recognized as potential feed ingredients long ago, they have recently made a re-entry in feeds at a commercial scale. Microbe-origin feedstuffs like **brewer's yeasts** in carp diets provide higher proportion of digestible nutrients than conventional plant-origin feedstuffs. Microalgae are also an ideal nutrient source. **Microalgal biomass**, including the **defatted meal**, can be a source of protein, micronutrients and pigments in the feeds of farmed fish. **Spirulina**-based carp feeds may provide a good alternative to fishmeal-free diet in the near future (reviewed in Kim et al., 2019).

Improving nutrient utilization from plant-based feedstuff

Protein and P from brewery wastes (like **malt protein flour** and **corn DDGS**) are better utilized by carp than non-fermented variants or conventional feedstuffs. Phosphorus digestibility from yeast or brewery wastes is also much higher than from the conventional plant-origin feedstuffs. For example, **brewery wastes** (cereals left out after fermentation in distillery) offer ~4–5 times more digestible P (and other minerals) than the parent cereals. Therefore, their inclusion in practical carp diets should be encouraged. Thermal processing (**roasting, cooking, expanding**) of plant-origin feedstuffs, mainly cereals, improves utilization of dietary protein resulting in higher weight gain in fish. Thermally processed and/or pressed cereals reportedly improve utilization of P in carp. With legumes-pulses, dry thermal processing is more effective than moist thermal processing (e.g. **steam extrusion, steam cooking**) in improving dietary protein utilization in carp. Hydro-thermal treatments (e.g.

normal autoclaving) or just water-soaking appear to improve the nutritional value of some plant feedstuffs, especially oilseeds, more than simple thermal processing. In general, **water soaking** followed by **thermal processing** helps to get rid of most of the anti-nutritional factors present in plant-origin feedstuffs. This improves the bioavailable nutrient profile of the plant-origin ingredients for carp. **Acidic pre-incubation** (pH 3–4) of the plant-origin feedstuffs **with phytases** (1,500–2,000 IU kg^{-1} feed) is a good option to hydrolyze phytate-bound P and render higher bioavailability of P for the skeletal growth of carp (reviewed in Roy et al., 2019).

Advantages and disadvantages of fish oil replacement

Polyunsaturated fatty acids (PUFA), especially n-3 long-chain fatty acids abundant in fish and seafood, have beneficial effect on human health, e.g. prevention of human coronary disease or weight reduction (Adamkova et al., 2011; Abedi and Sahari, 2014; Mráz et al., 2017; Linhartová et al., 2018). Two subclasses of PUFA, i.e. n-3 and n-6, are considered 'essential fatty acids' in human diet because humans lack the specific desaturases to sufficiently convert and synthesize these PUFA *de novo* (Adkins and Kelley, 2010), making dietary source their major source. Freshwater fish like common carp usually have higher content of n-6 PUFA, while marine fish (from which fish oil is primarily made) are rich in n-3 PUFA (NRC, 2011). Reducing fish oil levels in carp diet without proper knowledge of fatty acid profile of the alternative oil source (e.g. vegetable oils, animal tallow) may alter essential fatty acids content in the produced fish (Glencross, 2009), compromising the potential human health benefits of fish consumption. Fish muscle omega-3 fatty acid profile can be maintained to meet human requirements when feeding the fish with fish oil-free formulations, but sufficient knowledge of these alternatives is crucial (Mráz et al., 2011; Kwasek et al., 2020).

Present-day commercial carp feeds are mostly fish oil free and use vegetable oils like **rapeseed, sesame or sunflower**. In terms of fatty acids profile, most of the vegetable oils used in aquafeed provide 18:2n-6 fatty acid ratio or slightly more balanced 18:3n-3 fatty acid ratio. **Linseed oil** is an exception with the ratio of 18:3n-3 fatty acid. **Marine microalgae** are also rich in omega-3 (n-3) highly unsaturated fatty acids (HUFA), and **algal oils** are a suitable replacement of fish oil. However, the fatty acid profile in most vegetable oils provides more omega-6 PUFA. This is slightly different from the fatty acid profile of fish oil, which consists of long chain n-3 PUFAs like 20:5n-3 and 22:6n-3. Fortunately, unlike marine fish, non-carnivorous freshwater fish (e.g. common carp) have the capability to desaturate and elongate shorter (C-18) chain n-3 or n-6 series fatty acids (precursors) to highly unsaturated, long chain (C-22) PUFA (Tocher and Sargent, 1990; Glencross, 2009; Bláhová et al., 2020). Common carp is also more inclined to require greater amounts of n-6 fatty acids than n-3 fatty acids for maximum growth. High levels of n-3 PUFA (like in fish oil) actually might not be even useful for carp or carp feed (reviewed in Turchini et al., 2009), which makes the substitution of fish oil in carp feed with vegetable oils easier and not as disputable as replacing fishmeal.

9. APPLICATION OF THE CERTIFIED METHODOLOGY

Feed formulation tools and calculations involved

The best way to implement the nutritional calculations is by using animal feed formulation software(s). A list of some available options is provided in Tab. 6. The user can: (a) input animal nutritional requirements, including lower and upper limits; (b) fill the virtual feed store (i.e. a set of required ingredients), input price and digestible nutrient-energy profile of ingredients; (c) define (for mandatory items) or cap (for expensive items) the proportion of specific ingredient(s) in the formulation, and; (d) instruct the software to calculate the best combination (either least-cost, premium nutrient or stochastic formulation). The software notifies the uses of any potential limitations or bottlenecks of the selected ingredient combination (e.g. missing micronutrients, too much fiber or ash) are. However, the trial versions do not offer all these features and premium license of the software must be purchased to unlock all the functions.

A general formula for simple calculations without the software is provided below. For protein/lipid/energy/amino acids/fatty acids, ***remember to*** use 'digestible' values rather than crude values. This ensures precision of the nutrition provided to carp. For fiber and ash, use crude values but do not exceed the upper limits (see Tab. 4).

$$X_i = \frac{X_i \text{ x } B_i}{100}$$

X_i = Digestible nutrient supplied by ingredient ***i*** in the diet (value in % or g per 100 g).
A_i = Digestible nutrient content of ingredient ***i*** (value in % or g per 100 g).
B_i = Proportion of ingredient ***i*** in the total diet (value in % or g per 100 g).

$$\Sigma X = x_i + \ldots + x_n$$

ΣX = Total amount of available/digestible nutrient in the diet (value in % or g per 100 g) from the set of used ingredients (i_{th} to n_{th} ingredient).

X_i = Digestible nutrient supplied by ingredient ***i*** in the diet (calculated by the abovementioned formula).
X_n = Digestible nutrient calculated for each ingredient and added up to the last ingredient.

Tab. 6. *Example of available aquafeed formulation softwares.*

Category/ Level	Software name®	Website	License
Single user versions/ Intermediate level	WinFeed	www.winfeed.com	Trial, Premium
	AFOS	https://animalfeedsoftware.com/	Trial, Premium
	FeedAccess (online only)	http://www.feedaccess.com/	Premium
Enterprise versions/ Advanced level	Bestmix	www.adifo.be	Premium
	Alix[2]	www.a-systems.fr	
	Brill	www.feedsys.com	
	Format	www.formatinternational.com	

Using the database for fishmeal replacement

Step-by-step instructions in database use through feed formulation software are provided below (Fig. 1). For instance, we used **WinFeed®** and **ZeroFish CarpFeed** to generate four model formulations. The screenshots of the model formulations can be found in Fig. 2 and 3.

Complete replacement of fishmeal protein by other protein sources is a challenge. Meeting optimum digestible requirements of essential amino acids like lysine and methionine without using fishmeal is the main issue. The maximum digestible protein must be set (in the software) at ~42% to supply lysine and methionine adequately. Attempts to formulate feeds below this value without fishmeal often result in "failed formulation" notice from the software. To avoid such a scenario, (i) either supplement Lysine hydrochloride and/or DL-Methionine into the formulation and minimize protein use (Fig. 2A), or, (ii) accept a high protein + high energy formulation (~45% crude protein; Fig. 2B). In general, fishmeal-free diets are prone to be higher in energy content than fishmeal-based feeds. Based on experience, we suggest to nominally include **10% of fishmeal** with **methionine (+lysine)** supplementation and **other animal protein sources**. This helps to keep the formula cost low and still achieve lower crude protein level; this is otherwise unachievable with fishmeal-free formulas (Fig. 3B), making this option more practical. The scenarios associated with all the approaches are demonstrated in Figure 4. We examined **blood meal** (poultry origin; bovine blood is prohibited in EU), **poultry meal**, **meat and bone meal** (porcine origin) and **silk-worm pupae** or **meal worms** as supposedly good replacements of FM-protein in carp feeds.

10. SIGNIFICANCE AND TARGET AUDIENCE

This methodology presents a practical approach and links to available databases with the purpose, to scientifically assist to replace the fish derivatives in carp feed. Such practical guidelines and databases of alternative ingredients are not readily available to public knowledge. For commercial interests, these types of know-how and tools are almost certainly strictly confidential or subject to a charge. Therefore, the present methodology is expected to be of a considerable assistance to fish nutritionists, feed formulators, farmers and nutrition researchers. Especially small-scale farm managers preferring farm level feeds or small-scale feed manufacturers in Czechia and neighbouring countries may benefit from this methodology.

