Fakulta rybářství
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David Hlaváč

Czech Republic, Vodňany, 2015

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1. Sustainable aquaculture in ponds

Global aquaculture production has increased 40 times since 1970, and is again expected to increase fivefold over the coming 50 years (Avnimelech et al., 2008). Fish production from capture fisheries and aquaculture provides more than 15% of the animal protein consumed by the world's human population (FAO, 2002). Consequently, aquaculture is the fastest growing food sector globally and its economic importance is increasing concomitantly (FAO, 2009), with pond farming of common carp (*Cyprinus carpio* L.) being of great importance.

Freshwater aquaculture is an important and integral part of the Czech agricultural sector. In the Czech Republic, primary emphasis is on fish pond farming, which is traditional form of aquaculture with deep historical roots, consequentially there is a high degree of specialised knowledge. As such, fish pond farming represents a national heritage that should be promoted. Pond systems play a fundamental role in landscape water management and are important for water retention, flood prevention and in the preservation and protection of biodiversity. Ponds also have an important social, cultural and recreational function, and this contributes to the sustainable development of living conditions, not only locally but also in adjacent regions.

Aquacultural production in the Czech Republic is characterised by both extensive and semi-intensive fish farming in ponds. According to Adámek et al. (2012), the majority of farmed fish in the Czech Republic are produced using these types of systems; common carp is the main species, accounting for around 88% of total fish produced. Carp are an ideal species for pond farming as they are a hardy species, able to tolerate wide variations in abiotic and biotic environmental factors (Stankovic et al., 2010). Under this system, mosts of carp growth (70–75%) is achieved using natural food, such as zooplankton and zoobenthos, but in about 25–30% supplementary feeding is used which is mainly raw whole cereals. In recent years, artificial fertilisation has become an additional intensification measure, leading to eutrophication and algal blooms in pond ecosystems and receiving waters due to high nutrient loading. According the current Czech legislation (The Water Act 254/2001), the application of fertilisers to the pond environment presents an increasing problem and it should be avoided in the future. As a result, the use of supplementary feeding remains as the only available tool for intensification of fish production.

Fish culturists strive to transfer the energy from feed into the fish crop efficiently, while minimising adverse impacts on the environment and water quality (Filbrun et al., 2013). In the case of carp, it is extremely important to select the type of feed that will achieve the most profitable production. In other words, improvement of semi-intensive systems is achieved through better quality supplemental feed and improved feeding practises. The use of high-quality feed should minimise water pollution through high digestibility and low conversion rate, resulting in better fish growth (Ciric et al., 2015) and less organic waste loading per kilogram of fish produced (Cho et al., 2006).

In many cases, the sustainability of aquaculture has been questioned and, as a result, global and regional institutions have proposed Best Management Practice (BMP) to enhance sustainable production (FAO, 1997). The goal of BMP is to make aquaculture more environmentally responsible, while also considering social and economic sustainability (Bosma and Verdegem, 2011). The development of BMP should lead to further improvement in feed efficacy and water management regimes, ultimately leading to significant reductions in phosphorous discharge levels, thereby contributing to the sustainability of pond aquaculture systems and to improved environmental integrity. The current challenge in aquaculture is to raise productivity while maintaining environmental sustainability and, therefore, a large proportion of the production increase must be realised through low-cost technologies (Diana et al., 2013). Hence, fish feed must be of good quality to assure high utilisation, high growth rate and good health, while at the same time protecting the aquatic environment. This objective can best be achieved by using mechanically and thermally treated cereals as modified cereals are economically inexpensive (Másílko et al., 2014). Such dietary supplements could prove important for both sustainable pond management, improved carp growth performance and phosphorus budget in pond systems (Hlaváč et al., 2015).

1.2. Aims of this thesis

1) In the first study, the aim was to describe the main issues affecting pond water quality under supplementary feeding through a review of current knowledge, thereby presenting the essential backround for the following studies (Paper I).

2) In the second study, the aim was to assess, i) nutrient levels entering an experimental storage pond where fish are fed supplementary cereal, ii) conversion efficiency along the food chain resulting in carp growth, and iii) the impact of such inputs on overall nitrogen and phosphorous budget (Paper II).

3) In the third study, the aim was to assess the influence of different supplementary feeds (cereal grain and thermally treated cereal) on i) water quality, ii) the invertebrate community, and iii) nutrient budget in carp growing ponds over two consecutive growing seasons (Paper III).

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CHAPTER 2

EFFECTS OF SUPPLEMENTARY FEEDING IN CARP PONDS ON DISCHARGE WATER QUALITY: A REVIEW

Hlaváč, D., Adámek, Z., Hartman, P., Másílko, J., 2014. Effects of supplementary feeding in carp ponds on discharge water quality: a review. Aquaculture International 22, 299–320.

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Effects of supplementary feeding in carp ponds on discharge water quality: a review

David Hlaváč · Zdeněk Adámek · Pavel Hartman · Jan Másílko

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Abstract Discharge of aquacultural wastewater can lead to eutrophication and disruption of natural ecosystems in receiving water bodies. A controlled waste production strategy is necessary, therefore, in order to maintain sustainable aquacultural growth. Along with fertilisation, supplementary feeding is the major source of allochthonous matter in aquaculture and management of aquacultural waste, therefore, should be approached through diet formulation and/or feeding strategy. The introduction of highly digestible feed has reduced solid waste excretion, and further reductions can be achieved through careful selection of ingredients and processing to improve nutrient availability. Dissolved nitrogen waste can be reduced by ensuring a balance between protein and energy, such that fish use non-protein sources as energy, while phosphorous waste can be reduced through careful ingredient selection and processing to improve digestibility. Thermal and mechanical treatment of feed cereals prior to application can also help decrease the amount of poorly or undigested feed. Finally, feeding practices that minimise wastage should also be explored as they have a significant impact, not only on waste output but also on the overall economy of carp pond farming.

Keywords Cereals · Common carp · Environment · Nitrogen · Phosphorus · Pond aquaculture

Introduction

Aquaculture has developed rapidly over the past few decades (FAO 2010), with growth aimed at meeting two major incentives: food security and income generation. To achieve

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this, fish culture, in general, has become much more intensified. Such intensification has significant drawbacks, including increased environmental impact due to the large amounts of waste discharged as effluent (Cho and Bureau 2001; Tacon and Forster 2003; Saremi et al. 2013).

Central European fishponds represent unique, man-made aquatic ecosystems that are important and integral parts of the landscape (Kořínek et al. 1987; Adámek et al. 2012). Currently, the main function of most fishponds is the production of fish based on utilisation of the natural production potential of the pond ecosystem. Since the early twentieth century, however, carp (*Cyprinus carpio*) production in ponds has been systematically increased through a variety of management improvements. Current carp pond management practice (which includes fertilisation and supplementary feeding), together with the influence of agriculture and human settlements, has led to a state in which the majority of ponds in Central Europe are considered as eutrophic to hypertrophic aquatic ecosystems (Pechar 2000; Potužák et al. 2007; Všetičková et al. 2012).

Fishpond farmers must cope with many problems related not only to maintaining the health and nutritional status of fish but also to pond water quality (Dulic et al. 2010; Máchová et al. 2010a; Filbrun and Culver 2013) and pond ecosystem diversity in general. As such, water quality is likely to be one of the main limiting factors for Central European fishpond farming in future (Kolasa-Jamińska 2002; Wezel et al. 2013). Pond water quality is subject to increasingly stringent requirements, requiring pond managers to have a good understanding of the interactions between biological and chemical parameters within the aquatic environment, including fundamental factors at the trophic and saprobic levels such as nutrient and organic content (Máchová et al. 2010b). According to the level of allochthonous loading (i.e. *de facto* pollution), the shift in water quality can be positive in the case of strong organic loading of the inflow (Svoboda and Koubek 1990; Masseret et al. 1998; Všetičková and Adámek 2013) or negative in the case of good water quality inflow (Všetičková et al. 2012). A large amount of detailed information has been published on the impact of nutrients and other allochthonous substances on the pond environment. On the other hand, data on the release of nutrients by fish, particularly as affected by ingested food quality and feeding technique, are limited. Nutrients released in this manner have important implications for the pond's hydrochemical regime and development of natural food resources and for discharged pond water quality, both of which are extremely important for correct functioning of the pond ecosystem and for the efficiency and sustainability of fish farming. Thus, this review focuses on issues that contribute to better understanding of the interconnection between fish production, applied feeding technologies and feed quality with respect to water quality determinants.

General impacts of pond carp culture

In comparison with the existing voluminous literature on trout farm effluent and its impact on the environment, the characteristics of carp pond effluents are poorly documented. This is probably due to the fact that carp are mainly produced in extensive, semi-intensive or integrated systems on a worldwide basis (Kestemont 1995; Woynarovich et al. 2011). Such "traditional" systems are generally considered non-polluting and acting as stabilizing elements in the ecosystem (Manz et al. 1988; Szücs et al. 2007; Barszczewski and Kaca 2012). Pond may also contribute to the self-purification processes in surface waters (Lewkowicz 1996; Zygmunt 2006). Research carried out in Austria by Kainz (1985), for example, found no negative impact on receiving water quality from traditionally managed carp pond facility. As indicated by Butz (1988), Banas et al. (2002, 2008) and Vallod and Sarrazin (2010), however, nutrient outflow may not pose problems during the growing season, but pond draining preceding the annual harvest and during harvesting operations can significantly increase loading in receiving waters due to mobilisation of sediments.

Carp pond water quality

Concern for the natural environment, and with surface water quality in particular, has led to the imposition of requirements regarding the physical and chemical parameters of both input and output waters of ponds (Fournier et al. 2003). Recently, it has become the norm in most EU countries to allow fish cultivation, provided it does not have an adverse impact on water quality. Some scientists are even of the opinion that, in some cases, the natural significance of fishponds may outweigh their production role (Lymbery 1992; Duras and Potužák 2012). Pond water quality, however, varies throughout the production season, the scale of such changes depending to a large degree on the amount and quality of fertiliser and fish feed used (Milstein 1993; Diana et al. 1997; Hartman 2012). Intensifying pond fish cultivation by increasing stock density and supplying large amounts of supplementary feed has an obvious impact on environmental conditions in a pond (Kolasa-Jamińska 1994; Szumiec 2002; Abdel-Tawwab et al. 2007). Indeed, according to some researchers (e.g. Seyour and Bergheim 1991; Das et al. 2005; Sindilariu et al. 2009), it is the use of supplementary feed itself that poses the greatest threat to water quality and a number of studies have highlighted adverse impacts on the pond environment from the addition of large quantities of feed, especially where it is improperly balanced or of poor nutritional value (Horner et al. 1987; Poxton and Allouse 1987; Poxton and Lloyd 1989). Strategies aiming to reduce the impact of aquacultural waste, therefore, have to address feed composition, feeding technology and feeding strategy (Cho and Bureau 1997; Bureau and Hua 2010; Hua and Bureau 2012). Feeding strategy improvements are generally based on two main approaches: improving nutrient retention (Cho and Bureau 1997, 2001; Dalsgaard et al. 2012) and increasing waste removal efficiency (Amirkolaie et al. 2005a b, 2006; Lefrancois et al. 2010).

Fishpond water quality is affected through interactions between a range of physicochemical determinants, including temperature, oxygen regime, transparency, nutrient content, pH, alkalinity and hardness, along with the biological component, with fish as a final link in the pond food chain (Ponce et al. 1994; Das et al. 2005; Jana and Sarkar 2005). The physicochemical characteristics of both the pond bottom and the water column are not static, however, but also change with management measures applied, e.g. fish stock composition, supplementary feeding, manuring and fertilisation. Both bottom soil and sediments and pond water quality undergo complex changes due to all these factors (Milstein et al. 2001; Ali et al. 2006; Ahmed et al. 2013). Impacts of aquaculture on the surrounding environment tend to be more diverse and include physicochemical and biological changes not only due to the fish production system itself but also due to the use of available resources, which can generate conflicts with other end-users (Table 1). Finally, water quality attributes will also be influenced by inputs related to the metabolism of the fish (or other aquatic organism) being cultured (Milstein and Svirsky 1996; Chatterjee et al. 1997; Bechara et al. 2005; Rahman et al. 2010).

During the growing season, the physicochemical and biological parameters of inlet water have been changed by the time it is discharged due to a number of factors, including stocking density, species composition, management methods, climatic conditions,

Table 1 Negative impacts of pond aquaculture on the environment

Physicochemical and biological issues	References
1. Modification of water temperature and flow rate profiles	Billard and Perchec (1993), Beveridge (1984), Všetičková et al. (2012)
2. Increased concentration of suspended solids, BOD, COD, forms of N (including ammonia), phosphorus	Warrer-Hansen (1982), Muir (1982), Boyd and Tucker (1998), Kanclerz (2005), Petrovici et al. (2010), Kopp et al. (2012), Všetičková et al. (2012)
3. Reduced concentration of dissolved oxygen	Bergheim and Silvertsen (1981), Boyd and Tucker (1998), Všetičková et al. (2012)
4. Alteration of water quality due to the use of chemicals and antibiotics	Buchanan (1990). Boyd and Massaut (1999)
5. Generation of organic-rich sediments	Holmer (1992), Lin et al. (1998), Lin et al. (1999), Lin and Yi (2003), Vallod and Sarrazin (2010)
6. Occurrence of algal blooms in eutrophic waters	Gowen et al. (1990), Potužák et al. (2007), Jahan et al. (2010)
7. Modification of the biotic index (based on invertebrate communities) and of the index of biotic integrity (based on fish populations)	Gowen et al. (1988), Trigal et al. (2009), Petrovici et al. (2010)
8. Genetic pollution and escape of undesirable and invasive fishes (e.g. *Carassius gibelio* and *Pseudorasbora parva*)	Cross (1992), Kalous et al. (2004), Gozlan et al. (2005), Tsoumani et al. (2006), Musil et al. (2007, 2010), Lusk et al. (2010)
9. Increased risk of disease spread	Hepher and Pruginin (1981), Hubbert (1983), Akoll et al. (2012), Hoverman et al. (2012)

elevation, original quality and quantity of inflow water, hydraulic retention time, pond volume, morphology and area, outlet location (surface, bottom) and many others (Adámek et al. 2010). In general, water discharged from ponds supplied with good-quality water has a higher temperature, and increased nutrient loading (mainly in the form of ammonia and phosphates), dissolved substances and suspended solids content (Kanclerz 2005; Kopp et al. 2012; Všetičková et al. 2012). On the other hand, nitrate concentrations are usually lower due to water retention. Changes in biochemical oxygen demand (BOD) and chemical oxygen demand (COD) depend primarily on inflow water quality, the outflow having reduced BOD and COD when inflows have high organic substance loading (Svoboda and Koubek 1990; Masseret et al. 1998; Všetičková et al. 2012). On the contrary, inflow water poor in organic content will become enriched in the pond, and consequently, outflows will have increased BOD and COD (Všetičková et al. 2012). Elevated levels of BOD and COD, along with increased suspended solid and phosphorus (P) concentrations and decreased oxygen content, are typically associated with pond draining during harvesting.

Water retention in a pond will affect the oxygen regime, with a decline in dissolved oxygen (DO) concentration due mainly to respiration of water organisms and consumption of oxygen via decomposition of organic substances (including unused feed) and oxidation of inorganic substances. On the other hand, pond water can be saturated with oxygen from phytoplanktonic photosynthesis during the day. Supplementary feeding of pond fish requires a high input of organic matter and nutrients into the ecosystem; ponds with high fish stock densities, therefore, are particularly prone to low DO connected with high organic waste and feed levels. Excess organic material consumes oxygen during decomposition and can drive DO concentrations in ponds to dangerously low levels should sudden destratification occur. In addition, nutrients derived from organic waste and unused feed can enhance algal growth: blooms of planktonic algae resulting in extremely high DO concentrations during daylight and super-saturation of oxygen in the epilimnion. Intensive ecosystem respiration, which can consume substantial quantities of oxygen at night, may result in anoxic conditions in the predawn hours (Schroeder 1974; Boyd 1982; Jana and Sarkar 2005).

Pond aquaculture waste

The influence of supplementary fish feed on discharged water quality will depend on the composition and physical characteristics of the feed used, the technology used in its production, its digestibility, palatability, quality of the components supplied, and feeding technique. The waste produced can be divided into either solid or dissolved phase. Solid waste, consisting of settleable and suspended solids, mainly originates from uneaten and/or spilled feed and from excreted faeces. Part of the dissolved waste (i.e. organic substances, ammonia) originates from metabolites excreted by fish through the gills and in urine, the rest originating from disintegration/resuspension of nutrients from both the settleable and suspended solid waste fractions (Amirkolaie 2011; Adámek and Maršálek 2013). In the carp pond, the mud is mixed with water by carp in its search for food in the bottom (Hepher 1958).

In principle, supplementary feeding intensity in semi-intensive carp ponds is adjusted according to quantitative and qualitative measures of zooplankton development, which is ultimately controlled by fish predation (Schlott et al. 2011). In intensive aquacultural systems, between 20 and 40 % of dietary dry matter is incorporated into the fish body and the remaining part excreted (Siddiqui and Al-Harbi 1999; Verdegem et al. 1999; Brune et al. 2003). When feeding fish with cereals alone, the fraction of nutrient supply driving the bacterial–detrital food chain can rise to almost 95 %, with only 5 % directly utilised for fish growth (Olah 1986). The amount of faecal waste produced can range between 0.2 and 0.5 kg dry matter per kg feed, depending on feed composition, fish species and temperature (Chen et al. 1997). Whichever system is used, the proportion of uneaten or spilled feed will range between 5 and 15 % (Beveridge et al. 1997; Cho and Bureau 1997). In order to improve this situation, a number of studies have been undertaken that address fishpond management and feeding strategy, investigating such issues as new fish feed mixtures with lower nutrient content and improved utilisation of the feed supplied (Adámek et al. 1997).

In all aquacultural systems, waste is partially discharged with effluent water. Both the amount and composition of waste discharged, however, will differ depending on the system employed. In pond systems, the vast majority of total waste remains in the system, with part of the organic waste mineralised in situ (Fig. 1; Verdegem et al. 2001). For this reason, carp ponds can be of great ecological value, though sustainability can only be maintained if stocked at production intensities of at least 500–1,000 kg ha^{-1}, thereby guaranteeing minimum profit without obvious signs of pond degradation (Knösche et al. 1998; Knösche et al. 2000). In addition, ponds may trap nutrients from the outer catchment area and transform them through primary and subsequent production links within the pond food chain (Füllner et al. 2000). Many critics, however, point out that carp culture pollutes the environment as effluent water enters downstream watercourses (Petrovici et al. 2010).

In intensive aquaculture, most dissolved nitrogen (N) and P come from metabolic waste products excreted by fish (Hakanson et al. 1998; Lemarie et al. 1998; Gondwe et al. 2011), with levels of N and P in fish food (Table 2) and its efficiency of use influencing the amount excreted (Rodehutscord et al. 1994). Reducing dissolved N and P output is now considered a key element for long-term sustainability of aquaculture around the world (Phillips et al. 1993; Cho and Bureau 1997; Sugiura et al. 2006). Fish can retain 20–50 % of feed N and 15–56 % of feed P (Schneider et al. 2004; Rahman et al. 2008b; Adhikari et al. 2012), releasing the remainder into the water where it can then be converted to valuable products by phototrophic and heterotrophic organisms (Schneider 2006). The use of balanced diets, therefore, can significantly reduce the amount of these compounds in the water (Hasan 2001), with rearing size, fish species, rearing practice, alimentary handling and food characteristics also affecting the amount of alimentary residue (Mallekh et al. 1999). The use of extruded diets has proved to be an important advancement in fish nutrition. These possess higher stability and digestibility, resulting in a significant reduction in the amount of nutrients excreted into the rearing water (Johnsen et al. 1993). As feeding efficiency improves, the waste N and P concentrations decrease, and thus, ammonia concentration is often seen as the limiting water quality parameter in intensive aquaculture production systems (Thomas and Piedrahita 1998).

Nitrogen

Fish are able to utilise protein very efficiently, despite using a significant proportion of digestible protein for energetic purposes (Kaushik et al. 1982; Kaushik and Dabrowski 1983) and producing large amounts of nitrogenous metabolites (Dosdat et al. 1996). Results from a variety of culture systems indicate that, on average, about 25 % of N (ranging from 11–36 %; Fig. 2) supplied in feed or other nutrient input is retained by the target organism (Avnimelech and Lacher 1979; Hargreaves 1998; Rahman et al. 2008a; Nowosad et al. 2013). Little information is available regarding the effects of N input and

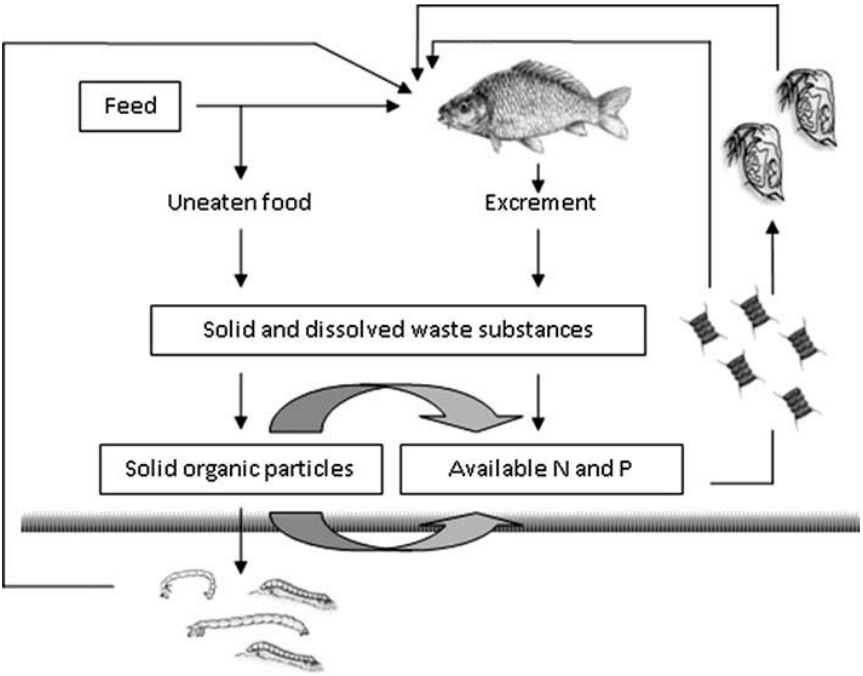

Fig. 1 The fate of feed in a semi-intensively managed fishpond (Rahman 2006; Adámek et al. 2010)

output on N dynamics in aquacultural ponds (Gross et al. 2000; Gál et al. 2003, 2013), and a more complete understanding of the factors regulating ammonia and nitrite concentrations and the exchange of nitrogenous compounds between sediment and water is needed (Hargreaves 1998). Uneaten feed and faeces, however, do contribute to the system's organic loading. Microbial decomposition of organic matter in the water and sediment (see Crab et al. 2007) leads to increased levels of ammonium (Read and Fernandes 2003), which in turn may be transformed into nitrite, nitrate and gaseous N (though formation of N gas is considered negligible in aquaculture ponds). Data on the amount of ammonia excreted by fed and starved fish as well as the dynamics of ammonia excretion by fish of different size are limited (Nowosad et al. 2013). Both ammonium and nitrite are harmful to fish, even at low concentrations. Regarding ammonium in water, it is in equilibrium with ammonia, depending on pH and temperature (Timmons et al. 2002). It would appear that ammonium is at least two orders of magnitude less toxic to the fish than ammonia, being toxic for commercial fish at concentration above 1.5 mg N l^{-1} (Eshchar et al. 2006). Ammonia can also cause a decrease in growth and feed utilisation in different fish species (Biswas et al. 2006). On the other hand, nitrite can cause severe damage in fish, even mortality at 0.43 mg l^{-1} (Koltai et al. 2002), mainly through the decrease in oxygen-carrying capacity of the blood and causing anoxic conditions in organs and tissues (Kroupova et al. 2005). However, the toxic effects of nitrite and ammonia depend on a large number of external and internal factors (Svobodová et al. 2005; Colt 2006).

The inherent nutrient utilisation efficiency of fish implies that N loading in fishponds may be limited by the pond capacity to assimilate nitrogenous excreta (Hargreaves 1998;

Table 2 Average phosphorus and nitrogen content of different fish feeds and in fish

Source	Total phosphorus ($g\ kg^{-1}$)	Total nitrogen ($g\ kg^{-1}$)	References
Corn	2.9	15.7	Kirchgessner (1982), Eeckhout and De Paepe (1994), Lád (2003), Jirásek et al. (2005)
Rye	3.5	16.1	Kirchgessner (1982), Eeckhout and De Paepe (1994), Jirásek et al. (2005), Steiner et al. (2007), and Kowieska et al. (2011)
Triticale	3.4	19.1	Knösche et al. (1998), Jirásek et al. (2005), Steiner et al. (2007), Kowieska et al. (2011)
Barley	3.7	17.9	Eeckhout and De Paepe (1994), Jirásek et al. 2005, Steiner et al. (2007), and Kowieska et al. (2011)
Wheat	3.3	19.2	Kirchgessner (1982), Cossa et al. (2000), Kim et al. (2002), Jirásek et al. (2005), Füllner et al. (2007), Steiner et al. (2007)
Oats	3.9	22	Eeckhout and De Paepe (1994), Jirásek et al. 2005, Steiner et al. (2007);
Dry feeds	9–11	60–72	Knösche et al. (1998), Knösche et al. (2000)
Fish	8.1	29	Sterner and George (2000), Tanner et al. (2000), Nwanna et al. (2010), Hartman (2012)

Paspatis et al. 2000). Factors affecting N excretion include fish species and time after food intake (Lupatsch and Kissil 1998), while an excess of amino acids in feed results in amino acid catabolism, which is associated with ammonia excretion and a loss of energy (Lloyd et al. 1978). In addition to protein content of feed, the balance between digestible protein and digestible energy in the diet can result in an increase in N retention efficiency and a decrease in ammonium waste excreted (Kaushik 1994, 1998; McGoogan and Gatlin 2000). The use of non-protein energy sources such as fat or carbohydrate, to meet energy requirements, can improve protein retention (Keshavanath et al. 2002), thereby also reducing ammonium waste. This phenomenon is commonly called the "protein-sparing effect" and has been demonstrated in a number of species (Kaushik 1998). In semi-intensive carp pond culture, ammonia and urea excretion by fish is of less importance as, under favourable conditions, both compounds are immediately incorporated in the pond ecosystem's "metabolism".