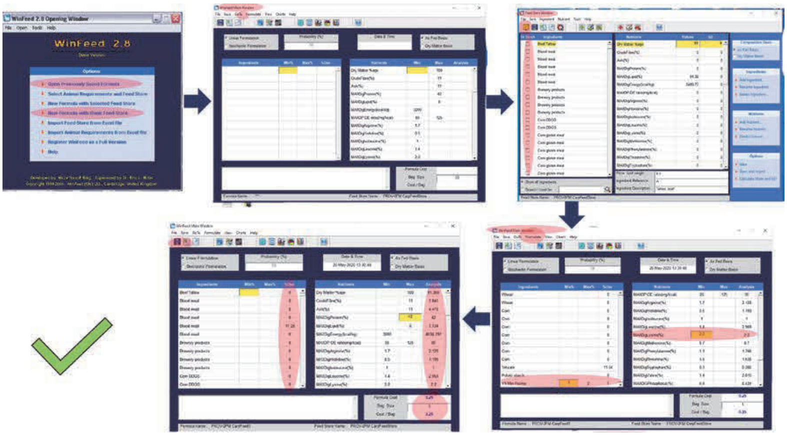

Fig. 1. *Steps (see enlarged images in the database): Load/import feed store file + animal requirement file > go to feedstore (window) > select required ingredients (as per digestible lysine) or all ingredients > confirm and go to main window > set mandatory ingredient limits (min-max), check/set amino acid limits, set/loosen maximum protein, set bag size > click formulate (if an error message appears, loosen limits) > save.*

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL AND FISH OIL IN CARP FEED

A

INGREDIENTS	FORMULA %	NUTRIENTS	ANALYSIS	CRUDE
Blood meal (poultry/ non-bovine)	15.2	Dry Matter(%)	90.9	90.9
Feather meal	0.15	Crude Fibre(%)	3.7	3.7
Linseed oil	1.73	Crude Ash(%)	4.7	4.7
Meat Bone meal	2.14	Digestible Protein(%)	38.0	40
Poultry meal	27.81	Digestible Lipid(%)	7.5	8.2
Wheat	51.01	Digestible Energy(kcal/kg)	4524.6	4918
Vit-Min Premix	1	Dig Protein-Dig Energy ratio(mg/kcal)	77.9	
DL-Methionine	0.14	Digestible Arginine(%)	2.1	
		Digestible Histidine(%)	1.1	
		Digestible Isoleucine(%)	1.0	
		Digestible Leucine(%)	2.9	
		Digestible Lysine(%)	2.2	
		Digestible Methionine(%)	0.7	
		Digestible Phenylalanine(%)	1.7	
		Digestible Threonine(%)	1.5	
		Digestible Tryptophan(%)	0.3	
		Digestible Valine(%)	2.0	
		Digestible Phosphorus(%)	0.6	
		Digestible Linoleic18:2n-6(%)	2.2	
		Digestible Linolenic18:3n-3(%)	1.0	
		Crude Phospholipids(%)	1.0	
Formula Cost /unit	0.24 USD/kg	**Or, 0.22 EUR/kg**		
Formula Cost /Bag	3.3 EUR	(Bag Size = 15 kg) **Or, 90 CZK/bag**		

B

INGREDIENTS	FORMULA %	NUTRIENTS	ANALYSIS	CRUDE
Blood meal (poultry/ non-bovine)	15.79	Dry Matter(%)	91.2	91.2
Linseed oil	1.42	CrudeFibre(%)	4.2	4.2
Meat Bone meal	3.7	Crude Ash(%)	5.9	5.9
Poultry meal	23.77	Digestible Protein(%)	42.0	45
Rapeseed meal	6.16	Digestible Lipid(%)	7.4	8
Silk worm pupae/ meal worm	6.6	Digestible Energy(kcal/kg)	4558.8	4955.2
Wheat	41.5	Dig Protein-Dig Energy ratio(mg/kcal)	86.6	
Corn	0.07	Digestible Arginine(%)	2.1	
Vit-Min Premix	1	Digestible Histidine(%)	1.2	
		Digestible Isoleucine(%)	1.0	
		Digestible Leucine(%)	2.9	
		Digestible Lysine(%)	2.2	
		Digestible Methionine(%)	0.7	
		Digestible Phenylalanine(%)	1.7	
		Digestible Threonine(%)	1.5	
		Digestible Tryptophan(%)	0.4	
		Digestible Valine(%)	2.0	
		Digestible Phosphorus(%)	0.6	
		Digestible Linoleic18:2n-6(%)	2.0	
		Digestible Linolenic18:3n-3(%)	1.0	
		Crude Phospholipids(%)	1.0	
Formula Cost /unit	0.3 USD/kg	**Or, 0.27 EUR/kg**		
Formula Cost /Bag	4.05 EUR	(Bag Size = 15 kg) **Or, 110 CZK/bag**		

Fig. 2 (A, B). *ZeroFish CarpFeed based fishmeal-free, nutritionally balanced and least-cost formulations for grower carps formulated via WinFeed™. The two sub-formulations are principally the same, but formula-A has lower crude protein content due to crystalline amino acid supplementation than formula-B without such supplementation.*

INGREDIENTS	FORMULA %	NUTRIENTS	ANALYSIS	CRUDE
Blood meal (poultry/ non-bovine)	14.29	Dry Matter(%)	90.1	90.1
Corn	17.28	CrudeFibre(%)	2.0	2
Fish meal	39.15	Ash(%)	10.8	10.8
Linseed oil	1.01	Digestible Protein(%)	34.0	37
Safflower oil	3.12	Digestible Lipid(%)	7.8	8.5
Wheat	24.13	Digestible Energy(kcal/kg)	4035.5	4386.4
Vit-Min Premix	1	Dig Protein-Dig Energy ratio(mg/kcal)	37.4	
		Digestible Arginine(%)	2.0	
A		Digestible Histidine(%)	1.0	
		Digestible Isoleucine(%)	1.0	
		Digestible Leucine(%)	2.9	
		Digestible Lysine(%)	2.3	
		Digestible Methionine(%)	0.7	
		Digestible Phenylalanine(%)	1.7	
		Digestible Threonine(%)	1.5	
		Digestible Tryptophan(%)	0.3	
		Digestible Valine(%)	2.0	
		Digestible Phosphorus(%)	0.6	
		Digestible Linoleic18:2n-6(%)	3.4	
		Digestible Linolenic18:3n-3(%)	1.0	
		Crude Phospholipids(%)	1.1	
Formula Cost /unit	0.33 USD/kg	**Or, 0.3 EUR/kg**		
Formula Cost /Bag	4.5 EUR	(Bag Size = 15 kg)	**Or, 123 CZK/bag**	

INGREDIENTS	FORMULA %	NUTRIENTS	ANALYSIS	CRUDE
Blood meal (poultry/ non-bovine)	16.35	Dry Matter(%)	90.5	90.5
Feather meal	0.9	CrudeFibre(%)	4.3	4.3
Linseed oil	1.53	Ash(%)	6.3	6.3
Meat Bone meal (porcine)	0.63	Digestible Protein(%)	37.0	40
Poultry meal	17.43	Digestible Lipid(%)	6.6	7.2
Wheat	52.04	Digestible Energy(kcal/kg)	4339.9	4717.3
Fish meal	10	Dig Protein-Dig Energy ratio(mg/kcal)	68.4	
Methionine	0.12	Digestible Arginine(%)	2.1	
Vit-Min Premix	1	Digestible Histidine(%)	1.1	
		Digestible Isoleucine(%)	1.0	
B		Digestible Leucine(%)	3.0	
		Digestible Lysine(%)	2.2	
		Digestible Methionine(%)	0.7	
		Digestible Phenylalanine(%)	1.7	
		Digestible Threonine(%)	1.5	
		Digestible Tryptophan(%)	0.3	
		Digestible Valine(%)	2.0	
		Digestible Phosphorus(%)	0.6	
		Digestible Linoleic18:2n-6(%)	2.0	
		Digestible Linolenic18:3n-3(%)	1.0	
		Crude Phospholipids(%)	1.0	
Formula Cost /unit	0.26 USD/kg	**Or, 0.24 EUR/kg**		
Formula Cost /Bag	3.6 EUR	(Bag Size = 15 kg)	**Or, 98 CZK/bag**	

Fig. 3 (A, B). *Screenshots of ZeroFish CarpFeed based least-cost, balanced formulation fishmeal for grower carps formulated via WinFeed™. Formula-A is a conventional fishmeal based formulation employing unrestricted use of fishmeal (without regard to sustainability or price concerns). Formula-B, on the other hand, addresses these concerns, allowing only nominal use of fishmeal.*

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL AND FISH OIL IN CARP FEED

11. ECONOMIC ASPECTS

Valuation of ingredient databases

Development of an up-to-date feed focused database is time consuming (man-hours requirement). It also requires certain degree of fish nutrition expertise to synthesize information (qualified personnel requirement). Besides, such projects are often unknown to non-academic (non-institutional) users. One of the aims of this methodology is to familiarize such users with the use of feed formulation databases. Despite these merits it is difficult to quantify the actual value of any database. Nevertheless, it is generally acknowledged that data is an expensive commodity whose valuation is often ignored.

Economic aspects of fishmeal and fish oil replacement

Fish derivatives constitute up to 60% of the cost of a feed formulation. Replacing them with cheaper, widely available plant/microbial protein-lipid sources would most likely reduce the cost of feed. Even if the most expensive plant/microbial feedstuffs cost 3/4 of the fishmeal-fish oil price, it would still mean saving 25% of the cost. Our model formulations suggest (Fig. 2 and 3), fishmeal (FM) free formulations can be ~10–27% cheaper than a conventional FM-based formulation. The FM-free feeds, with or without amino acid supplementation (formula cost 0.22–0.27 EUR.kg^{-1}), have either lower or comparable formula cost to that of a conventional FM-based feed (formula cost 0.3 EUR.kg^{-1}; Fig. 3).

The fishmeal-free formulation can be further economized by supplementing pure essential amino acids like methionine and lysine. Our formulations suggest 18% reduction in protein cost of a fishmeal-free carp feed by supplementing just 0.14% DL-Methionine. The formula cost with nominal FM use (10%) + methionine supplementation is even more economical (0.24 EUR.kg^{-1}), which makes it cheaper than unrestricted FM use (0.3 EUR.kg^{-1}) and FM-free + EAA-free formulations (0.27 EUR.kg^{-1}). While replacing FM, a formula cost of **0.22–0.25 EUR kg^{-1}** can be considered reasonable.

If we multiply the 'reasonable formula cost' by two to account for manufacturing + packaging + manpower + logistics + sales expenses, the final market price (~0.44–0.54 EUR kg^{-1} or ~0.66–0.81 EUR kg^{-1}) should be at least 37% lower than present-day commercial carp feeds. The final prices of present-day commercial carp feed (with ≤15% fishmeal included) usually range between 0.7–1.3 EUR.kg^{-1}. Thus, fishmeal and fish oil replacement can be potentially beneficial in terms of savings and/or higher profit margin.

Economic scenario analysis of different formulations

Scenario analysis of different formulations is given in Fig. 4. It is quite clear that the cost of a balanced carp feed with unrestricted FM use is higher than FM-free formulation(s). However, cost can be expected to lower dramatically, if EAA supplementation is not allowed in a FM-free formulation. Besides, the crude protein content of such FM-free + EAA-free formulation is bound to be much higher, raising question on environmental responsibility. In terms of formula cost, 'FM-free + EAA supplement' and '10% FM + EAA supplement' feeds are comparable. They also do not raise environmental concerns, since they reach similar but still lower crude protein content in the end.