Vegetable protein, the primary protein source in carp ponds, affects feed utilisation and N waste differently according to its origin. Generally, vegetable protein has a poor amino acid balance, which reduces N retention and, consequently, increases N excretion. In common carp, total N loading calculated based on whole-body N retention is 31–86 kg N per 1 tonne of fish produced (Jahan et al. 2002). Thus, alternative protein sources, such as fish meal and soya bean meal, have been suggested in order to improve N assimilation and utilisation efficiency (Hargreaves 1998).

However, ponds have a large nitrogen retention capacity due to denitrification and fixation in sediments (El Samra and Olah 1979; Olah et al. 1983; Pokorný et al. 1999). Several data for nutrient budget of freshwater fishponds operated under various climatic conditions (Avnimelech and Lacher 1979; Boyd 1985; Foy and Rosell 1991) and carp-based ponds in the temperate zone (Olah et al. 1994; Schreckenbach et al. 1999; Knösche et al. 2000) have been reported. According to Knösche et al. (2000), fishponds retain on average 78.5 kg N ha^{-1} $year^{-1}$. However, nitrogen retention of 93 kg N ha^{-1} $year^{-1}$ was

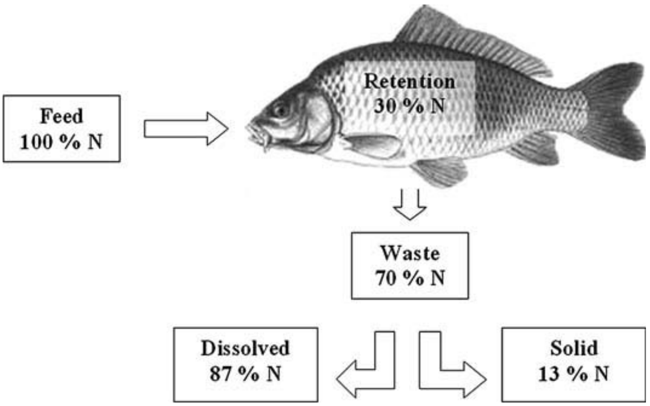

Fig. 2 Nitrogen retention in fish (adapted from Jirásek et al. 2005)

reported by Olah et al. (1994), as a result of an analysis of nitrogen input and output data for a 20-year period. The average nitrogen retention in Germany and Hungary was 43 kg ha^{-1} year1 in a number of fishponds (Schreckenbach et al. 1999).

Phosphorus

While fish can absorb P from water, dietary supplementation is usually necessary due to low waterborne P concentrations (Phillips et al. 1958). Under traditional commercial aquacultural feeding regimes, retention of dietary P is around 20 %, the rest (68–86 %) being excreted (Avnimelech and Lacher 1979; Crab et al. 2007; Lazzari and Baldisserotto 2008; Rahman et al. 2008a). Excess P in fish diet results in higher levels of excreted P, and this is one of the main causes of eutrophication in ponds (Kim et al. 1998; Jahan et al. 2003), often also resulting in impaired water quality downstream. Sediment-bound P is a major problem in carp ponds, and about half the P in a pond can be controlled by monitoring the output of solids (Pursiainen 1988; Vallod and Sarrazin 2010).

With the global concern for reducing water pollution, it is becoming imperative that the fish food industry reduces P excretion in fish (Rodehutscord et al. 2000). One way of addressing this is in the formulation of new fish feeds that produce less P pollution while maintaining adequate levels of available P to support growth (Jahan et al. 2003; Satoh et al. 2003; Bueno et al. 2012). The bioavailability of P from fish meal is lower for carp than rainbow trout *Oncorhynchus mykiss* (Table 3), such differences probably originating from the lack of gastric digestion in stomach-less carp (Lall 1991; Satoh 1991; Jahan et al. 2001). Feed composition, therefore, has a great impact on P digestibility, retention and loss (Amirkolaie 2005a).

The level of unretained P is largely a function of the amount of P in the feed and its bioavailability (Buyukates et al. 2000). In general, P is found in all plant and animal components used in formulate feeds; its bioavailability varies greatly, however, depending on the particular component (Table 3). Plant protein ingredients, such as corn gluten or soya bean meal, have a lower P content compared to fish meal or other animal by-products: a desirable characteristic for low-polluting diet formulation (Cho et al. 1994;

Sarker et al. 2011; Yang et al. 2011). Vegetable sources, however, generally possess larger amounts of phytate form P, a form unavailable to fish as they do not possess the enzyme phytase (NRC 1993; Cho and Bureau 2001; Kumar et al. 2012). As a consequence, most phytate-P ends up being excreted into the water and may cause algal bloom pollution (Baruah et al. 2004). The use of high-protein ingredients with a high percentage of digestible P, therefore, should help to reduce the concentration of unavailable P in feed (Cho et al. 1994). In common carp, total P loading calculated based on whole-body P retention is 8.9–26.4 kg P per 1 tonne of fish produced (Watanabe et al. 1999; Jahan et al. 2000, 2001, 2002).

Reduction of carp pond waste discharge into the environment

Feed quality improvement

Ingredient digestibility and nutrient composition are among the main factors affecting total waste output in aquacultural production, and therefore, efforts at minimising further waste discharge from aquaculture should aim to improve diet formulation and processing. Solid waste in aquaculture is mainly composed of undigested starch and fibre from grain and plant ingredients: undigested protein and fat being low in solid waste as they are highly digestible (Cho and Bureau 2001). Application of highly digestible feed, however, cannot solve the issue of faeces production completely as digestion in fish is naturally limited and a certain fraction of the feed will always remain undigested and excreted in faeces (Cho et al. 1994). Furthermore, as availability of fish meal and fish oil becomes limited in future (Hardy 1996; Gatlin et al. 2007; Amirkolaie 2011), modern farming systems for herbivorous, omnivorous and carnivorous fish are all expected to rely more on supplementary diets containing a high percentage of plant ingredient (Naylor et al. 2000; Hardy 2008; Hua and Bureau 2012).

The composition of feed and the way it has been processed can alter the physical properties of faeces, thereby influencing the efficiency of solid waste sedimentation (Amirkolaie et al. 2005b). Starch is a cheap source of energy, and its inclusion in fish feed can influence faeces stability (Han et al. 1996; Brinker and Friedrich 2012). Stable faeces have a larger particle size, settle more quickly and are more efficiently incorporated into decomposition and bioturbation processes at the pond bottom. Plant ingredients always contain a fraction of starch and the addition of starch to an aqua diet can reduce the dissolved nitrogenous waste of many fish species by increasing the non-protein dietary energy content (Steffens et al. 1999; McGoogan and Gatlin 2000).

Over the past few decades, there have been many changes in feeding and feed technology aimed at reducing the production of solid waste through uneaten or spilled feed (Bergheim and Asgard 1996; Kiang 1999), including technological treatments (e.g. extrusion and expansion) that have improved the physical characteristics (e.g. water stability, leaching) of fish feeds (Kearns 1993; Wilson 1994; Misra et al. 2002). The feeding habits of benthivorous fish (such as carp) are of special importance in this context as regards their role in ingestion of feed sinking to the bottom. In searching for spilled feed, they release large quantities of nutrients into the water column, thus enhancing phytoplankton production (Adámek and Maršálek 2013). This may be especially important in aquacultural carp ponds (Avnimelech et al. 1999), particularly in those with older carp that receive supplementary food from the onset of the growing season (Kloskowski 2011).

Table 3 Comparison of bioavailability (%) of phosphorus from different feed ingredients for rainbow trout and common carp (adapted from Kaushik 1993)

Ingredient	Rainbow trout	Common carp
Brewer's yeast	91	93
Casein	90	97
White fish meal	60–72	10–26
Brown fish meal	70–81	24
Rice bran	19	25
Wheat germ	58	57
Phytate-P	0–19	8–38

Supplementary feeding with cereals

Common carp are the most frequently cultivated fish in Central and Eastern European ponds (Mráz and Picková 2009; Mazurkiewicz et al. 2011; Mráz et al. 2012). As a traditional culinary fish, its economic importance is enhanced by its ability to adjust to changeable environmental conditions (e.g. water quality), to accept diverse fish feed components, and its high feed conversion ratio (Jauncey 1982). Production in Central Europe is typically achieved using a combination of semi-intensive farming, based on natural food, and supplementary feeding with cereals (Hepher and Pruginin 1982; Moore 1985, Horváth et al. 1992; Kaushik 1993), which represents from around 25–30 % (Adámek et al. 2012) to more than 50 % (Tacon and De Silva 1997) of total yield. Supplementary feed has proved a useful tool for providing the nutrients and energy required for improved fish growth and production (Abdelghany and Ahmad 2002), though it has raised concerns as allochthonous substances (feed) are released into the ponds during the production season.

Cereals are one of the most frequently used supplementary feeds in semi-intensive aquacultural ponds (Turk 1995; Zajic et al. 2013), the main components including rye, triticale, maize, wheat and barley (Edwards 2007). While these cannot fully cover the needs of complete carp nutrition, they represent a cheap and readily available source of energy (Turk 1994, 1995; Mráz and Picková 2009). The cereals used tend to have a high proportion of carbohydrates, the primary source of energy for cultured fish (Smith 1989; Sadowski and Trzebiatowski 1995; Sargent et al. 2002). Carp have enzyme systems with high amylase and maltase activity, enabling the fish to utilise large amounts of carbohydrate, though oversupply can result in deposition of fat (Yamamoto et al. 2003; Urbánek et al. 2010).

Cereal grains provide the majority of carbohydrate in feeds used in carp nutrition, the proportion amounting to 35–45 % of the diet on average (Przybyl and Mazurkiewicz 2004). The larger part, however, consists of starch (60–70 %), of which the carp is able to digest around 60–80 % in the raw state, depending predominantly on the cereal species (Hernández et al. 1994; Medale et al. 1999; Cirkovic et al. 2002; Krogdahl et al. 2005).

However, when grain is subjected to the thermal treatment (roasting, boiling, extrusion), starch becomes gelatinous and its subsequent digestibility may reach up to 90 %. Such high carbohydrate digestibility makes grain a basic source of energy in the diet, and this, in turn, allows better utilisation of dietary protein for fish weight gain (Sadowski and Trzebiatowski 1995), in addition to lowering the environmental loading of undigested waste particles. Total protein content in cereal grain varies depending on the species, but ranges between 7 and 15 % (Füllner et al. 2000; Kowieska et al. 2011). The protein is poor in essential amino acids needed by fish, however, and thus is of poor biological value (Przybyl and Mazurkiewicz 2004; Másílko and Hartvich 2010).

Wheat and other cereals contain antinutritional substances (e.g. albumin) that inhibit α-amylase activity (Hofer and Sturmbauer 1985). Others include protease inhibitors, phytoestrogens, goitrogens, antivitamins, phytase and various oligosaccharides and antigenic (allergenic) proteins (Tacon and Jackson 1985; Hendricks and Bailey 1989; Friedman 1996; Alacrón et al. 1999). These are undesirable as they reduce both feed intake and nutrient bioavailability, which leads to slower growth and higher loading of excreta in the water (Van der Ingh et al. 1996; Alacrón et al. 1999). Due to the thermal instability of some antinutritional factors, however, it is possible to use heat treatment to reduce, limit or inactivate the enzymes responsible without impairing the feed (Másílko and Hartvich 2010). Some antinutritional factors can be present in the hulls of cereals, and therefore, removal of the grain's hull prior to heat treatment can also significantly reduce the impact of these factors with little or no effect on the feed's digestibility (Robaina et al. 1995; Glencross et al. 2007).

Mashing has been shown to improve the feed conversion rate of cereals by between 11 (Másílko et al. 2009) and 18 % (Urbánek 2009). Tacon and Jackson (1985) have shown that grinding into meal improves the digestibility of feed by breaking the grain surface, which can also result in a reduction in undesirable antinutritional factors.

The use of good-quality pelleted, and especially extruded, feeds is less expensive and minimises water pollution and the spread of disease through high digestibility and low conversion rate, resulting in better fish growth (Ćirić et al. 2013) and less organic waste loading per kg of fish produced (Cho et al. 2006). The addition of thermal and mechanical treatment prior to their application in carp ponds should further contribute to decreasing the load of undigested or poorly digested supplementary feed.

Conclusion

Both existing and future environmental regulations are driving development and research towards increased sustainability in freshwater carp aquaculture. Numerous studies have examined nutritional strategies as a means of reducing waste production and minimising the environmental impact of aquacultural waste. In recent years, feed quality and feeding method have also been improved to meet this goal.

Diet composition has a profound impact on the quantity and quality of waste in an aquacultural production system, which subsequently affects water quality both inside the system and in receiving water bodies through waste discharge. Waste output into the aquatic ecosystem may be reduced, but not completely eliminated, as fish cannot retain all the food they consume and part of the feed always remains uneaten. Pollution from carp fishpond farms can be significantly reduced, however, by using highly digestible feeds, appropriate feeding strategies, feed processing and an appropriate balance of energy and nutrients (particularly N and P, the main contributors to eutrophication). Thermal and mechanical treatment of feed cereals prior to their application can also help to decrease the amount of poorly or undigested feed and improve water quality and also nutrient balance in rearing ponds. These diets can be helpful for farmers, not only as a source of nutrients for carp growth but also indirectly as a management tool for maintaining ecological quality and stability in ponds.

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CHAPTER 3

EFFECTS OF COMMON CARP (*CYPRINUS CARPIO* LINNAEUS, 1758) SUPPLEMENTARY FEEDING WITH MODIFIED CEREALS ON POND WATER QUALITY AND NUTRIENT BUDGET

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Effects of common carp (*Cyprinus carpio* Linnaeus, 1758) supplementary feeding with modified cereals on pond water quality and nutrient budget

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Summary

This 4-month study (10 May – 6 September 2012) evaluated the effects of supplementary feeding on common carp (*Cyprinus carpio* Linnaeus, 1758) growth, water quality, natural food availability and nutrient balance under different pond treatment regimes (eight rectangular earthen ponds; 0.03 ha surface area; 3-year-old carp). The nitrogen and phosphorus budgets were calculated as the difference between input (food, fish stocked and influent water) and output (effluent water and fish harvested). Three types of supplementary feeds were used: wheat, thermally-treated wheat, and thermally-treated and pressed wheat. An additional test group was provided with naturally available forage only and served as a control. The type of supplementary feed did not influence the water quality, with the exception of dissolved oxygen. No significant differences among experimental ponds were observed in zooplankton abundance. The use of modified cereals (especially thermally-treated and pressed wheat) improved carp growth performance and resulted in lower nutrient concentrations in effluent water via improving their digestibility. Both thermally-treated and thermally-treated and pressed cereals improved the balance of phosphorus; hence these diets could be beneficial, not only from the fish production point of view but also as a tool to reduce the deterioration of pond water quality.

Introduction

The influence of intensive aquaculture on water quality has been frequently studied since the second half of the 20th century (e.g. Ketola and Harland, 1993; Maillard et al., 2005). Impact of common carp (*Cyprinus carpio* Linnaeus 1758) pond production on fishpond ecosystems has been extensively studied in Central and Eastern Europe (Hrbáček et al., 1961; Kořínek et al., 1987; Pechar et al., 2002) Currently, the evaluation of possible positive as well as negative effects of fishery management on surface water quality (e.g. see Všetičková et al., 2012) is another important issue, often linked to integrated aquaculture systems. The majority of carp production in the Czech Republic originates from semi-intensive fish pond operations, where yields rely to a large extent on the natural food resources produced in the pond itself, supplemented by feed at around 25–30% of fish biomass (Adámek et al., 2012). Cereals are the main supplementary feed used within the Czech carp pond farming industry (Mráz et al., 2012; Zajic et al., 2013)

Current carp pond management practice (which includes fertilisation and supplementary feeding), together with the influence of agriculture and human settlements, has led to the majority of ponds in Central Europe being considered as eutrophic to hypertrophic (Pechar, 2000; Knud-Hansen et al., 2003).

This current unsatisfactory state in surface water quality is a growing problem for many water users, including fish farming although Oláh et al. (1994) demonstrated that this has not necessarily to be the case. In most EU countries, present legislation allows fish farming only if effluent quality does not exceed the national quality standards and thus will not adversely affect water quality in the receiving waters (Všetičková et al., 2012; Wezel et al., 2013). High concentrations of nitrogen (N) and, particularly, phosphorus (P) in pond waters are the main reason for water quality deterioration, initial hyper-nutrification and subsequent eutrophication (increase of primary production) in these ecosystems (Boyd et al., 1998; Gross and Boyd, 1998). Reducing dissolved N and P output through better retention of nutrients is now considered a key element for long-term sustainability of aquaculture operations around the world (Sugiura et al., 2006; Verdegem, 2013).

Strategies aimed at reducing the impact of nutrients on water quality in receiving waters have focused on optimizing feed composition, improving feed and feeding technology as well as feeding strategy (Cho and Bureau, 1997; Bureau and Hua, 2010). For example Avnimelech (1999) reported that the addition of carbohydrate in feed to the production systems will reduce the total ammonia nitrogen concentration through immobilization by bacterial biomass in ponds. Increased attention has also been given to the nutrient balance of ponds under different levels of intensification (Verdegem, 2007; Nhan et al., 2008), with the aim not only of preventing excessive surface water pollution (with P as the main element responsible for increased eutrophication and heavy cyanobacterial blooms) but also to take full advantage of the pond's ability to retain nutrients (Oláh et al., 1994; Knösche et al., 2000).

In order to reduce the excessive nutrient loading associated with pond fish farming, the amount of feed and fertiliser should be adjusted in line with fish production such that a zero balance for phosphorus is achieved ($P_{\text{fish feed}} + P_{\text{fertiliser}} + P_{\text{fish stocked}} = P_{\text{fish harvested}}$). This would mean that all phosphorus applied to the pond in connection with fish farming is removed with the harvested biomass. In this way, surface waters will not be subjected to extra P loading that otherwise would increase in concentration and result in enhanced primary production and pond eutrophication. Recent studies have suggested that thermal and mechanical treatment of cereal feeds prior to their application could further decrease the load of undigested or poorly digested supplementary feed (Hlaváč et al., 2014), thus supporting the pond's nutrient balance.

The aim of this paper is to assess (i) nutrient levels entering into a pond where fish are fed supplementary cereals, (ii) conversion efficiency along the food chain resulting in carp growth, and (iii) the impact of such inputs on overall N and P budget.

Materials and methods

Experimental design and management

The experiment was conducted in storage ponds of the Třeboň Fisheries Ltd. (Czech Republic) located at 48° 59′ N, 14° 46′ E and which took place over 120 days from 10 May to 6 September 2012 in eight rectangular earthen ponds, each with a surface area of 0.03 ha. Before stocking, the experimental ponds were drained and dried. The ponds were then refilled with water from the Svět carp marketing pond (215 ha) located upstream. Depth was set at 1 m, thereby providing a uniform pond volume of 300 m^3. The bottom of the storage ponds consisted of a sandy substrate with no regular occurrence of macrozoobenthos, thus zoobenthos sampling was omitted. The ponds were stocked with 3-year-old common scaly carp (Třeboň strain) with a mean individual weight of 1220 ± 157 g ind^{-1}, corresponding to a density of 363 fish per hectare and a biomass of approximately 450 kg per hectare. This density is typical of semi-intensive carp culture in the Třeboň region as it allows maintenance of a sufficient level of large zooplankton (Urbánek et al., 2010). All fish used in the study were of uniform genetic origin and of the same age. Each individual was marked with a microchip in the dorsal musculature using a DataMars Needle Kit with a simple implanter.

Three groups of fish in separate ponds were fed with different supplementary cereal feeds: (i) wheat (W), (ii) thermally-treated wheat (WT), and (iii) thermally-treated and pressed wheat (WTP). As a control (C), one additional group without supplementary feed was based exclusively on natural food. Each treatment was run in duplicate.

WT cereals were heated whole at 100°C for 90 s using a Bühler AG dryer (Switzerland) and then optionally pressed using the Himel GQ 43 processor (Germany). Feed cereals were supplied three times a week (Monday, Wednesday and Friday) at the same feeding site (a 1 × 1 m bankside concrete panel) at 08:00–11:00 hours at an initial rate of 5% of fish stock biomass. Feeding rates were adjusted during the experiment according to fish weight and feed intake, as recorded during control monitoring. When appropriate, the water level was adjusted to a stable 1-m depth from the upstream source in order to compensate for losses through evaporation. The experimental ponds were drained monthly and all fish weighed individually to the nearest 0.2 g, whereupon the ponds were again refilled and fish restocked.

Water quality analysis

During the experiment, important water quality parameters were determined fortnightly between 7:00 and 10:00 hours. In addition, water variables were measured before harvesting each month and after refilling the ponds, restocking and ensuring zero flow-through (after 24 h). Water temperature (T), pH, dissolved oxygen (DO) saturation (O_2Sat) and conductivity (Cond) were measured *in situ* using a YSI Professional Plus multimeter (YSI Incorporated, Yellow Springs, OH, USA). Turbidity (Turb) was measured *in situ* using a WTW Turb 430T/SET (WTW, Weilheim, Germany). Total alkalinity (TA) was determined by titrimetric method *in situ* (Stirling, 1985). Water samples were collected fortnightly for laboratory chemical analysis by taking a 2-L sample from three different locations (two in the middle of the experimental pond and one close to the outflow) at each pond using a Patalas sampler. Samples were kept at 4°C until analysis. Concentrations of NH_4-N, NO_3-N, and dissolved reactive phosphate (DRP) were determined using a Tecator flow injection analyser (FIA Star 5010, Tecator, Sweden; Růžička and Hansen, 1988) in samples filtered through Whatman GF/C filters. Total nitrogen (TN) and total phosphorus (TP) were determined in samples filtered through 100 μm mesh after mineralisation by persulphate as NO_3-N and DRP. Alkalization of the sample by 1 M NaOH and gas diffusion method was used for NH_4-N estimation (Karlberg and Twengstrom, 1983). Reaction of nitrite with sulfanilamide and N-(l-naphthyl)- ethylenediamine was used for determination of NO_2-N. NO_3-N was determined as nitrite after reduction of the sample in a Cd-Cu column. The standard phosphomolybdenum complex method was used for the DRP estimation (Parsons et al., 1984). The FormacsHT Analyzer (Skalar, The Netherlands) was used for Total Organic Carbon (TOC). TOC is converted to carbon dioxide at temperatures up to 950°C by catalytic (Co) oxidation and subsequently measured by a non-dispersive infrared detector (NDIR). TOC concentrations were converted to COD_{Cr} by the empirical relationship validated for fishpond water. The biological oxygen demand (BOD_5) was determined by the standard diluting method (APHA, 1998). TSS content was analysed on Whatman GF/C glass-fibre filters after drying at 105°C and determined according to Stirling (1985). Chlorophyll-*a* concentration was estimated spectrophotometrically following extraction with a mixture of acetone and methanol (Pechar, 1987).

Zooplankton sampling and analysis

Zooplankton were sampled fortnightly at the same time as water quality measurements (between 7:00 and 10:00 hours), samples being taken from the centre of each experimental pond by hauling a 35 cm diameter plankton net of 100-μm mesh-size. The net was drawn vertically from the bottom to the water surface at each location and the collecting funnel contents preserved in 4% formalin. Zooplankton was determined under a binocular microscope (Olympus BX51, Japan) and the quantitative composition determined by direct counting using a Sedgewick-Rafter cell.

Fish growth and production

Individual weight of fish was measured at the beginning and end of the experiment in order to assess individual growth characteristics over the entire rearing period.

Specific growth rate (SGR) was calculated as:

$$SGR(\%.d^{-1}) = (lnW_T - lnW_0) \times 100/T$$

where W_T is the final body weight (kg), W_0 is the initial body weight (kg) and T is the culture period (days).

Phosphorus and nitrogen balance

All management activities (fish stocking, harvesting, feeding) were monitored throughout the study. In order to calculate N- and P-balance, the input of these elements through cereals supplied as supplementary feed was calculated as dry matter, TN and TP according to the Czech National Standard for feed analysis (ČSN 46 7092, 1998).

Average TP and TN contents in 1 kg of fish biomass (Table 1) were taken from literature sources (Sterner and George, 2000; Tanner et al., 2000; Hartman, 2012). For TP and TN balance, cumulative inputs of nutrients immediately after pond refilling and outputs in the discharge were calculated in relation to experimental pond volume. Input of phosphorus and nitrogen in incoming zooplankton during pond refilling was calculated according to empirical relationships between TP, TN and dry weight of zooplankton biomass. The zooplankton biomass was monitored in the Svět fishpond (215 ha), which was the source of water (L. Pechar, unpublished data). A stylised representation of nutrient mass balance assessment is presented in Fig. 1.