Additionally, the FM-free diets are higher in energy content than FM-based diets, meaning that the condition of the fish must be monitored to prevent lowering the market price by producing 'fatty carps'. This can be accomplished by lowering feed ration at the farm. From economic perspective, we do not recommend using FM-free + EAA-free formulations due to excessive energy content and unjustified formula cost. Instead, we recommend using nominal FM + EAA supplemented feeds or FM-free + EAA supplemented formulations as responsible choices.

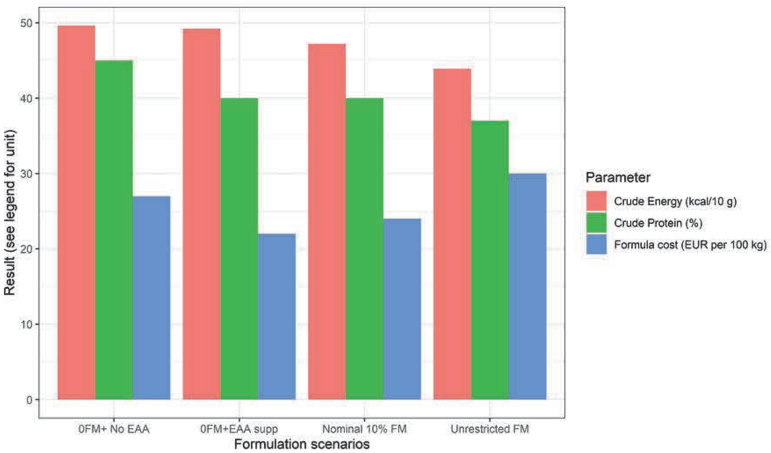

Fig. 4. *Scenario analysis of different formulations (0FM+No EAA = No fishmeal, no amino acids; 0FM+EAA supp = No fishmeal, amino acid supplementation; Nominal 10% FM = Nominal 10% inclusion of fishmeal, EAA supplementation; Unrestricted FM = unrestricted use of fishmeal).*

ALTERNATIVE FEED COMPONENTS TO REPLACE FISHMEAL AND FISH OIL IN CARP FEED

11.1. Implications at farm level

The present-day prices of commercial carp feed range between 0.7–1.3 EUR.kg^{-1} with minimal inclusion of fish derivatives (≤15%). Most commercial carp feeds have an FCR (food conversion ratio) around 1.2 units. Thus, the effective feed cost ranges between 0.8–1.6 EUR.kg^{-1} carp produced. Presently, the farm gate prices of carp generally range between 1.7–2.3 EUR kg^{-1}. For the farmers, this means a profit margin of only +0.7 to +0.9 EUR.kg^{-1} carp produced using artificial feed (without regard to the other expenses). This situation can be an opportunity for the farmers to further lower the prices of carp feed (at least below 1 EUR.kg^{-1}) by using the right combinations of plant-microbial-animal-origin feedstuffs and maintain a profit margin of +1 EUR.kg^{-1} carp produced. This methodology and ZeroFish CarpFeed database provide guidelines on the improvement at feed formulation level.

12. LIST OF PUBLICATIONS THAT PRECEDED THE METHODOLOGY

Roy, K., Vrba, J., Kaushik, S.J., Mráz, J., 2019. Feed-based common carp farming and eutrophication: is there a reason for concern? Reviews in Aquaculture 12: 1736–1758. raq.12407. https://doi.org/10.1111/raq.12407

13. CZECH SUMMARY

Rybí moučka a olej jsou v současné době díky svému vyváženému obsahu esenciálních aminokyselin a lipidů dvěma nepostradatelnými složkami pro oblast rybích krmiv. V blízké budoucnosti nebude výroba rybí moučky a rybího oleje schopna pokrýt rostoucí poptávku po těchto složkách pro výživu zvířat. Zvyšující se náklady a environmentální otázky spojené s použitím těchto složek přiměly firmy zabývající se výrobou krmiv pro ryby, aby hledaly levnější, snadno dostupné, vysoce stravitelné a ekologicky odpovědné krmné komponenty rostlinného a mikrobiálního původu. To vedlo k rozvoji výzkumu se dvěma hlavními cíli. Jedním z nich je snížení hladiny proteinů v krmivu zvýšením obsahu tuků a sacharidů z jiných zdrojů. Druhým cílem je možnost změny částečným nebo úplným nahrazením rybí moučky a rybího tuku z hlediska jejich stravitelnosti a rovnováhy živin.

Poslední čtyři desetiletí byl u kapra obecného prováděn výzkum vhodnosti různých složek krmiva, které mohou nahradit rybí moučku a rybí tuk. Účelem této metodiky je nahradit rybí moučku a rybí olej v krmivu pro kapry – a) informováním o rozsahu dostupných alternativních krmiv, b) shrnutím rozsahu stravitelnosti živin různých kategorií krmiv a optimálních požadavků na výživu

kapra; c) demonstrací metodologie (t.j. rybí moučka, olejová náhrada) pomocí databáze složek krmiv (obsahující informace o stravitelných živinách a energii), d) představením technických možností, problémů a vyhlídek na nahrazení rybí moučky a rybího oleje pomocí softwaru pro komerční přípravu krmiv. Metodika představuje postup vytváření receptur krmných směsí pro kapra s využitím alternativních krmných ingrediencí, shromažďuje informace o jejich nutričních hodnotách, stravitelnosti a potenciálního dopadu na životní prostředí. Představuje 3 alternativní přístupy pro vytváření krmných směsí nahrazujících rybí moučku a olej a vysvětluje jejich limitace. Modelové formulace odvozené z databáze naznačují, že formulace bez rybí moučky (RM) mohou být o 10–27 % levnější než konvenční formulace na bázi RM. Krmiva bez RM, s nebo bez přídavku aminokyselin (náklady na recepturu 0,22–0,27 EUR.kg^{-1}) mají nižší náklady na recepturu než konvenční krmiva na bázi RM (náklady na recepturu 0,3 EUR.kg^{-1}).

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15. DEDICATION

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CHAPTER 9

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
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TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

1. General discussion

1.1. Minimizing losses

Closing the loop of nutrients in controlled, intensive aquaculture systems

Aquaculture's rapid growth has attracted widespread criticism for its environmental and social impacts (Bacher, 2015; Barrett et al., 2002; Krause et al., 2020; Osmundsen and Olsen, 2017; Regueiro et al., 2021; Whitmarsh and Wattage, 2006). Much of this criticism has arisen around the provision of feed, mainly marine ingredients (proteins and oils, mostly from fisheries), and the release of nutrients from farm sites (Deutsch et al., 2007; Martinez-Porchas and Martinez-Cordova, 2012; Naylor et al., 2000; Naylor et al., 2021; Regueiro et al., 2021). Our second chapter (Lunda et al., 2019) focuses on this latter part. The concept of circularity aims to reduce resource consumption and emissions to the environment by closing the loop of materials and substances. Under this paradigm, losses should be prevented and recovered for reuse (de Boer and van Ittersum, 2018). Nitrogen (N) and phosphorus (P) wastes are two major excretory end products of concern in aquaculture. The primary route of N excretion occurs via gills and urine, thus in soluble form. In contrast to N, a major proportion of P loss occurs through feces and particulate form (Prabhu et al., 2019). Chapter 2 (Lunda et al., 2019) showed that the feed and fish-derived effluents from commercial-scale intensive aquaculture operations are potent enough to support plant growth or, if released to receiving waters, may trigger eutrophication (algal growth). It is not just N and P, but there are many other nutrients of concern if allowed to be released to the environment and not re-valorized. Such examples were Mg, Ca, S, Fe, Zn, Cu, and Ni; partly K (Lunda et al., 2019). Our work underpins a definite contribution of feed and wastes production by fish to the environmental footprint of our food systems (Lunda et al., 2019). Furthermore, the work also emphasizes the indispensable need of adopting end-of-pipe treatments in intensive, low-exchange, high water reuse efficiency aquaculture systems like recirculatory aquaculture systems (RAS). Because the choice of daily pH adjustment buffers in RAS (like sodium bicarbonate, calcium carbonate, or hydroxides) causes accumulation of sodium (Na) or calcium (Ca) in the concentrated sludge and wastewater from these systems. The daily pH management itself in RAS or aquaponics should be changed to better alternatives in the future; managing the nutritional and excretion dimensions alone might not fulfill all the goals of a future sustainable circular bioeconomy. Even to the levels that it may be considered toxic for the plants. Merely re-using such aquaculture sludge directly on agricultural fields over a long-term period would pose threats of soil salinization in a future circular food system. Even in the regions of the EU where no to low risk of salt salinization exist (Tóth et al., 2008).

Presently, aquaculture nutrient inputs outweigh extracted nutrients. N-loading from fed aquaculture represents ~0.9% of the human input to the N-cycle on a planetary scale. While P loading from fed aquaculture represented about ~2.5% of the global P fertilizer supply (Verdegem, 2013). Circular aquaculture models would need to explore creative designs to minimize losses (see introduction). But the valorization of wastes should generate additional food for humans, reducing the pressure on conventionally used resources like land, water, fertilizers (de Boer and van Ittersum, 2018; Muscat et al., 2021; Regueiro et al., 2021). Some of the advancements of waste valorization strategies in intensive aquaculture involving microbial digestion processes such as the anaerobic ammonium-oxidizing (Anammox) technology, which converts TAN directly into nitrogen gas (Martins et al., 2010), may also be the source of very potent GHGs like nitrous oxide (Hu et al., 2012; Williams and Crutzen; 2010); and be counterproductive to mitigate climate change (*i.e.,* climate ambitions) in a future circular bioeconomy. Existing bio-based solutions are also not optimum. For example, the efficiency of denitrifying dephosphatation microbes in removing P is often lost due to fluctuations in pH of wastewater or sludge due to their inconsistent or heterogeneous nature (Kuba et al., 1997; Marcelino et al., 2011). As a result, the P removal (dephosphatation) techniques are still largely reliant on classic chemical flocculants and are much more expensive (Martins et al., 2010). Additionally, they are not eco-friendly or pose public health concerns (Okaiyeto et al., 2016).

Our work (Lunda et al., 2019) highlighted the opportunities that exist in this direction. Commercial intensive aquaculture farms may reduce their environmental footprint, valorize (fish effluents to plants) and re-valorize (plants to aquafeed) their wastages, and produce both fish and plants as edible food from a single feed input. The study (Lunda et al., 2019) also emphasized that such processes should be more encouraged in the future. Future initiatives must not be deterred by nutrient insufficiency arguments claiming that enough nutrients are not available to operate such eco-based aquaculture designs sustainably. Therefore, in continuation to our previous work (Lunda et al., 2019), our latest effort (Chapter 8; Roy et al., 2021/2022 unpublished) has gone into developing bio-based solutions explicitly targeting the nutrition-excretion processes of fish for biomanipulation in futuristic eco-based designs of aquaponics; a hallmark of circular food production systems (discussed later).