Table 1
Chemical composition of the different experimental diets (own results) and fish chemical composition obtained from Hartman (2012) for total phosphorus; Sterner and George (2000) and Tanner et al. (2000) for dry matter and total nitrogen. All values expressed with the same units (g kg^{-1})

Components	W	WT	WTP	Fish
Dry matter	872.2	878.1	879	260
Total phosphorus (wet mass)	3.15	3.25	3.48	8.4
Total nitrogen (wet mass)	16.8	19.9	19.05	29

W = wheat only; WT = thermally treated wheat; WTP = thermally treated and pressed wheat.

Statistical analysis

Data were processed using the STATISTICA CZ, v. 10 data analysis software (StatSoft CR s.r.o., Prague, Czech Republic). All values, except those for the nutrient budget, are expressed as mean ± standard deviation. One-way ANOVA and Tukey's *post-hoc* test, with statistical significance set at $\alpha = 0.05$, were used throughout, with Tukey's *post-hoc* test applied in cases of significant difference. All data were checked for normality and homogeneity of variance before analysis.

Results

Effect of supplementary feeding on water quality

Throughout the 120-day fish culture, T ranged around the optimal for carp growth performance (i.e. 20.6 ± 2.4°C; Table 2). DO concentration ranged between 5.79 and 7.00 mg L^{-1}, the highest mean values being in the control (Table 2). Mean O_2Sat ranged between 64.00 and 77.45%, with highest values also in the control. Mean Cond showed low variation, from 192 to 195 μS cm^{-1}, and mean pH fluctuated between 7.91 and 8.33, with highest values being again in the control ponds. Mean Turb ranged between 12.23 and 12.76 NTU with highest levels occurring in the WT treatment. Mean TA was identical in all experimental ponds (1.1 mmol L^{-1}), as was the mean NH_4-N concentration (0.04 mg L^{-1}). Mean NO_3-N and TN concentrations ranged from 0.04 to 0.06 and 1.88 to 1.93 mg L^{-1}, respectively; while mean PO_4-P and TP concentrations ranged from 0.007 to 0.008 and 0.18 to 0.19 mg L^{-1}, respectively. Higher values were found in all treatments with supplementary feeding (Table 2). Mean BOD_5 ranged between 7.39 and 7.46 mg L^{-1} with highest levels occurring in the control, while mean COD_{Cr} values ranged from 56.36 to 58.35 mg L^{-1} with highest levels occurring in the W treatment. Mean TSS concentration ranged between 19.27 and 20.40 mg L^{-1} and mean Chl*a* concentration between 77 and 90 μg L^{-1} with highest levels occurring in both WT and WTP treatments. The application of supplementary feed did not significantly (P > 0.05) affect any of the parameters under study, with the exception of DO and O_2Sat (Table 2).

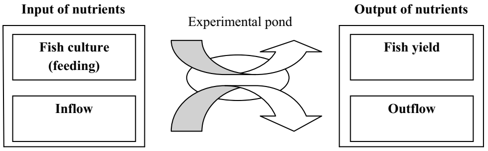

Fig. 1. Nutrient balance evaluation chart (adapted from Duras and Potužák, 2012)

Table 2
Water quality variations in experimental carp ponds operated under four different feeding regimes: C = control (no supplemental feeding); ponds supplemented by: W = wheat only; WT = thermally treated wheat; WTP = thermally treated and pressed wheat; Data represent F-values of ANOVA and mean values ± standard deviation (n = 24 samples; time = 120 days) of the environmental variables measured

Variable	F-value	Treatment mean ± SD C	W	WT	WTP
T [°C]	0.04 ns	20.5 ± 2.4	20.6 ± 2.4	20.6 ± 2.4	20.6 ± 2.4
DO [mg L^{-1}]	3.62*	7.00 ± 1.73^{a}	6.08 ± 1.07	6.29 ± 0.98	5.79 ± 1.38^{b}
O$_2$Sat [%]	3.77*	77.45 ± 19.46^{a}	67.54 ± 11.98	69.80 ± 10.32	64.00 ± 14.02^{b}
pH	2.05 ns	8.33 ± 0.83	8.06 ± 0.56	8.05 ± 0.49	7.91 ± 0.48
Cond [μS cm^{-1}]	0.01 ns	193.7 ± 12.8	192.4 ± 17.8	195.0 ± 12.0	194.2 ± 11.9
Turb [NTU]	0.01 ns	12.23 ± 8.98	12.52 ± 8.64	12.76 ± 9.21	12.59 ± 8.92
TA [mmol L^{-1}]	0.06 ns	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.2
NH$_4$-N [mg L^{-1}]	0.01 ns	0.04 ± 0.08	0.04 ± 0.08	0.04 ± 0.07	0.04 ± 0.08
NO$_3$-N [mg L^{-1}]	0.30 ns	0.04 ± 0.07	0.06 ± 0.11	0.04 ± 0.07	0.04 ± 0.07
TN [mg L^{-1}]	0.03 ns	1.88 ± 0.77	1.91 ± 0.69	1.88 ± 0.75	1.93 ± 0.74
PO$_4$-P [mg L^{-1}]	0.15 ns	0.008 ± 0.006	0.007 ± 0.007	0.008 ± 0.006	0.008 ± 0.006
TP [mg L^{-1}]	0.09 ns	0.18 ± 0.08	0.19 ± 0.08	0.19 ± 0.09	0.19 ± 0.09
BOD$_5$ [mg L^{-1}]	0.00 ns	7.46 ± 4.52	7.44 ± 4.34	7.43 ± 4.19	7.39 ± 4.12
COD$_{Cr}$ [mg L^{-1}]	0.04 ns	56.36 ± 22.86	58.35 ± 23.00	56.99 ± 25.46	58.34 ± 23.56
TSS [mg L^{-1}]	0.03 ns	19.27 ± 13.02	19.91 ± 13.52	20.04 ± 13.36	20.40 ± 14.56
Chl*a* [μg L^{-1}]	0.24 ns	77 ± 59	80 ± 61	90 ± 74	90 ± 75

T: water temperature; DO: dissolved oxygen; O2Sat: oxygen saturation; Cond: conductivity; Turb: turbidity; TA: total alkalinity; NH$_4$-N: ammonium nitrogen; NO$_3$-N: nitrate nitrogen; TN: total nitrogen; PO$_4$-P: phosphate phosphorus; TP: total phosphorus; BOD$_5$: biochemical oxygen demand; COD$_{Cr}$: chemical oxygen demand; TSS: total suspended solids; Chl*a*: chlorophyll-*a*.
The F-values correspond to the results of ANOVA, and if significant, a Tukey's *post-hoc* test was performed. Superscripts a, b represent outcome from Tukey's *post-hoc* test. Mean values in same row with different superscript differ significantly ($P < 0.05$).
*$P \leq 0.05$, ns: not significant.

Zooplankton

No significant differences ($P > 0.05$) were noted in zooplankton density among the experimental ponds (Table 3). Rotifers showed low mean density (ranging from 4 to 12 ind L^{-1}) and low species richness (from 2 to 4 species at all stations; mainly from the genera *Keratella* and *Brachionus*). Following the second sampling of July, rotifers no longer appeared at any of the sampling stations. The most abundant group were copepods in all treatments (varying from 31 ind L^{-1} in C and WTP, to 44 ind L^{-1} in W and WT), mainly juvenile stages (nauplii and copepodites) on most of the sampling dates. In total, one calanoid and four cyclopoid copepods species were found. Regarding cladocerans, individuals smaller than 0.7 mm (mainly genera *Bosmina*, *Chydorus* and small *Daphnia longispina*) achieved similar mean density in all treatments (minimum of 13 ind L^{-1} in W and maximum of 29 ind L^{-1} in WT), except for WTP, which had similar values (15 and 16 ind L^{-1}) in both replicates.

Table 3
Main zooplankton groups in experimental carp pond culture using different supplemental feeding regimes over a period of 4 months

Variable	F-value	Treatment mean ± standard deviation C	W	WT	WTP
Rotifera	0.36 ns	12 ± 30	4 ± 8	4 ± 7	6 ± 14
Copepoda	0.16 ns	37 ± 30	44 ± 36	44 ± 39	37 ± 20
Cladocera	0.35 ns	23 ± 28	22 ± 18	37 ± 63	31 ± 16
Total zooplankton	0.14 ns	72 ± 61	70 ± 42	86 ± 80	73 ± 40
Cladocera <0.7 mm	0.41 ns	17 ± 20	13 ± 12	29 ± 63	15 ± 11
Cladocera >0.7 mm	1.78 ns	7 ± 11	8 ± 8	9 ± 9	16 ± 8
Copepoda >0.7 mm	0.39 ns	12 ± 12	19 ± 16	18 ± 23	14 ± 12

C = control (no supplemental feeding); ponds supplemented by: W = wheat only; WT = thermally treated wheat; WTP = thermally treated and pressed wheat; Data represent F-values of one-way ANOVA and mean density values ± standard deviation (ind $^{-1}$) for the main zooplankton groups observed in the experimental ponds over the entire study period (n = 16). F-values correspond to results of ANOVA, ns: not significant.

Specific growth rate

Values for SGR in those ponds with supplementary feeding were significantly different ($P < 0.05$) from the control at the end of the experiment (Fig. 2). Highest SGR was recorded in the WTP treatment, followed by WT ($P > 0.05$) and W treatments ($P < 0.05$).

Phosphorus and nitrogen budgets

Table 4 summarizes the amount of nitrogen and phosphorus retained in fish biomass in all treatments. The balance of TP calculated as the increase of TP in fish biomass minus the input of TP in the feed was the highest in the control treatment with zero feed input (63.7 g). The lowest value was observed in the ponds receiving supplementary feed W (25.4 g) and WT (25.9 g), respectively. P concentrations in inflow and outflow waters indicated a release of this nutrient

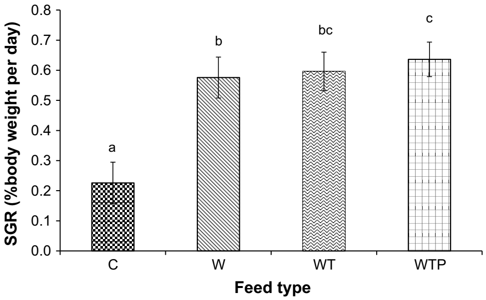

Fig. 2. Specific growth rate (SGR) of common carp (means and standard deviations, n = 22) calculated for different supplemental feeding treatments after 120 days. Letters = results of a one-way ANOVA and Tukey's *post-hoc* test: treatments with different letter are significantly different ($P < 0.05$). C: control without supplementary feed; W: wheat; WT: wheat thermally treated; and WTP: thermally treated and pressed wheat

for all treatments, including the control ponds, with effluent water having higher P than inflow water. TP loading from the WTP treatment was significantly ($P < 0.05$) lower compared to the W treatment.

According to these results the final budget of total phosphorus input and output indicates another unexplored source of this element in all treatments. Considering the input of phosphorus by zooplankton (about 36 g in each treatment) ± 50 g is still unexplained. The balance of total phosphorus input and output in the control treatment indicated no significant differences with supplementary W and WTP feeding treatments (supplementary feeding with these feeds has an insignificant effect on the total budget). Conversely, WT treatment significantly ($P < 0.05$) improved the total phosphorus budget.

Nitrogen was less retained in fish than supplied in feed except in the control (Table 4). N-budget values for inflow and effluent waters indicate N loss from all experimental ponds, with significant ($P < 0.05$) differences between each treatment. Total retained N was highest in the control pond receiving no supplementary feed. The balance of total nitrogen inputs and outputs indicated the retention of this nutrient in all treatments.

Discussion

In this study we compared water quality in experimental carp ponds with and without supplementary treated and untreated wheat feed. Water quality parameters measured during the experimental period corresponded to common values recorded in Czech ponds and remained within the ranges necessary for good growth performance of carp (Svobodová et al., 1993). All parameters, except for DO and O_2Sat, showed no significant differences from the control ($P > 0.05$), indicating that supplementary feeding had no effect on the hydrochemical parameters in experimental ponds. The addition of modified cereal diets, however, did improve the final balance of P, especially the addition of WTP.

Carp are omnivorous fish feeding mainly on benthic macroinvertebrates and zooplankton (Spataru et al., 1983; Rahman et al., 2006). Without supplementary feeding, carp prefer feeding on benthic organisms; however, in the absence of benthic macroinvertebrates, carp can shift their feeding niche to the water column and graze principally on zooplankton (Rahman et al., 2010). However, carp readily accept artificial feed when provided (Spataru et al., 1980; Milstein and Hulata, 1993). Carp are capable of having a strong effect on aquatic habitats through regulation of the freshwater ecosystem structure (Hrbáček et al., 1961). Carp do this in two ways: through feeding activity and nutrient recycling via physical resuspension, i.e. bioturbation (Adámek and Maršálek, 2013), and through predation on

Table 4
Phosphorus and nitrogen budgets (g) of carp pond culture systems with four different supplemental feeding regimes

	Phosphorus				Nitrogen			
Treatment	C	W	WT	WTP	C	W	WT	WTP
Stocked fish	219.9	221.8	219.5	219.6	759.2	765.7	757.8	758.1
Feed	–	208	214.6	229.6	–	1108.8	1313.4	1257.4
Harvested fish	283.6	455.2	460	493.2	979.3	1571.5	1587.6	1702.8
Balance	63.7^{a}	25.4^{b}	25.9^{b}	44^{c}	220.1^{a}	-303^{b}	-483.6^{c}	-312.7^{b}
Inflow water	459	458.9	467.7	472.9	5054.8	5013.7	4974.5	4956
Effluent water	484.8	524	518	516.6	4681.2	4706.2	4804.2	4702.5
Balance	25.8^{a}	65.1^{b}	50.3^{bc}	43.7^{c}	-373.6^{a}	-307.5^{b}	-170.3^{c}	-253.5^{d}
Total input	678.9	888.7	901.8	922.1	5814	6888	7045.7	6971.5
Total output	768.4	979.2	978	1009.8	5660.5	6277.7	6391.8	6405.3
Total balance	89.5^{a}	90.5^{a}	76.2^{b}	87.7^{a}	-153.5^{a}	-610.5^{b}	-653.9^{b}	-566.2^{b}
Differences*	–	1	−13.3	−1.8	–	−764	−807.4	−719.7

C = control (no supplemental feeding); ponds supplemented by: W = wheat only; WT = thermally treated wheat; WTP = thermally treated and pressed wheat.

*Difference between control and supplemented treatments. Data represent cumulative values for input and output as well as total nutrient balance for each treatment regime.

Superscripts a, b, c, d = outcome from Tukey's *post-hoc* test when one-way ANOVA was significant. Values in same row with different superscript differ significantly ($P < 0.05$).

large zooplankton such as *Daphnia* (Angeler et al., 2002). By stirring up sediments, carp can enhance the nutrient concentration, which in turn increases natural food availability in ponds (Milstein et al., 2002). This is probably the reason why water quality in all treatments, including the control, showed no significant differences. Thus, this bioturbation had larger effects in the nutrient concentration than does the input of nutrients through cereals (albeit with a small volume of sediment on the bottom). Similar results were obtained by Dulic et al. (2010) and Ćirić et al. (2015), who showed that different supplementary feeds (cereals or pelleted and extruded diet) had no effect on water quality in fish ponds. On the contrary, water quality can be strongly influenced by fish biomass: higher densities of carp increase turbidity and nutrient concentration, especially P (Chumchal et al., 2005; Driver et al., 2005). Carp size and age can also influence water parameters, rising chlorophyll concentration or decreasing transparency (Driver et al., 2005; Kloskowski, 2011).

The application of artificial feeds in semi-intensive carp farming systems increases specific growth rate as fish are capable of retaining 20–50% of N and 15–56% of P (Siddiqui and Al-Harbi, 1999; Schneider et al., 2005), releasing the remainder into the water where it can then be converted into valuable products by phototrophic and heterotrophic organisms (Schneider, 2006). In our study, supplementary feeding resulted in increased carp growth performance. This was especially so when using treated wheat (WT and WTP), the thermal treatment of cereals apparently improving their digestibility (Przybyl and Mazurkiewicz, 2004; Hlaváč et al., 2014) by increasing starch gelatinization (Svihus et al., 2005). In addition, supplementary feeding provides nutrients (14% N and 14% P) for phytoplankton growth (as well as for bacteria, fungi and protozoa) through decomposition processes (Moriarty, 1997), which, in turn, increases zooplankton abundance. Several other studies have also shown that the use of cereals as a supplementary feed in carp ponds can affect zooplankton composition (e.g. Adámek et al., 2004; Ćirić et al., 2015). In the present study, however, different artificial diets had no significant influence on natural food availability (i.e. zooplankton comparison to control as well as to each other). The absence of any obvious impact of different feeds upon zooplankton in our study might be caused by regular monthly pond draining and re-filling from the same source.

Numerous studies on pond nutrient budgets have been undertaken, both for freshwater fish ponds under various climatic conditions (Boyd, 1985; Foy and Rosell, 1991) and for carp ponds in the temperate zone (Oláh et al., 1994; Schreckenbach et al., 1999; Knösche et al., 2000; Gál et al., 2003). Ponds have a large N retention capacity due to denitrification and fixation in sediments (Oláh et al., 1983; Pokorný et al., 1999). According to Knösche et al. (2000), carp ponds retain 78.5 kg N ha^{-1} $year^{-1}$ and 5.71 kg P ha^{-1} $year^{-1}$ on average. N-retention corresponding to 93 kg N ha^{-1} $year^{-1}$ was reported by Oláh et al. (1994) following extensive analysis of N input and output data covering a 20-year period. Monitoring performed on a number of fishponds in Germany and Hungary indicated that average nutrient retention corresponded to 43 and 5.1 kg ha^{-1} $year^{-1}$ of N and P, respectively (Schreckenbach et al., 1999). According to Gál et al. (2003), a significant amount of the TN input was removed by the fishery, with just a small percentage of TN input being discharged into the environment during fish removal. Our own data suggests that the use of WT and WTP modified cereal diets may further improve nutrient retention efficiency in carp ponds due to their higher digestibility, which reduces the amount of undigested or poorly digested food left over. However, the budget of total phosphorus input and output in our experiment indicates another unexplored source of this element in all treatments. According to our results, as well as considering the amount of phosphorus in the zooplankton, there is still ± 50 g (6%) of the total phosphorus unexplained. Verdegem (2007) has mentioned that even in closed recirculation systems, it is normal to find 15–25% of the budget unexplained. In our study, this 50 g can be attributed to the potential impact of sediment (Boyd, 1995) through its bioturbation by fish (Adámek and Maršálek, 2013), fallout (Kopáček et al., 1997), insects (Cole et al., 1990), rainfall (Holas et al., 1999) and dust (Newman, 1995) in open ecosystems.

Despite regularly draining of the ponds, modified cereals showed differing nutrient retention efficiencies throughout the study period. According to our results, both WT and WTP cereals improved the budget of P and N. In the WT and WTP treatment ponds, SGRs were higher than in the W and control ponds, indicating higher P output in harvested fish biomass. Large carp are known to retain more P per unit weight than smaller fish (Lamarra, 1975; Vanni, 1996), resulting in lower concentrations in effluent water. N is one of the most important, although not necessarily the decisive, factors in the eutrophication of surface waters. N-balance, however, is also influenced by fixation of N from the air, caused by cyanobacteria and bacterial denitrification. Both processes are likely to have a significant effect in carp ponds as they influence the N-balance in an opposite way (Oláh et al., 1983).

In conclusion, the use of modified cereals (especially thermally-treated and pressed wheat) improved carp growth performance and resulted in lower nutrient concentrations in effluent water. Both WT and WTP cereals improved the balance of phosphorus; hence these diets could be beneficial, not only as a source of nutrients for the fish, but also indirectly as a management tool for maintaining a good quality of the fishpond water. It is also necessary to mention that small storage ponds may not fully behave like large-scale ponds, which have a longer response time to environmental changes and a larger buffer capacity. This issue needs to be further investigated and verified.

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CHAPTER 4

SUPPLEMENTARY FEEDING WITH MODIFIED CEREALS IN COMMON CARP (*CYPRINUS CARPIO* L.) POND FARMING AND ITS EFFECTS ON WATER QUALITY, NUTRIENT BUDGET AND ZOOPLANKTON AND ZOOBENTHOS ASSEMBLAGES

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Abstract

To test the influence of supplementary feeding with modified cereals on nutrient budget and environmental and biotic variables, three different treatments were applied in four experimental ponds: two replicates with thermally treated cereals, one with raw cereal and a control with no added food. Water parameters, zooplankton and zoobenthos were analysed from May to October over two consecutive years (2012 and 2013). In addition, nitrogen and phosphorus budget were calculated as the difference between input (food, stocked fish) and output (harvested fish). The results showed that, aside from water transparency, type of supplementary feed did not influence water quality (in ponds with thermally treated cereals were significantly ($P < 0.05$) lower turbidity and suspended solids, and higher Secchi depth compared with the other two treatments). No significant differences were observed in zooplankton assemblage between the experimental and control ponds. Macrozoobenthos density and biomass were considerably lower in the control pond. High seasonal fluctuations, however, meant that significant differences in density were only recorded in 2012. The use of thermally treated cereals results in improved carp growth and a better nutrient budget, with an increase in carp biomass harvested and nutrient removed per hectare of water surface. The result is a win–win situation with a 10% lower feed conversion ratio, increased profits and lower environmental impact.

***Keywords:** common carp, nutrient budget, supplementary feeding, thermally treated cereals*

1. Introduction

Fish ponds are the most common form of stagnant water habitat in the Czech Republic and play an important role in the hydrological system (Pechar, 2000; Pokorný and Hauser, 2002). The majority of these ponds are several hundred years old and have become important elements of the countryside, with large regions around the ponds and their associated ecosystems affected (Potužák et al., 2007; Vsetičková and Adámek, 2013). Ponds are capable of providing a range of services, including fish production, water supply, flood protection, nutrient retention, carbon sequestration, biodiversity conservation and recreation (EPCN, 2007). Despite such high service potential, however, evaluations of management practice typically focus on a limited number (e.g. fish production), other benefits being frequently overlooked (Pechar, 2000). Several studies have highlighted the importance of ponds for aquatic biodiversity at regional scales (e.g. Robin et al., 2014), and many ponds primarily designed for fish farming also serve as pollution sinks, typically handling high nutrient loads from waste water treatment plants (Všetičková et al., 2012) and agricultural runoff. In other words, fish ponds represent a managed aquatic ecosystem in which the water level, fish stocks and, to some extent, nutrient input are under human control. In some countries, however, the size of ponds and their more-or-less natural character do not allow for large-scale technical measures to control pond environment quality.

In the 20th century, fish production in Czech fish ponds increased markedly, reaching its present stable level of about 450–500 kg.ha^{-1} in the 1980s (Potužák et al., 2008). At the same time, many of these ponds became highly eutrophic due to catchment nutrient inflow, addition of fertilisers and excess supplementary feed. Despite fish production levelling off, pond eutrophication continues to increase (Potužák et al., 2007; Hlaváč et al., 2014). Phosphorus (P) is the most damaging of these nutrients due to its high bioavailability, resulting in excessive phytoplankton growth and cyanobacterial blooms, fluctuations in oxygen and pH and presence of organic biodegradation products such as ammonia (Pechar, 1995). Despite this, the relationship between pond aquaculture and environmental damage has only been addressed relatively recently and, as such, water quality has tended to be accentuated and the pond's role as part of an integrated catchment management system ignored. Many countries have now started to implement strict regulatory guidelines, therefore, that address environmental and social issues in an effort to ensure sustainability (Read and Fernandes, 2003; Cai et al., 2013). In the Czech Republic, this is covered in by The Water Act 254/2001 on the application of fertilisers to the pond environment, which explicitly states that eutrophication is an increasing problem that should be avoided in the future. Under such laws, the only means of intensifying pond fish production is through supplementary feeding.

Common carp (*Cyprinus carpio* L.) are the most frequently cultured fish in Central and Eastern European countries (Mráz et al., 2012), with production typically semi-intensive, utilising a combination of supplementary feeding with cereals and occasional fertilisation with animal manure to increase natural phyto- and zooplankton food levels (Mráz et al., 2012; Másílko et al., 2014a). Supplementary feed usually consists of cheap and locally available raw ingredients (Markovic et al., 2009; De Silva, 2012), such as wheat, triticale or rye. Feed is generally the most costly input in aquaculture (Diana et al., 2013) and overfeeding is not only costly but is the main source of waste material, which leads to deterioration in local water quality. In order to reduce such waste, as well as the amount of nutrient lost with discharged water, it is essential that accurate nutrient budgets are calculated to assess the ultimate fate of nutrients introduced into the pond system. Estimation of such budgets allows for the quantification of potential pollution impact from specific pond management strategies (Bosma and Verdegem, 2011). In the long run, this would reduce feed use, increase profitability and lead to improved water quality, both as effluent and in the pond itself (De Silva, 2012). Ideally, feed should be added at levels that match consumption demand without reducing fish growth or survival through reduced habitat quality (Robinson et al., 2004). Fish retain only 20–50% of nitrogen (N) and 15–56% of phosphorous (P) from feed (Schneider et al., 2005; Rahman et al., 2008), however, releasing the remainder into the water where it becomes available for conversion into valuable by-products by phototrophic and heterotrophic organisms (Schneider, 2006). Stable isotope studies have shown that 50–80% of fish production in tilapia ponds is based on natural food (Schroeder, 1983). If correct, then about half the feed put into ponds represents nothing more than 'an expensive organic fertiliser' (Filbrun et al., 2013). Furthermore, it has been estimated that only 0.4–0.8% of primary production is retained in fish production (Verdegem, 2013). Increasing this value by just a tenth of a percent would be a big step in pond aquaculture. In recent years, fish farmers have developed new methods to enhance the production effectiveness of cereals by mechanical treatment, such as pressing, grinding and/or thermal treatment (Másílko et al., 2014b; Hlaváč et al., 2015). A number of studies have suggested that thermal and mechanical treatment of cereal feeds could decrease the load of undigested or poorly digested supplementary feed in the water by improving its digestibility (Hlaváč et al., 2014). Clearly, therefore, there are substantial opportunities for improving pond feeding strategies.