Minimizing nutrient footprint from natural, semi-intensive aquaculture systems

On a relative scale, the area and scale of impact by intensive RAS-based aquaculture or aquaponic units is much less than aquaculture in outdoor, closed- or semi-open waters. Chapters 3 (Roy et al., 2020a) and 5 (Roy et al., 2020b) focused on European carp farming in ponds. Despite being an old and economically fading venture, this system alone contributes about half of today's Central Eastern European Region's (CEER) inland fish production (Roy et al., 2020b). In order to achieve the circularity and sustainability of food systems in CEER, such a huge socially and ecosystem services relevant system (i.e., ponds or pondscapes; (Hill et al., 2018)) deserves a significant focus. The impact of pollution caused by aquaculture has increased public concern in the past (Dauda et al., 2019). Constraints on the use of public waters have emerged due to nutrient pollution caused by aquaculture, often forcing poor producers or poor production ventures out of the food production sector (Naylor et al., 2021). In China, aquaculture pollution accounts for more than 20% of the total nutrient input into freshwater environments, leading to the prohibition of aquaculture in many public water bodies essential for drinking water and ecosystem services (Cao et al., 2007, Naylor et al., 2021). Similar sensitivity presently exists around carp farming and ponds of Europe, especially CEER, which is predominantly landlocked. Our work (Roy et al., 2020a) portrayed and analyzed such controversies from the perspective of the animal in question, the common carp (*Cyprinus carpio*).

Chapter 3 (Roy et al., 2020a) analyzed carp's natural ability to digest and defaecate different food items, ranging from conventional feedstuffs to natural food. The range, limitations, and dependencies of digestibility, metabolic losses, and retention of N and P in general and under different feedstuff characteristics were summarized. The meta-analyses revealed the data deficiency surrounding alternative or circular feedstuffs of regional relevance (e.g., brewery wastes, rapeseed oil cakes, lupines, peas, faba beans), especially in terms of P, which is essential for commenting on eutrophication potential caused by these feedstuffs if applied directly in the fishponds. Presently the regional fishponds are protected by law, and animal protein feedstuffs, pelleted compound feed, fertilizers require special permission or are not permitted in general. Only plant-origin, locally sourced, and cheap feedstuffs, most commonly cereals, are put in the fishponds (Hlaváč et al., 2016). Lastly, considering the N and P processing limits by carps from a diverse range of feedstuffs, it was concluded that carps should not be vilified for nutrient emissions in European ponds. Although there are some biological limitations surrounding P digestibility (e.g., agastric fish, gut pH above 6, lack of ability to break plant-origin phytate P) from terrestrial- or plant-origin feedstuffs, organic P in their natural prey are surprisingly well digested. Therefore, carp's eutrophication potential depends on the selection and pre-processing of any future circular feedstuff and balancing it well with natural food to reduce P (and N) emissions. Such solutions are discussed, e.g., acidic pre-incubation with phytase or controlling nutrient (N, P) input through supplementary feed itself. Providing them with more nutrients than they need for optimum growth should be avoided. Such limits were quantified in our study (Roy et al., 2020a).

Most aquaculture LCAs highlight that feeds solely contribute to most LCA impact categories (Bohnes et al., 2019; Newton and Little, 2018; Pelletier et al., 2009; Regueiro et al., 2021). However, eutrophication potential (one of the LCA impact categories) is caused equally by feed and aquaculture farm emissions (Regueiro et al., 2021). It highlights the animal (fish) dependent processes; how the feed itself is good for the fish or a nutrient source for the aquatic environment. That is nutrients for fish or nutrients from fish. How the fish processes the food, in terms of intake, digestibility, metabolizability, and retention, ultimately contributes to such aquaculture farm emissions. Not just the feed alone. The focus of our works in chapter 3 (Roy et al., 2020a) and chapter 5 (Roy et al., 2020b) covers both these aspects. Presently, the efficiency of feed conversion per unit of production or human edible production (measured by feed conversion ratio, FCR) is sometimes taken as a proxy for environmental impacts of aquaculture and is a key target for reducing impacts (Fry et al., 2018; Regueiro et al., 2021). There are even emerging doubts whether the *status quo* measurement of FCR in aquaculture is indeed the right approach (Fry et al., 2018). The recent popularity of fish-in fish-out ratio (FIFO) is aimed to offset some of the drawbacks of FCR in reflecting sustainability indicators of aquaculture practice (Kok et al., 2020; Tacon and Metian, 2009). FIFO demonstrates the relationship between the quantity of wild-caught fish required to produce farmed fish (Boyd et al., 2020; Regueiro et al., 2021). However, the FIFO may reach near zero in a future circular bioeconomy or circular aquaculture, which will target re-valorizing its wastes and even using the aquaculture by-products in aquafeed (e.g., slaughtering discards, insects grown on sludge, algae or plants or microbes grown on aquaculture wastes). The concept of FIFO is also considered fundamentally flawed in many aspects (Kaushik and Troell, 2010; Turchini et al., 2019). In such a situation, where FCR or FIFO alone could not adequately address the environmental footprint of aquaculture, more precise focused lenses need to be developed.

As an alternative to the lenses like FCR or FIFO for quantifying aquaculture's environmental footprint, chapter 5 (Roy et al., 2020b) focused on the nutrient footprint of carp farming in European ponds considering carp's eutrophication potential through feeding and excretion processes (previously examined in (Roy et al., 2020a)). Using the knowledge of digestibility, metabolizability, and retention (i.e., nutrient partitioning) of actively feeding carps in European ponds, (Roy et al., 2020b) attempted to quantify precisely the autochthonous nutrient footprint caused by the farming management (mainly, feeding) and/or the fish (carp) itself. Thus, we excluded catchment nutrient run-offs or sewage nutrient contributions that end up sinking in the ponds and later emerging in nutrient loads; leading to unjustified vilification of aquaculture at times (Roy et al., 2020a,b). For circular food systems of the future, if the linear nutrient flow is to be closed (or looped), a precise firsthand knowledge of nutrient emissions and the causal effects driving them is necessary. Besides, putting nutrient emissions in a comparative perspective with other food sectors or expressing them in monetary terms against production (commercial) or regulatory (ecosystem) services helps understand the bigger picture, *i.e.,* weak links of the food systems or degree of damage to the environment. Findings of our study (Roy et al., 2020b) showed the cleanliness of carp farming in European fishponds compared to EU agriculture or livestock sectors. It also showed the trade-offs of minimizing nutrient footprint or risking the loss of valuable services (production and ecosystem) rendered by fish farming in fishponds. Calibrating supplementary nutrition (feeding) in synchrony with the base nutrition (available natural food) in fishponds is the key to neutralizing nutrient footprint further and increasing carp's bioremediation potential of allochthonous nutrients received in ponds from the catchment or sewage (Roy et al., 2020b). It was also outlined that carp also contribute to ecosystem services (bioremediation services) provided by ponds. Besides, the digestible and metabolic losses caused by the feeding activity of carp biomass (density) in most CEER fishponds cause little ecological cost via N, P footprint. Comparably, the positive regulatory ecosystem services provided by the ponds themselves are far greater (Frélichová et al., 2014; Roy et al., 2020b).

Negative public perception remains an essential factor for the future of aquaculture expansion (Kuempel et al., 2021); tackling it with the knowledge of fundamental biological processes like fish nutrition and excretion is a novelty. Chapters 3 and 5 (Roy et al., 2020a,b) touched on this aspect and tried to tackle some negative public perceptions surrounding carp farming in regional fishponds; using the lenses of in-vivo nutrient partitioning, in-situ nutrient throughput (e.g., N, P), and so on. However, it is imperative to note that public perceptions may often be blindly, unjustly motivated for natural, outdoor aquatic systems practicing aquaculture (or semi-intensive fish farming). For example, nutrient effluents from ponds, whether contributing to downstream nutrient enrichment (pollution) or not, are often straightforwardly blamed on aquaculture being the causal driver. Rarely, the nutrient run-offs from the catchment (especially during storms, heavy rains), agricultural land, and domestic sewage which sink in the pond basin are given a thought (Roy et al., 2020a,b). Until and unless the nutrient run-offs from the catchment, agricultural land, or domestic sewage entering fishponds are checked, the apparent nutrient footprint of aquaculture may never be 'neutralized'; although the true nutrient footprint of aquaculture might have been already 'neutralized' but overshadowed (using the same analogy as in fish, i.e., apparent digestibility coefficient versus true digestibility coefficient). Despite best efforts in optimizing status quo nutrient management or RUE of fishponds, the desired results may not be realized in this case. Therefore, future circular food systems should take these peripheral aspects or side streams of nutrients into account. Some indigenous adaptive strategies from around the world may be needed to be adopted, for example, the establishment of green belts around ponds, macrophyte beds in water inlet and outlet channels, artificial wetlands in large seasonally closed portions of the ponds, floating islands, artificial periphyton beds (Park et al., 2018; Roy, 2016; Sarkar et al., 2018).

1.2. Circularity issues in aquaculture nutrition

Problems and prospects in switching to sustainable feedstuffs

Aquaculture has become the largest consumer of global fishmeal (FM) and fish oil (FO) production, accounting for 68% and 89%, respectively (Hua et al., 2019). At the same time, most modern aquafeed are now predominantly composed of terrestrial plant materials and animal by-products; and the use of FM and FO have been reduced very much to even negligible amounts (≤10%) for omnivorous and herbivorous fish species like different cultured cyprinids (Colombo and Turchini, 2021a; Naylor et al., 2021; Turchini et al., 2019). Some of these alternative feedstuffs to FM and FO are also not without environmental impact. For example, a heavy reliance on terrestrially derived agriculture products has sustainability issues, such as pressure on land use, freshwater use, deforestation, areal footprint, pesticide and fertilizer use, irrigation, and polluting runoff. Many plant-based aquafeed ingredients, often promoted as sustainable to FM and FO, may also directly compete with human food streams (Colombo and Turchini, 2021b). In circular agriculture or aquaculture, it is referred to as food-feed conflict (from a human perspective) (de Boer and van Ittersum, 2018; Van Zanten et al., 2018). Especially from the perspective of a fish nutritionist (fish nutrition is a generic term covering all aquatic animals (Hardy and Barrows, 2003)), many of the terrestrial plant ingredients present certain nutritional challenges for farmed aquatic species (Colombo and Turchini, 2021a; Turchini et al., 2019). The challenges range from inadequate amino acids balance, skewed or undesirable fatty acids balance to complex or even toxic anti-nutritional factors, non-starch polysaccharides, and non-bioavailable nutrients (Kokou and Fountoulaki, 2018; Lall and Kaushik, 2021; Turchini et al., 2019). All of them have repercussions on either growth or physiology of fed aquatic animals and how they interact with the environment in terms of nutrient loading.