In this study, we assess the influence of two different supplementary feed types (cereal grain and thermally-treated cereal) on water quality, invertebrate community and nutrient budget in carp rearing ponds over two consecutive growing seasons. We also assess the effect of these two diets on carp growth and production.

2. Material and methods

Experimental design and management

This study was performed on the Horák (2.2 ha), Fišmistr (2.8 ha), Baštýř (1.7 ha) and Pěšák (2.7 ha) ponds, which all form part of the Naděje pond system (Třeboň region, Czech Republic, 49°12′N, 14°74′E). Sampling was carried out from 25 April to 4 October 2012 and from 2 May to 3 October 2013 (i.e. two consecutive growing seasons). All ponds are supplied with water in spring from the larger Rod pond (36.1 ha) upstream (Fig. 1). When necessary (e.g. to compensate for losses by evaporation), water levels were adjusted in the study ponds by adding water from the same source. Before stocking, the experimental ponds were drained and dried over the winter; subsequently, the ponds were managed under standard semi-intensive operating conditions (see Introduction) without extra fertilisation. Each year, the ponds were stocked with three-year-old scaled common carp (Třeboň strain; mean individual weight 1026 ± 250 g) at a density of 363 ind.ha^{-1} and a biomass of approximately 370 kg.ha^{-1}. This is a typical density for semi-intensive carp culture in the Třeboň region as it allows for sufficient production of large zooplankton (Urbánek et al., 2010). All fish in the study were of uniform genetic origin and of the same age. At the end of each year's experiment (first week in October), the ponds were harvested and the fish measured (nearest mm) and weighed (nearest 0.1 g).

Fish in separate ponds were fed with two different supplementary feeds: (i) cereal grain (CG; 20 EUR/100 kg), and (ii) thermally treated cereal (TTC; 21.5 EUR/100 kg). One additional group, which served as a control (C), received no supplementary feed, its diet being based exclusively on naturally occurring food. The TTC treatment was run in duplicate each year. TTC cereals were heated at 100 °C for 90 seconds using a Bühler AG drier (Switzerland). In both treatments, food was supplied at an initial rate of 2% fish stock biomass between 08:00 and 11:00 at the same feeding site three times a week (Monday, Wednesday and Friday). Feed rates were adjusted during the experiment according to fish weight and feed intake (recorded during control monitoring).

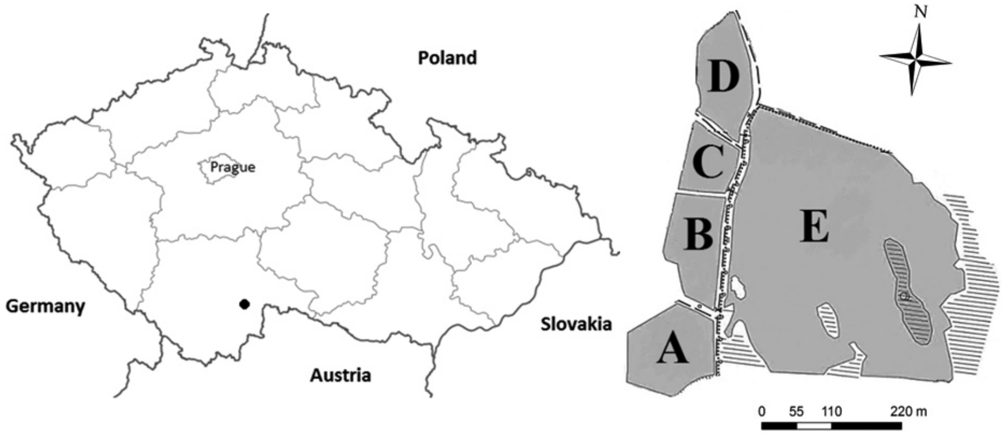

Figure 1. *Location and plan of the experimental ponds (A: Horák; B: Fišmistr; C: Baštýř; D: Pešák and E: Rod pond (the reservoir feeder pond).*

Water quality analysis

During the experiment, water quality parameters were determined between 7:00 and 10:00 each month. Water temperature (T), pH, dissolved oxygen (DO) and conductivity (Cond) were measured *in situ* using a YSI Professional Plus multimeter (YSI Incorporated, Yellow Springs, USA). Turbidity (Turb) was measured *in situ* using a WTW Turb 430T/SET (WTW, Weilheim, Germany). Alkalinity (TA) was determined by the titrimetric method and water transparency was measured *in situ* using a Secchi disk. Water samples were collected as a three litre pooled sample from three different locations in each pond using a modified tube sampler (Opting Service, Ostrava, Czech Republic). All samples were kept at 4 °C until analysed in the laboratory.

Hydrochemical variables (i.e. ammonium N, nitrate N, total N, phosphate P, total P, total suspended solids and chlorophyll-a) were assessed according to accredited methods (see Hlaváč et al., 2015).

Zooplankton and zoobenthos sampling and analysis

Zooplankton were sampled monthly at three different locations near the outlet by hauling a 22 cm diameter plankton with a 80-µm mesh funnel. The net was drawn for five metres three times at each location and the organisms retained were preserved in 4% formalin. The fixed volume of zooplankton biomass was later determined in the laboratory. Based on Faina (1983), we assumed zooplankton of > 0.7 mm to be preferred by carp; hence, we used a 0.7 mm sieve to filter the sample. The fraction obtained was then transferred to a graduated cylinder and, after 30 minutes of sedimentation, the volume of zooplankton was noted and converted to millilitres of zooplankton per litre of water (Schlott et al., 2011). The qualitative taxonomic composition of zooplankton was subsequently determined under an inverted microscope (Olympus CKX41, Japan).

Macrozoobenthos samples were collected monthly using a 225 cm^2 Eckman grab from four randomly selected sites in each pond. The substrate collected was pooled and sieved through a

500 µm mesh sieve to remove the mud. The raw substrate with benthic organisms was placed into a plastic bottle and preserved with 4% formalin. In the laboratory, the macroinvertebrates were separated out, sorted, weighed to the nearest 1 mg and determined to the lowest possible level (exceptionally family). A separate evaluation of midge fly (Chironomidae) larval density was performed due to their importance in the diet of marketable carp diet in Central European ponds (see Anton-Pardo et al., 2014).

Fish growth and production

Specific growth rate (SGR) and average individual fish weight were calculated on a monthly basis. Four control evaluations of fish growth were performed on 20-50 fish each year, the number of fish checked depending on the success of seine netting. Fish were weighed using a digital scale with 0.1 g precision.

SGR was calculated as: SGR ($\%.d^{-1}$) = ($\ln W_T - \ln W_0$) x $100/T$, where W_T is average final body weight (kg), W_0 is average initial body weight (kg) and T is the culture period in days.

Feed Conversion Ratio (FCR) was calculated as: FCR = $\frac{F}{w_t - w_o}$, where w_t is final body weight (kg), w_o is initial body weight (kg) and F is the feed consumed (kg).

Phosphorus and nitrogen budget

All management activities (fish stocking, harvesting, supplementary feeding) were monitored throughout the study. In order to calculate the N- and P-budget, input *via* supplementary feed was calculated as dry matter, total N and total P, according to the Czech National Standard for feed analysis (CSN 46 7092, 1998).

Fish P and N was analysed in 21 samples randomly selected during stocking and harvesting. The fish were starved until the digestive tract was empty, whereupon the fish were mechanically killed and ground whole in a commercial food grinder (Seydelmann K40, Germany) three times to ensure homogenisation. The homogenate was then dried in a convection oven for 24 h at 60 °C and ground with a mortar and a pestle. The samples were then stored in a freezer until further analysis. After thawing, body composition was analysed according to Czech National Standard (CSN 46 7092, 1998). Dry matter was analysed by drying the samples to constant weight at 105 °C.

The amount of nutrient retained in the sediment was not taken into account in this study. As such, the nutrient-balance model used was based on nutrient input (feed added) and nutrient removed (harvested fish) only. Nutrient input (feed) each year was calculated as: Nutrient (N, P) in feed [$g.ha^{-1}$] = nutrient concentration in feed × total amount of feed supplied.

Nutrient input/output (fish) was calculated as: Nutrient (N, P) in fish [$g.ha^{-1}$] = nutrient concentration in stock/harvested fish carcasses × total fish biomass.

Data analysis

All data were processed using STATISTICA CZ v. 12 data analysis software (StatSoft CR s.r.o., Prague, Czech Republic). All values are expressed as mean ± standard deviation (SD). Differences in feed conversion ratio and zooplankton and zoobenthos in between years and between chemical composition of stocked and harvested fish were analysed using the non-parametric Mann-Whitney U test. All other data were assessed using the non-parametric Kruskal-Wallis H test (statistical significance set at $\alpha = 0.05$), with a non-parametric multiple comparison test applied when results were significant.

3. Results

Water quality in experimental ponds

Aside from September, when T dropped to 14.8 ± 2.0 °C due to low air temperatures, T remained within the optimal range for carp growth (20.8 ± 4.1) throughout the experiment (Table 1). Similarly, DO stayed within the recommended range for carp pond culture throughout, with a range of 6.88 to 8.43 mg.l^{-1}, the highest mean value being observed in the control pond. While conductivity and pH both showed low variation between treatments, turbidity, Secchi depth (transparency) and TSS were significantly lower (7.51, 6.34 & 5.99 respectively; $P < 0.05$; Table 1) in ponds with TTC compared with the other two treatments. Despite these results, nutrient concentration showed only minor variation between treatments, with no significant differences.

Table 1. *Kruskal-Wallis H-values and mean ± standard deviation (n = 12) for environmental variables related to water quality assessment during the two years of sampling at the experimental ponds.*

Variable	H-value	**C**	**CG**	**TTC#**
T [°C]	0.04 ns	20.81 ± 4.14	21.01 ± 4.49	20.69 ± 4.13
DO [mg.l^{-1}]	4.52 ns	8.43 ± 2.10	7.50 ± 2.36	6.88 ± 1.79
pH	1.94 ns	8.04 ± 0.69	7.87 ± 0.74	7.68 ± 0.54
Cond [µS.cm^{-1}]	0.49 ns	174.90 ± 29.45	173.64 ± 36.66	176.40 ± 29.53
Turb [NTU]	7.51*	25.52 ± 15.15^a	13.76 ± 11.27^a	12.86 ± 9.62^b
Secchi [cm]	5.99*	43.42 ± 27.51^a	66.58 ± 44.18^a	75.79 ± 39.81^b
TA [mmol.l^{-1}]	0.53 ns	1.03 ± 0.14	1.08 ± 0.20	1.06 ± 0.19
NH_4-N [mg.l^{-1}]	0.74 ns	0.24 ± 0.27	0.23 ± 0.23	0.26 ± 0.27
NO_3-N [mg.l^{-1}]	0.19 ns	0.10 ± 0.08	0.08 ± 0.07	0.09 ± 0.08
TN [mg.l^{-1}]	0.33 ns	2.76 ± 1.14	2.98 ± 1.50	2.69 ± 1.24
PO_4-P [mg.l^{-1}]	1.32 ns	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
TP [mg.l^{-1}]	0.79 ns	0.16 ± 0.09	0.16 ± 0.1	0.15 ± 0.1
TSS [mg.l^{-1}]	6.34*	31.67 ± 18.27^a	20.24 ± 16.57^a	16.56 ±13.55^b
Chl*a* [µg.l^{-1}]	0.32 ns	66.88 ± 62.30	72.51 ± 76.18	60.46 ± 69.59

T = water temperature; DO = dissolved oxygen; Cond = conductivity; Turb = turbidity; Secchi = transparency; TA = total alkalinity; NH_4-N = ammonium nitrogen; NO_3-N = nitrate nitrogen; TN = total nitrogen; PO_4-P = phosphate phosphorus; TP = total phosphorus; TSS = total suspended solids; Chl*a* = chlorophyll-a; C = control without supplementary feed; CG = cereal grains; TTC = thermally treated cereals. Mean values in the same row with different superscripts differ significantly ($P < 0.05$); * = $P \leq 0.05$; ns = not significant. The TTC treatment was run in duplicate (n = 24).