The future circular food systems envisage developing innovative practices that involve conservation, restoration, integration, remediation, and even recycling, reuse, or remanufacturing (Colombo and Turchini, 2021a; de Boer and van Ittersum, 2018; Roy et al., 2021). It presents new opportunities for the next generation of protein and lipid sources for aquafeed, presently being dubbed as a new evolution that is coming ('Aquafeed 3.0'). One such opportunity is the production of nutritional resources created through the circular bioeconomy (Colombo and Turchini, 2021a). Under the paradigm of circular bioeconomy, losses or discards should be prevented or otherwise be recovered for reuse. Two recent reviews (Khanjani and Sharifinia, 2020; Robles-Porchas et al., 2020) provided an updated account on the usefulness of biofloc technology (BFT) in valorizing aquaculture system wastes into something useful. Indeed, how BFT can be seen instead as an end-of-pipe waste treatment option (not just an aquaculture opportunity) for intensive aquaculture farms is hinted in a recent review (Robles-Porchas et al., 2020). Even decoupled FLOCponics is emerging as an alternative solution to minimize artificial feed-based nutrition in intensive aquaculture facilities (Pinho et al., 2021). Indeed, microbial biomass grown on dissolved and particulate wastes (e.g., biofloc) is a circular-origin nutrition source. Besides, excess biofloc must be drained daily or periodically from such systems to keep the bioremediation processes running optimally, preventing the water quality parameters from going haywire, and avoiding non-welfare threats, toxicity dangers for aquatic animals reared therein (Schveitzer et al., 2013).

Chapter 4 (Lunda et al., 2020) lays out the nutritional strengths and weaknesses of such circular-origin nutrition sources (discussed here) while attempting to standardize the nutrition of an aquatic species that has a negative connotation for being invasive in many parts of the world (discussed below). Our study (Lunda et al., 2020) showed that biofloc, in general, despite having significant inconsistencies in its nutritional composition ((Emerenciano et al., 2013); a weakness), have attracted market interests. Presently being sold as commercial feedstuff for the feed industry under generically termed categories like 'single-cell protein' or 'microbial protein' (Jones et al., 2020), with different brand names and even using patented technologies to get rid of some inherent nutritional problems associated with normally produced biofloc biomass (reviewed in (Lunda et al., 2020)). Our work (Lunda et al., 2020) highlight that these circular feedstuffs may not be without problems either. Especially if seen as an alternative to completely replace the use of unsustainable ingredients like FM or food-feed conflict ingredients (e.g., soybean, peas) in aquafeed, biofloc may not provide the same gold standard to the aquafeed industry as FM or soybean had provided over decades (Turchini et al., 2019). In the context of evolving thoughts (Turchini et al., 2019), biofloc may be a 'complementary feedstuff' of circular origin, rather than an 'alternative'; ultimately minimizing the use of contemporary protein sources having footprint (e.g., not only FM but also soybean, peas; (Colombo and Turchini, 2021a; Naylor et al., 2021)). Our work (Lunda et al., 2020) also pointed out some inherent limitations of biofloc protein inclusion in aquafeed, owing to its amino acids balance which deteriorates with the aging of microbial culture (especially arginine limitation), low lipid content, high ash content, and insufficient non-protein energy to protein balance that might impact overall aquafeed formulation if incorporated at higher inclusion levels. Aquatic animals feeding on such ingredients, especially at higher inclusion levels, over a longer time (e.g., from juveniles to marketable size) may pose a risk of mineral stress or heavy metal cumulation (Lall and Kaushik, 2021; Lunda et al., 2020); not to critical or lethal levels though (Lunda et al., 2020). However, our work also showed the future promise of such circular-origin feedstuffs to replace one-third to half of the conventional feedstuffs presently used in aquafeeds without any compromises in production.

Many of the present-day alternative feed ingredients (e.g., listed in the International aquaculture feed formulation database or our developed ZeroFish CarpFeed database (Roy and Mráz, 2021b); TilaFeed database Chapter 7 – Roy et al., 2021/2022 unpublished) may not fit the image of 'sustainable aquaculture feedstuffs' in a future circular bioeconomy due to their pressures on land occupancy, water use, terrestrial ecotoxicity, and freshwater eutrophication (Boissy et al., 2011, Colombo and Turchini, 2021a). Some alternative feedstuffs would also pose higher food-feed conflict from a human food system perspective (e.g., legumes, cereals gluten). Since assessing the digestibility of nutrients and energy from diets and ingredients provides one of the most straightforward ways of unambiguously defining the nutritional value of an ingredient to an animal, future aquafeed formulations should be based on digestible nutrient content than crude nutrient content (Glencross, 2020). If applied universally, such a strategy would improve the overall resource use efficiency of the aquaculture sector. For this purpose, open-access, aquaculture species-specific databases of feedstuffs (or feed ingredient inventories) containing data on digestible nutrient supply are necessary, enabling resource-efficient aquafeed formulations in the future. Chapters 3 (Roy et al., 2020a), 7 (Roy et al., 2021/2022 unpublished), and 8 (Roy and Mráz, 2021a) provided such databases for carp and tilapia; two of the highest fed group of freshwater fish species in global aquaculture (Boyd et al., 2020). These chapters (3, 7, and 8) layout the platform of visualizing digestible nutrient supply of feedstuffs that could be of circular origin (like brewery wastes, insects and insect larvae, algae, bacteria, slaughterhouse wastes) and compare their nutritional strengths or weaknesses at par with conventional alternative feedstuffs that need large land and water use patterns to cultivate. These chapters (3, 7, and 8) also highlighted the lack of digestibility data for many easy, circular-origin ingredients; needed to be addressed immediately for future circular aquaculture bioeconomy to use such feedstuffs.

Complementary resource use, minimizing human inedible resources

Fish-derived ingredients for aquafeed have long served as 'gold standards' for the aquaculture nutrition industry (Kaushik and Seiliez, 2010; Turchini et al., 2019). Fish meal contains a considerable amount of highly digestible, well-balanced protein matching the amino acid requirements of aquatic livestock and an "unknown growth factor"; also rich in phospholipids (Hardy, 2010; Turchini et al., 2019). Fish meal is also highly palatable to cultured species, contains no antinutritional factors, and has limited carbohydrate and fiber content (Hardy, 2010; Turchini et al., 2019). Fish oil is a triglyceride-rich oil with a unique fatty acid composition, typically comprising roughly equal amounts of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and long-chain polyunsaturated fatty acids (LC-PUFAs), particularly those in the n-3 series (Tocher, 2015; Turchini et al., 2009). Because of their distinctive composition and other attributes, few if any raw materials match the feeding value of FM and FO in aquafeeds (Turchini et al., 2019). Despite the utility of FM and FO in aquafeed formulation, the incorporation of wild-caught fish in aquafeeds has attracted considerable criticism from scientists and the public, consumers, and markets (Naylor et al., 2000; Naylor et al., 2021; Turchini et al., 2019). Over the last two decades, most aquaculture nutrition researchers have focused exclusively on FM replacement, alternative protein sources, FO replacement, and alternative lipid sources. From the time of review of 2000 (Naylor et al., 2000) till 2021 (Naylor et al., 2021), the use of FM and FO have considerably decreased in aquafeed; and replaced by ingredients of plants, algae, animal by-products, insects, and microbial protein origin (Boyd et al., 2020). But not all of them are environmentally sustainable or fit the principles of circularity, e.g., additional pressure on land and water resources, food-feed conflicts, does not valorize any waste, or having individual environmental footprints (Colombo and Turchini, 2021a; Roy et al., 2021). In a future circular bioeconomy, the aquafeed formulation should be the process of identifying different combinations of "complementary" raw materials, preferably of circular origin but also including FM, FO, and others; that collectively meet the established criteria for the fed aquatic species or aquafeed in question (Turchini et al., 2019).

Between 1997 and 2017, the volume and share of freshwater fish produced with compound feed, such as fed carps, tilapia, and catfish, increased substantially, but FCR also improved (Boyd et al., 2020; Naylor et al., 2021). Meanwhile, fishmeal inclusion rates dropped for carps, tilapia, and catfish to below 10%. Besides, there is almost no fish oil used in most types of freshwater aquafeed (Naylor et al., 2021). Nevertheless, the finfish and crustacean aquaculture sector consumed over 69% of the total global fishmeal production and 75% of the total global fish oil production in 2016 (Boyd et al., 2020; Hua et al., 2019). Feed for carps still has the highest share of fed aquaculture globally; approximately 13.55 million tons of carp feed or 26.4% of the global aquafeed production (Boyd et al., 2020). Chapter 8 (Roy and Mráz, 2021a) developed a certified methodology for local carp feed producers to aid in their quest for further replacement of fish meal and fish oil using digestible nutrient content of alternative or complementary feedstuffs (or categories) and at par with carp's digestible nutrient considerations for optimum growth. An FM-FO free aquafeed ingredient database was developed based on carp's digestibility of alternative ingredients or alternative ingredient categories, which can be directly used for feed formulation using feed formulation software (ZeroFish CarpFeed database; (Roy and Mráz, 2021a)).

Chapter 8 (Roy and Mráz, 2021a) also demonstrated the tricky parts or difficulties to replace FM and FO in carp feed formulations completely (e.g., too high crude protein level, too high energy diets, costs, high requirements of crystallized free amino acids) but hinted that 'complementary' feed formulation (with nominal FM-FO in feed) should be a safer, better approach in the future than complete exclusion of FM-FO from carp feed. Therefore, complementary resource use in aquaculture nutrition is advocated even from a perspective of optimizing fish nutrition or future feed formulations. Our methodology document (Roy and Mráz, 2021a) also highlighted some potential ingredients (e.g., most vegetable oils, algal oil, poultry by-product meal, animal meat, and bone meal, earthworm, silkworm or mealworm meals, poultry blood meal) on a digestible nutritional quality basis that can replace FM and FO. However, lower P digestibility of bone or keratin-containing ingredients should also be paid attention to; and avoid eutrophication threats (Hua and Bureau, 2010; Roy et al., 2020a).

A future circular food system would try to maintain the value of any biological resource with efforts to minimize or avoid inedible human losses (de Boer and van Ittersum, 2018; Roy et al., 2021); for example, successfully invaded aquatic species presently regarded as 'societal discards' having growth, the nutritional potential to serve as food or feed. As shown in Chapter 4 (Lunda et al., 2020), the crayfish aquaculture has started to gain momentum globally and the studied red swamp crayfish owing to their attainable body size and high growth-reproduction potential, could be potentially re-valorized for aquaculture (Haubrock et al., 2021; Lunda et al., 2020). However, aquaculture of an invasive species must be dealt with caution and advocated in regions where natural populations might have been established successfully despite remedial measures (unfortunately) after decades of invasion. For captive, fed aquaculture at intensive scales to occur, knowledge of the nutritional requirements of the farmed aquatic species is of paramount importance. Our study (Lunda et al., 2020) show the lack of established nutritional requirements for crayfish (in general) despite some nutrition research conducted in the last decades, especially nutritional knowledge on the red swamp crayfish. Hence, not only some nutritional requirements were summarized, but also their growth and nutritional dependencies (also, physiology) behind growth were summarized (Lunda et al., 2020). Taking hints from these, aquaculture nutrition for red swamp crayfish may be tailored accordingly, and potential future crayfish aquaculture (Haubrock et al., 2021; Tönges et al., 2021) may thrive better.

1.3. Improved resource use efficiency (RUE)

Bio-based solutions for improved RUE from in-vivo to in-situ

As clarified in the introduction, the bioeconomy part of circular food systems would focus more on finding innovative, bio-based solutions for the future. The eras of physical solutions (physics), synthetic chemical solutions (chemistry) would be replaced by biological solutions (*i.e.,* an era of biology) (de Boer and van Ittersum, 2018; Roy et al., 2021). It is rather an untapped area in the EU food (system) 2030 pathways, aiming to achieve four goals at once: climate neutrality, bio-based innovations, circularity, and nutrition security (Commission, 2020). A decade ago, it was predicted that a major future challenge (problem) for aquaculture would be to influence the composition and ratios of nutrients in aquaculture effluents and facilitate further water purification processes through proper diet formulation or nutrient provisioning to fish (Verdegem, 2013). Chapter 6 (Roy et al., 2021/2022 unpublished) and chapter 7 (Roy et al., 2021/2022 unpublished) provide some advancement of knowledge and potential bio-based solutions to this anticipated problem.

In the end, circularity aims to optimize the resource use efficiency (RUE) of a system and is not limited to just focusing on individual fish stocks or a farm (de Boer and van Ittersum, 2018, Roy et al., 2021). Chapter 6 (Roy et al., 2021/2022 unpublished) contributes to this direction. Using an advanced understanding of fish or animal nutrition (*in-vivo* nutrient partitioning, bioconversions; *de-novo* nutrient biosynthesis; nutritional bioenergetics; the flow of nutrients through the fish body), and connecting it with the present knowledge of shallow-lakes hydrobiology (dynamics of food availability; revisited plankton ecology group model; the unified concept of ecosystem RUE), Roy et al. (2021/ 2022 unpublished) describe how fish nutrition-excretion shapes the autochthonous nutrient turnover and trophic status of large-sized European carp ponds (presently). The work highlights the importance of balanced fish nutrition in such ponds in a future circular aquaculture-centric bioeconomy of CEER to improve RUE in these ecosystems, further decrease nutrient emissions, stepwise excavation of nutrient legacy from ponds, maintain ecosystem services at an optimum level, and promote eco-based carp farming. Presently carp ponds have the largest horizontal (area) and vertical (production) coverage in CEER's inland aquatic protein or LC-PUFA production. Besides, the intangible ecosystem services offered by the fishponds are more significant than the tangible production services realized per production cycle of carp (AAC, 2021, Roy et al., 2020b).

However, **chapte**r 3 (Roy et al., 2020b) and chapter 6 (Roy et al., 2021/2022 unpublished) also show that these services complement each other, i.e., optimum ecosystem services help optimizing production services, and well-managed production (pond nutrition) helps to optimize overall services (ecosystem + production) of the system. It hints that the RUEs at *in-vivo* and *in-situ* levels are not separated but rather synchronized and unified. Perhaps no other aquaculture models in CEER have such broad social and ecosystem relevance (Roy et al., 2020a,b) and are one of the cleanest food production units of the EU food systems (Roy et al., 2020b). Therefore, any slight improvement in RUE of these systems or decreased losses from these systems would have an enormous impact, contributing to EU food 2030 pathways goals (mentioned above), water framework directive, and Europe's living within planetary health boundaries (EEA/FOEN, 2020).

Bio-based innovations for improved RUE of the overall food system unit

The concept of circularity aims to reduce additional resource consumption through integration and emissions to the environment by closing the loop of materials and substances (de Boer and van Ittersum, 2018; Roy et al., 2021). Thus, one of the core principles of circular food production is the recycling of by-products or nutrients. Recycling or reuse should be done in a way that reduces pressures on land, water, manufacturing, energy and ensures safety and biosecurity (Muscat et al., 2021). Chapter 7 (Roy et al., 2021/2022 unpublished) shows aquaponics is a hallmark of such a system, where fish excreta is a waste (but resource for plants) but without worries of safety, rather insufficiency (see chapter 2; (Lunda et al., 2019)). Both fish and plants under one unit produce food for humans (Folorunso et al., 2021), reduce pressures on arable land, water use, and environmental nutrient emissions (Baganz et al., 2021). However, supplementary plant fertilizers (metaphorically, 'feed' for plants) and commercially formulated feed for fish (without thoughts to tailor its mineral contents for aquaponics) make these systems less circular. Although much effort has gone into improving aquaponics' mineralization efficiency, little effort has gone to tailor fish nutrition (and excretion) to complement aquaponic (plant) nutrient needs. So far, the central focus was on upgrading the presently low efficiency of microbial degradation processes (Goddek et al., 2018; Martins et al., 2010). But these microbial processes in aquaponics, when they occur inappropriately for specific nutrients (e.g., microbial reduction of carbon or microbial oxidation of nitrogen), may also result in highly potent GHGs (e.g., methane or nitrous oxide). Their emissions (e.g., from aquaponic units) to the atmosphere would contribute to global warming (Hu et al., 2012; Williams and Crutzen, 2010; Yuan et al., 2019). Therefore, using proper feed formulation, if extra nutrient inputs into systems like aquaponics are avoided and plant growth is sustained simultaneously, the overall RUE of aquaponics would improve. The abovementioned threats to planetary health boundaries would also be minimized.

Chapter 7 (Roy et al., 2021/ 2022 unpublished) describes a different direction by applying the knowledge of fish nutrition and excretion to complement the needs of aquaponics system nutrients planning. On the one hand, the work aims to reduce the inherent food-feed conflict of aquaponics systems itself, where 'one formula' (*i.e.,* one feed) could be used to produce two foods (fish, plants) with least to no additional nutrient input (or feed for plant) for the aquaponic system. On the other hand, through the novel database (TilaFeed) connecting aspects like *in-vivo* digestibility, requirements, retention, and excretion of plant-essential nutrients in feedstuffs and how nutrient partitioning or flow happens through the fish body (from feed to microbial sludge digesters, plants), which feedstuffs used in aquafeed are rich or deficient in what plant-essential nutrients, a new direction is expected to emerge in the commercial aquafeed formulation. Using the approaches, tools, and database presented in

Roy et al. (2021/ 2022 unpublished), the aquafeed industries may also begin to solve the issues of nutrients in aquaponics systems by tailoring aquafeed for aquaponics' needs beyond just fish. Thus, through tailored fish nutrition and fish as a pump of nutrients, significant strides may be made to strengthen the circular image of hallmark systems like aquaponics.

Lastly, it is well understood that fish nutrition alone cannot solve all the problems or address all the puzzles of a sustainable and circular blue-based (fisheries and aquaculture-centric) bioeconomy of Europe (Commission, 2020). However, the multi-faceted, innovative applications of fish nutrition-excretion knowledgebase to address some circular and sustainable development goals is an example for the future researchers. In the same analogy of improving our resource use efficiency for achieving circular bioeconomy goals, an improvement of our knowledge use efficiency is also needed. Knowledge is also a resource. More complementary exchange(s) of knowledge or inter-disciplinary applications of specific expertise (as in the present dissertation) would be needed for developing bio-based innovations in a future circular bioeconomy.

Conclusions

Chapter 2 concluded by generating applied information that can aid in future conversions, rather 'upgrades', of operational RAS farms to semi-commercial Aquaponic ventures. Intensive aquaculture effluents, generated mostly by feed and feeding fish, contain adequate N, P, Mg, Ca, S, Fe, Zn, Cu, Ni to meet most aquaponic crop needs. K is generally deficient, requiring full-fledged fertilization. Micronutrients B, Mo is partly sufficient and may be ameliorated by more efficient sludge removal. No threat of heavy metal accumulation in aquaculture sludge due to aquafeed was observed. However, pH adjustment measures in the intensive RAS systems have even higher consequences (than fish feed) in terms of soil salinization if accumulated sludge is re-used long-term on land.

Chapter 3 concluded that recent eutrophication of the European carp fishponds might have been rather 'management driven' than caused by 'biological limitations' of common carp. Eutrophication potential from feeding seems linked to P digestibility followed by the bad protein profile of diets. Circular food items like brewery wastes, microbial protein, and natural prey offer high P digestibility (75–90%), but large knowledge gaps still exist in the P digestibility of various alternative feedstuffs. Thermal processing does not always improve P digestibility; acidic pre-incubation with phytases (optimum: 1,500–2,000 IU kg^{-1} feed) is worth exploring. Under a semi-intensive system, digestible 'supplementary' nutrients (N: 3.3–4.9%, P: 0.2–0.5%; even lower) can support at least 0.6–1.2 thermal growth coefficient (reasonable growth) and be regarded as ecologically responsible supplementary feeding.

Chapter 4 concluded that raising aquatic animals solely relying on circular-origin feedstuffs like biofloc biomass (single cell or microbial protein) may not realize full growth potential, and some invasive aquatic species like red swamp crayfish may provide aquatic food production opportunities if their nutritional requirements are addressed. High biofloc biomass inclusion in feed could deteriorate growth due to high ash content (exceeding physiological limit > 14% for crayfish), arginine deficiency (~ 14–20% lower than an optimum requirement for crayfish), and insufficient non-protein energy: protein ratio (3.7 cal mg^{-1} for crayfish). However, the work also showed the promise of these circular origin feedstuffs to replace 33% to 50% of conventional feedstuffs presently used in aquafeed (many of which may not be considered sustainable in future circular bioeconomy) without hampering production.

Chapter 5 concluded that carp production in fishponds has the least nutrient burdens to the environment compared to other food production sectors in Europe. Existing feed provisioning in carp ponds and production intensity cannot thus be considered as a pollution-causing activity. The focus should be on the actual management of the fishponds. The ecosystem and production services offered by carp farming in fishponds have immense societal and economic advantages. However, opportunities exist to calibrate the current feeding practices to achieve environmental (minimized footprint) and aquaculture goals (uncompromised production). Despite the best efforts to optimize *status quo* nutrient management of carp farming in fishponds, nutrients contributed through side streams (catchment, agricultural, municipal nutrient run-offs) would eventually be manifested in the effluents (footprint) from fishponds.

Chapter 6 concluded highest ecosystem resource utilization efficiency and least N, P loading by fish are related to balanced nutrition and managing fishes' satiety to graze (or spare) zooplankton-zoobenthos, enabling maintenance of clear-water phase and ecosystem services. Improved ecosystem resource utilization efficiency (RUE) and tackling eutrophication may be achieved by 'bio-manipulating' temperate shallow lake ecosystems like large European ponds towards balanced fish nutrition (≈proposed approach). Besides extrinsic factors, the highest ecosystem RUE, highest ecosystem services, and least internal N, P loading depend much on nutrition availability for fish in ponds. However, the unchecked allochthonous nutrient influx from the catchment, agricultural land, and municipal sewage must also be addressed simultaneously. Otherwise, optimizing the RUE of pond systems may be a never-ending goal.

Chapter 7 concluded by targeting nutrition-excretion processes of fish in aquaponics systems, having the highest daily in-system throughput of nutrients. A novel database, 'TilaFeed' and its associated utility tools, was developed to provide aquaponics a 'one formula' solution. The objectives of 'TilaFeed' are: (a) to solve nutrient constraints in aquaponic systems, both for fish and plants; (b) avoid or strongly limit artificial fertilizer use in aquaponics by smartly tailored aquafeeds; (c) equip system managers with decision-making tools for nutrient planning of their aquaponic systems.

Chapter 8 concluded a practical approach and links to a digestible nutrient-based database of 'alternative' feed ingredients for fulfilling the nutrient requirements of common carp to replace the fish derivatives in carp feed. Such practical guidelines and databases of alternative ingredients are not readily available to public knowledge. For commercial interests, these types of know-how or tools are almost certainly strictly confidential or subject to a charge. Therefore, the certified, open-access methodology may greatly assist fish nutritionists, feed formulators, farmers, and nutrition researchers. Especially small-scale farm managers preferring farm level feeds, or small-scale feed manufacturers in Czechia and neighboring countries may benefit from the methodology and ZeroFish CarpFeed database containing digestibility and digestible nutrients data for improved resource use efficiency.

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English summary

Circular and sustainable fish nutrition

Koushik Roy

The present doctoral dissertation aims at circular and sustainable applications of fish nutrition (and resultant excretion) knowledge in improving resource (nutrient) utilization efficiency of aqua food systems. The objectives were to address the multidisciplinary issues in the future, embracing circular blue-based (fisheries and aquaculture centric) bioeconomy and environmental sustainability, using fish nutrition-excretion knowledge. The question of why nutrition was chosen as a tool lies in the presumption that perhaps nutrition (and resultant excretion) is the most dynamic and regular process in living organisms (besides respiration), which involves the exchange of nutrients or matter to and from the environment. In order to address circularity and sustainability from the animal to farm level, such processes may be increasingly targeted in the future for assessment and bio-manipulation of nutrients flow (resource use efficiency).

The linear flow of nutrients from aquafeed, feeding fish to excretory products released by the fish if closed by integrating with plants, then the excretory waste does not seem to be a waste, but rather a resource. Whether a nutrient molecule is going in fish (*i.e.,* nutrition) or coming from fish (*i.e.,* excretion) depends a lot on the interactions of management decisions in aquaculture and the biological or physical environment of a feeding fish. If circular-origin feedstuffs are used in future aquaculture or species that are societal discards are integrated into aquaculture, both offer prospects as a nutrition source either for humans (food) or for farmed animals (feed). However, they are not without problems either- nutritionally. On the one hand, the nutritional requirements of raising such new circular origin 'food' may not be well known. On the other hand, the nutritional value of the circular origin 'feed' may not be perfect. Thus, completely integrating everything (feed and food) within an umbrella of 'circularity' would bring their own, completely new challenges.

Feeding decisions or nutrition provisioning in aquaculture greatly impact neutralizing nutrient footprint and achieving sustainable production. But in semi-intensive pond aquaculture, the repercussions of feeding decisions and its resultant nutrient footprint are nothing compared to the many times higher positive value of intangible ecosystem services such systems provide. Therefore, the focus for sustainable production in the future should focus more on ecosystem functioning than blindly curbing production intensity and assuming it would make a significant difference in environmental sustainability. By using fish nutrition and excretion knowledge, there are possibilities to manipulate in-vivo systems to maximize nutrient retention efficiency and minimize losses *in-situ*. If these pieces of knowledge are applied in line with contemporary ecological principles in outdoor semi-intensive aquaculture systems, future adaptation strategies may be intelligently formulated to achieve improved resource use efficiency of a farming system. The entire system (*in-vivo* and *in-situ* nutrients pool) must be visualized as a unit, functioning individually but synchronized. The synchronization mechanisms should be targeted for future biomanipulation.

The applications of fish nutrition (and excretion) can also be beyond the nutrition (growth and physiology) of farmed animals or emissions (and re-valorization) of nutrients. Knowledge of the digestibility of different nutrients in a wide range of feed ingredients by a particular fish species, and its established digestible nutrient requirement, can help find more precise replacements of finite, unsustainable, and conventionally overexploited or even non-circular resources presently used in aquafeed of a given species. The knowledge of nutrient

partitioning (digestibility, metabolic losses), its retention or total loss limits and repercussions on growth and excretion by fish, composition of excreted products itself (suspended losses versus reactive losses) can further make the recycling and re-use of nutrients (in circular food system models like aquaponics) more precise and more efficient. Even using the knowledge, the *in-vivo* system of fish can be taken advantage of through tailored feed formulation (crude intake levels) that would result in manipulated levels of excreted nutrients *in-situ*, available to microbial processes or plants.

In order to materialize a paradigm shift to circular aquaculture regionally or globally, increased awareness regarding the circular bioeconomy concepts needs to be developed first. Then, through collective leadership and brainstorming, more inter-disciplinary exchanges or multi-disciplinary applications of knowledge are necessary. Knowledge of fish nutrition and excretion is just a small part of the bigger puzzle. The present dissertation thus demonstrated a limited overview of how targeted use of knowledge may help address multiple future puzzles of achieving a sustainable and circular aquaculture-centric bioeconomy.

Czech summary

Cirkulární a dlouhodobě udržitelná výživa ryb

Koushik Roy

Hlavním tématem předložené dizertační práce je cirkulární a udržitelná aplikace výživy ryb (a jejich výsledného vylučování). Dizertační práce se zaobírá řešením multidisciplinárních otázek zabývajících se cirkulární bio-ekonomikou (zaměřenou na rybářství a akvakulturu) a environmentální udržitelností s využitím znalostí o výživě a vylučování ryb. Otázka, proč byla výživa vybrána jako nástroj, spočívá v předpokladu, že příjem potravy (a výsledné vylučování) je nejdynamičtějším a nejpravidelnějším procesem v živých organizmech (kromě dýchání), který zahrnuje výměnu živin/hmoty do a z prostředí. Právě tok živin bude pro řešení cirkularity a udržitelnosti od úrovně organizmů až k farmám stále více cílený, a to pro hodnocení stavu životního prostředí a biomanipulaci (např. manipulace toku živin, zefektivnění využití zdrojů).

Živiny vstupující do ryb krmivem jsou rybami vyloučeny jako odpadní produkty, nicméně, pokud jsou živiny integrovány do rostlin a tok živin je uzavřen (cirkuluje), pak lze odpadní produkty ryb pokládat za zdroj živin, nikoli za odpad. Zda jsou živiny pro ryby (tj. pro jejich výživu) nebo pocházejí z ryb (tj. vylučování), závisí převážně na interakcích managementu akvakultury, fyziologii ryb a prostředí, kde jsou ryby chovány. Použití krmiv produkovaných v souladu s cirkularitou nebo začlenění druhů, které nejsou společností akceptovány jako zdroje potravy/živin, jsou pravděpodobně budoucí zdroje výživy buď pro člověka (potraviny) nebo pro hospodářská zvířata (krmivo). Nicméně, u zmíněných možností se lze setkat s problémy. Na jednu stranu nemusí být známé či dostupné živinové nároky pěstované „potraviny“, na stranu druhou nutriční hodnota „krmiva“ nemusí být plně optimální. Úplná integrace krmiva a potravin pod deštník „cirkularity“ by tedy přinesla úplně nové výzvy.

Management krmení či zajišťování výživy v akvakultuře má vliv na neutralizaci živinové stopy a dosažení udržitelné produkce. Nicméně, živinová stopa managementu krmení v polointenzivním rybničním chovu ryb je minimální ve srovnání s mnohonásobně vyšší kladnou hodnotou ekosystémových služeb, které právě rybniční ekosystémy přinášejí. Udržitelná produkce by se proto měla v budoucnu zaměřit spíše na fungování ekosystému samotného než slepě omezovat intenzitu produkce a předpokládat, že takové rozhodnutí by mělo významný pozitivní vliv na udržitelnost životního prostředí. Aplikace znalostí o výživě a vylučování ryb přináší možnost manipulace systému *in vivo* k dosažení maximální účinnosti zadržování živin a minimálních ztrát *in situ*. Pokud jsou výše zmíněné znalosti aplikovány v souladu se současnými ekologickými principy ve venkovním polointenzivním chovu ryb, budoucí adaptační strategie mohou být formulovány tak, aby bylo dosaženo lepší účinnosti využívání zdrojů (angl. *resource use efficiency*). Celý systém (*in vivo* a *in situ nutrients pool*), fungující jak jednotlivě, tak synchronizovaně, musí být vizualizován jako jedna jednotka. Takový synchronizační mechanizmus by měla být v budoucnu cílený při jakékoliv biomanipulaci (např. manipulace toku živin).

Aplikace výživy ryb (a jejich vylučování) může mít mnohdy mnohem větší dosah. Znalost stravitelnosti různých živin širokého spektra ingrediencí krmiv a nutriční požadavky konkrétního druhu ryby můžou pomoci při hledání lepšího zdroje živin, než je původní konvenční, který se většinou neslučuje se zásadami cirkularity a udržitelnosti. Znalosti procesů transformace živin organizmem (stravitelnost, metabolické ztráty), retenční limity živin nebo jejich limity ztrátové, dopady na růst ryb, vylučování ryb a forma ve které jsou odpadní produkty vyloučeny (suspendované versus rozpuštěné) můžou dále přispět k přesnější a efektivnější recyklaci a opětovnému využití živin (například v cirkulárním systému, jako je akvaponie). V *in vivo*

systému ryb lze navrhnout krmivo (přístupem tzv. na míru), jehož transformací v organizmu lze dosáhnout úrovně živin, která by v *in situ* systému odpovídala živinovým nárokům rostlin nebo bakterií.

Aby se nynější paradigma posunulo více k cirkulární akvakultuře regionálně či globálně, je třeba nejprve zvýšit povědomí o konceptu cirkulárního bio-hospodářství. Prostřednictvím kolektivního vedení a brainstormingu je poté potřeba prohloubit spolupráci mezi různými obory a multidisciplinární znalosti aplikovat. Znalost výživy a vylučování ryb je jen malou částí větší skládanky. Tato dizertační práce tedy ukazuje malý přehled o tom, jak s použitím dosavadních znalostí řešit otázky udržitelné a cirkulární bio-ekonomiky zaměřené na akvakulturu.

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List of publications

Peer-reviewed journals with IF

Jia, H., **Roy, K.**, Pan, J., Mraz, J., 2022. Icy affairs: Understanding recent advancements in the freezing and frozen storage of fish. Comprehensive Reviews in Food Science and Food Safety: 1–25. https://doi.org/10.1111/1541-4337.12883 (IF 2020 = 12.811)

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Folorunso, E.A., **Roy, K.,** Gebauer, R., Bohatá, A., Mraz, J. 2021. Integrated pest and disease management in aquaponics: A metadata-based review. Reviews in Aquaculture 13: 971–995. (IF 2020 = 10.592)

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Sarkar, U.K.*, **Roy, K.*,** et al., 2021. Assessing vulnerability of freshwater minnows in the Gangetic floodplains of India for conservation and management: Anthropogenic or climatic change risk? Climate Risk Management 33: 100325. (IF 2020 = 4.09) *(Sarkar and Roy share joint first authorship).

Sarkar, U.K.*, **Roy, K.*,** et al., 2021. Reproductive environment of the decreasing Indian river shad in Asian inland waters: disentangling the climate change and indiscriminative fishing threats. Environmental Science and Pollution Research 28: 30207–30218. (IF 2020 = 4.223) *(Sarkar and Roy share joint first authorship).

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Roy, K., Vrba, J., Kaushik, S.J., Mraz, J., 2020. Nutrient footprint and ecosystem services of carp production in European fishponds in contrast to EU crop and livestock sectors. Journal of Cleaner Production 270: 122268. (IF 2020 = 9.297)

Lunda*, R., **Roy*, K.,** Másílko, J., Mráz, J., 2019. Understanding nutrient throughput of operational RAS farm effluents to support semi-commercial aquaponics: Easy upgrade possible beyond controversies. Journal of Environmental Management 245: 255–263. (IF 2019 = 5.647) *(Lunda and Roy share joint first authorship).

Nag, S.K., Nandy, S.K., **Roy, K.,** Sarkar, U.K., Das, B.K., 2019. Carbon balance of a sewage-fed aquaculture wetland. Wetlands Ecology and Management 27: 311–322. (IF 2019 = 1.221)

Sarkar, U.K., Naskar, M., Srivastava, P.K., **Roy, K.,** et al. 2019. Climato-environmental influence on breeding phenology of native catfishes in river ganga & modeling species response to climatic variability for their conservation. International Journal of Biometeorology 63: 991–1004. (IF 2019 = 2.680)

Sarkar, U.K., **Roy, K.,** Naskar, M. et al. 2019. Minnows may be more reproductively resilient to climatic variability than anticipated: Synthesis from a reproductive vulnerability assessment of Gangetic pool barbs (*Puntius sophore*). Ecological Indicators 105: 727–736. (IF 2019 = 4.229)

Application of methodologies, verified technologies

Roy, K., Kajgrova, L., Dvorak, P., Mraz, J., 2021/2022. Výživa reofilních kaprovitých ryb. Edice Metodik, FROV JU, Vodňany, no 192, 34 pp. (in press)

Roy, K., Mráz, J., 2021. Alternative feed components to replace fishmeal and fish oil in carp feed. Edice Metodik, FROV JU, Vodňany, no. 184, 22 pp.

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Others

Kajgrová, L., **Roy, K.,** Vrba, J., Mráz, J., 2020. Něco pro povzbuzení našich rybářů, kteří odvádějí dobrou práci! Rybníkářství 31: 43.

Training and supervision plan during study

Name	Koushik Roy, M.F.Sc.	
Research department	2018–2022 – Laboratory of Nutrition of FFPW	
Supervisor	Assoc. Prof. Jan Mraz (head of lab; head of RP3 CENAKVA)	
Period	12th February 2018 until 22nd March 2022	

Ph.D. courses	**Year**
Czech language for foreigners	2020
Basic of scientific communication	2019
Biostatistics	2019
Pond aquaculture	2018
Applied hydrobiology	2018
Ichthyology and fish taxonomy	2018
English language	2018
Scientific seminars	**Year**
Ph.D. seminar of FFPW	2019
Ph.D. seminar of FFPW	2020
Ph.D. seminar of FFPW	2021
USB conference of doctoral students	2021
International conferences	**Year**
Aquaculture Europe 2019 by European Aquaculture Society	2019
FAO Workshop 2021	2021
Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
4 days visit and carp farming related consultation with Dr. Martin Oberle (Head of carp pond economy sub-station IFI2, Bayerische Landesanstalt für Landwirtschaft – LfL) in Hoechstadt, Bavaria (Germany)	2019
4 days visit and aquaponics related consultation with Prof. Werner Kloas (Head, Aquaculture division IGB and Leader, EU funded INAPRO) and Dr. Hendrik Monsees (Post-doc, INAPRO), Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB Berlin, Germany)	2019
4 days visit to Paris for Horizon 2021 cluster-6 circular bioeconomy farm2fork strategy project partners meeting (France, Netherlands, Poland, Hungary, Belgium, Czech Republic) and work package development for CENAKVA	2021
Pedagogical activities	**Year**
International Summer School Project 2019: student –Sara Ahani (Iran)	2019
International Summer School Project 2021: student – Anil Axel Telbuscher (Germany)	2021
Consultation of bachelor’s thesis	2018-2019
Teaching and training of ERASMUS exchange students (feed pereparation, RAS, Biofloc operation, digestibility trials, fish nutrition)- >50 hours	2018–2021

Curriculum vitae

PERSONAL INFORMATION

Name:	Koushik
Surname:	Roy
Title:	M.F.Sc.
Born:	22nd November 1989, Haripal Hooghly, India
Nationality:	Indian
Languages:	English (FCE Level C1), Hindi, Bengali.
Contact:	kroy@frov.jcu.cz

EDUCATION

2018–present	Ph.D. (Fishery), Faculty of Fisheries and Protection of Waters, University of South Bohemia, Ceske Budejovice, Czech Republic.
2011–2014	M.F.Sc. (Aquaculture) "with distinction", Department of Aquaculture, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India.
2008–2011	B.Sc. (Industrial Fish and Fisheries) "with distinction", Department of Industrial Fish and Fisheries, Asutosh College, University of Calcutta, West Bengal, India.
2014	Qualified Indian Council of Agricultural Research (ICAR) National Eligibility Test (NET) in Aquaculture. Eligible for nationwide university lecturership.

EMPLOYMENT

2014–2015	Guest faculty (teaching), Department of Industrial Aquaculture and Fisheries, Asutosh College, University of Calcutta.
2015–2018	Senior Research Fellow, Project NICRA (climate change, inland fishes, and fisheries), ICAR-Central Inland Fisheries Research Institute (Government of India), Barrackpore, West Bengal, India.

AWARDS

2011	University order of merit in B.Sc., First rank holder, University of Calcutta.
2014	University order of merit in M.F.Sc., First rank holder, Indira Gandhi Agricultural University.
2019–2021	Best presentation awards in FROV PhD seminars (3 years, 3 seminars).
2021	FROV Dean's best publication award.
2020–2021	Recipient of two one-year grants (GAJU projects) by university grant agency.

TRAINING

2016	Training Program on Climate Change Impact and Adaptation strategies for Wetland Fisheries (1 day).
2015	Training on impact of climate variability on inland fisheries and strategies for adaptation at ICAR- CIFRI, Barrackpore (2 days).
2014	Training on fish nutrition and feeding strategies at Central Institute of Fisheries Education, Kolkata (7 days).
2013	Training on disease diagnosis, prevention, and control measures in aquaculture at Central Institute of Fisheries Education, Kakinada (8 days).
2010	Training in fish processing and preservation at National Institute of Fisheries Post Harvest Technology and Training, Kochi (30 days).
2010	Training on freshwater aquaculture at KVK Nimpith, Sunderbans, West Bengal, India (10 days).

COLLABORATIONS

2018	Dr. Francis Murray, Institute of Aquaculture, University of Stirling.
2018	Dr. Salin Krishna, Asian Institute of Technology, Thailand.
2018–2020	Prof. Sadasivam Kaushik, University of Las Palamas Gran Canaria, Spain.
2018–2020	Dr. Uttam Kumar Sarkar, Central Inland Fisheries Research Institute, India
2019	Prof. Werner Kloas, IGB, Berlin, Germany.
2019–2021	Dr. Martin Oberle, Lfl-Bayern, Bavaria, Germany.
2020–2021	Prof. Giovanni Turchini, Deakin University, Australia.
2021	Dr. Serhat Turkmen, University of Birmingham, USA.
2021	Assoc. Prof. Stefanie Colombo, Dalhousie University, Canada.
2020–2021	Prof. Johan Verreth, Wageningen University and Research, Netherlands
2021	Dr. Joel Aubin, INRAE France