Zooplankton and zoobenthos

Throughought the study, zooplankton in all ponds was dominated by small *Daphnia* spp. (*Daphnia longispina* and *D. galeata*), which occurred in all samples at a dominance of generally > 40% (Fig. 2). On occaision, other species co-dominated in the zooplankton community at different ponds, usually copepods (*Thermocyclops crassus, Acanthocyclops trajani*) and small cladocera (*Ceriodaphnia* spp.). Later in the study, larger species were found at higher abundance (e.g. *D. pulicaria* or *Chaoborus* sp.).

Average biovolume over the two-year study ranged between 3.59 and 5.17 $ml.m^{-3}$, with comparison among treatments revealing no significant differences (Fig. 2). Highest values were observed in the control pond in 2013, this being caused by a strong increase in the biomass of *Daphnia* sp.. Zooplankton biovolume was significantly higher in 2013 compared to 2012 (5.91 in 2013 and 2.90 $ml.m^{-3}$ in 2012; $P < 0.05$).

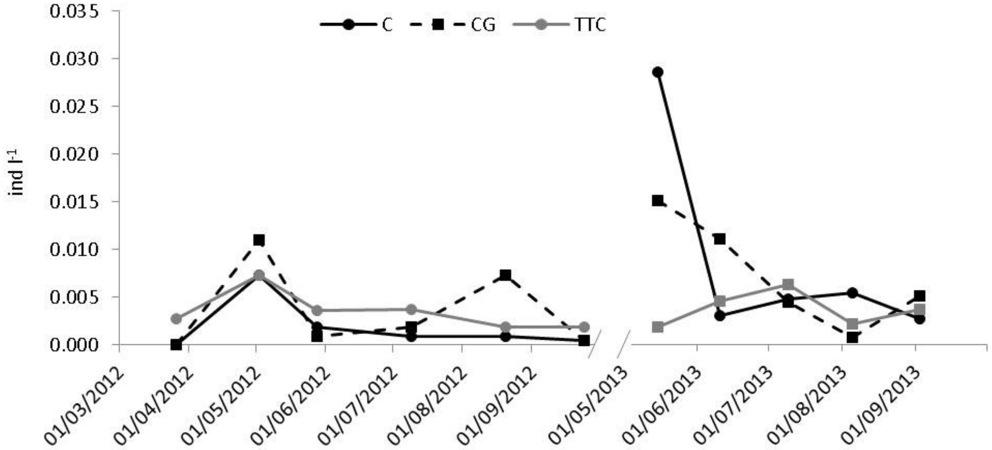

Figure 2. *Biovolume of zooplankton species > 0.7 mm observed during different treatments in the experimental ponds over the two years of sampling. C = control without supplementary feed; CG = cereal grains; and TTC = thermally treated cereals.*

Average macrozoobenthos density in the control pond was 411 ± 124 and 430 ± 193 $ind.m^{-2}$ in 2012 and 2013, respectively; being significantly lower than the CG and TTC treatments in 2012 ($P < 0.05$; Fig. 3). Though differences between treatments were not significant, macrozoobenthos density and biomass were generally lowest in the control pond. Midge fly larvae (Chironomidae) density was considerably higher in the CG and TTC ponds; though again, the differences were not significant. Oligochaetes and dipteran larvae prevailed in the macrozoobenthos, *Limnodrilus hoffmeisteri* (Tubificidae), *Chironomus plumosus* (Chironomidae), *Chaoborus flavicans* (Chaoboridae) and Ceratopogoninae g. sp. being most numerous.

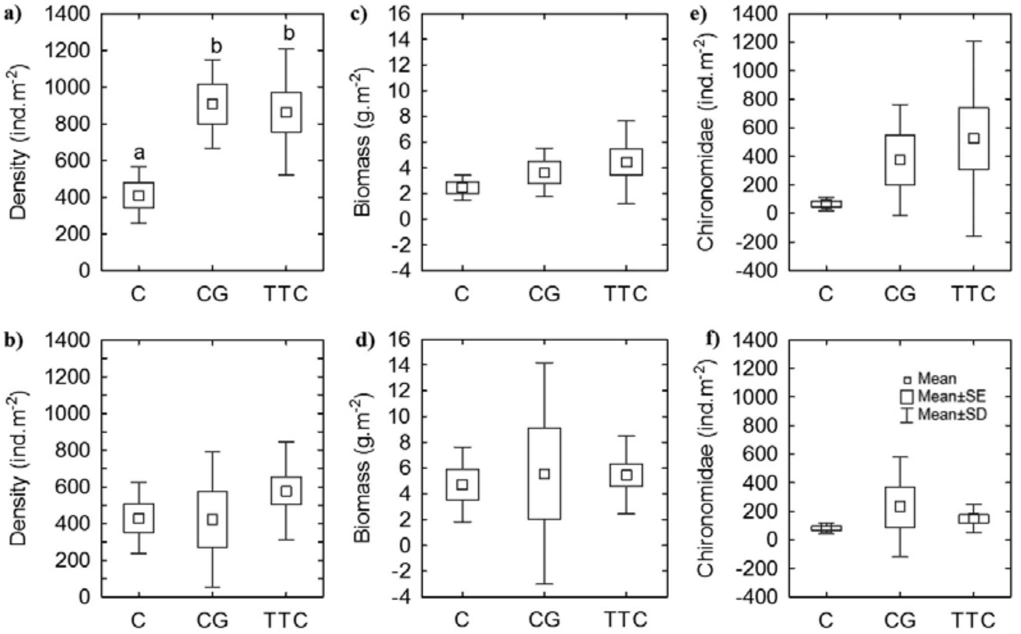

Figure 3. *Mean values for macrozoobenthos quantitative determinants during the 2012 (top) and 2013 (bottom) growing seasons: a) and b) = total invertebrate density; c) and d) = total biomass; e) and f) = Chironomidae larval density; C = control without supplementary feed; CG = cereal grains; TTC = thermally treated cereals; superscripts a & b = results of non-parametric multiple comparison tests (Kruskall-Wallis and multiple comparison tests): mean values in the same row with different superscripts differ significantly ($P < 0.05$).*

Fish growth

A significantly higher FCR ($P < 0.05$) was obtained in the CG ponds (1.94 ± 0.01) compared to those with TTC treatment (1.74 ± 0.07). Values for SGR were significantly higher than the control in the TTC ponds ($P < 0.05$) in both 2012 and 2013 (Fig. 4). No significant differences in SGR were recorded between CG and the control.

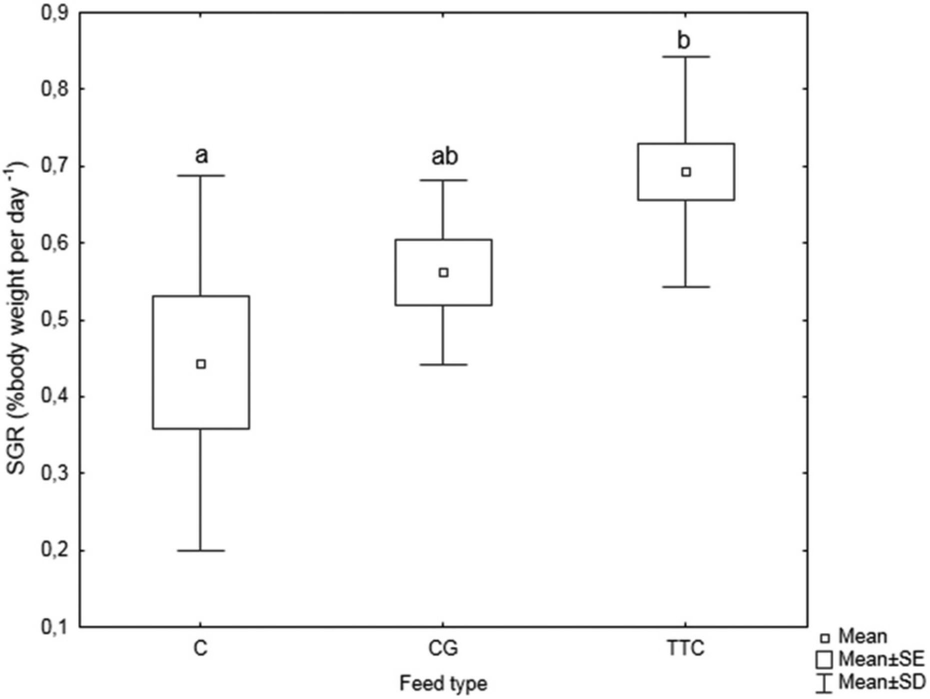

Figure 4. *Mean and standard deviation for specific growth rate (SGR) of common carp (n = 8), calculated for different experimental treatments undertaken over the two-year experiment. Letters correspond to the results of Kruskal-Wallis and multiple comparison tests: treatments with different letters are significantly different (P < 0.05); C = control without supplementary feed; CG = cereal grains; TTC = thermally treated cereals. The TTC treatment was run in duplicate (n = 16).*

Feed and fish chemical composition

While percentage of dry matter and nutrient concentration (P and N) were higher in the TTC pond, the differences were not significant (Table 2). In ponds with supplementary feeding, harvested fish had significantly higher dry matter and P and N content compared to fish at the time of stocking (all P < 0.05; Table 2). Despite fish in the control ponds having significanly lower dry matter values than those in the CG ponds (P < 0.05; Table 2), there was no significant difference between treatments regarding the chemical compositon of harvested fish.

Table 2. *Chemical composition of the different experimental diets and fish body (mean ± standard deviation) during the two years sampling.*

Treatment	*n*	C	CG	TTC
Feed				
Dry matter (%)	2	–	864.6 ± 0.85	873.1 ± 1.27
P (g.kg^{-1} wet mass)	2	–	3.27 ± 0.17	3.37 ± 0.16
N (g.kg^{-1} wet mass)	2	–	16.56 ± 0.34	18.00 ± 2.68
Stocked fish				
Dry matter (%)	5	21.04 ± 1.78	21.04 ± 1.78†	21.04 ± 1.78†
P (g.kg^{-1} wet mass)	5	4.88 ± 0.75	4.88 ± 0.75†	4.88 ± 0.75†
N (g.kg^{-1} wet mass)	5	25.28 ± 0.92	25.28 ± 0.92†	25.28 ± 0.92†
Harvested fish				
Dry matter (%)	4	24.54 ± 4.09^{a}	34.54 ± 0.73^{b‡}	30.36 ± 4.70^{a‡}
P (g.kg^{-1} wet mass)	4	6.96 ± 3.5	7.24 ± 1.44‡	7.28 ± 1.32‡
N (g.kg^{-1} wet mass)	4	27.12 ± 2.58	27.29 ± 0.49‡	27.20 ± 1.12‡

Note: C: control without supplementary feed; CG: cereal grains; and TTC: thermally treated cereals. Superscripts a, b represent outcomes from the non-parametric multiple comparison test (Kruskall-Wallis and multiple comparison test). Mean values in the same row with different superscript differ significantly ($P < 0.05$). Superscripts †, ‡ represent outcomes from the non-parametric Mann-Whitney U Test between stocked and harvested fish. Mean values in the same column with different superscript differ significantly ($P < 0.05$). The TTC treatment was run in duplicate (for harvested fish n = 8).

Nutrient budget

The P balance (calculated as increase in P in fish biomass minus input of P in feed) was highest in the control treatment (with zero feed input), while lowest values were found in those ponds receiving supplementary feed (Table 3). Total P budget was significantly higher in the C treatment than in the CG treatment ($P < 0.05$). Aside from the control, less N was retained in harvested fish than was supplied in feed (Table 3). No significant differences were recorded between supplementary feed treatments ($P > 0.05$).

Table 3. *Phosphorus and nitrogen budget [g.ha^{-1}] for the different input and output sources and the total balance for each nutrient in the different treatments performed in the experimental ponds during two years of sampling. Data represent mean ± standard deviation.*

Treatment	**C**	**CG**	**TTC**
Phosphorus			
Input			
Amount of nutrient in stocked fish	1592 ± 256	1556 ± 237	1674 ± 364
Amount of nutrient in total feed suplied	–	3560 ± 185	3668 ± 145
Output			
Amount of nutrient in harvested fish	4573 ± 125	6370 ± 338	7051 ± 411
Total budget	**2981 ± 132^{a}**	**1254 ± 83^{b}**	**1710 ± 150^{ab}**
Nitrogen			
Input			
Amount of nutrient in stocked fish	8240 ± 938	8054 ± 865	8606 ± 1633
Amount of nutrient in total feed suplied	–	18027 ± 261	19624 ± 2065
Output			
Amount of nutrient in harvested fish	17811 ± 343	24016 ± 901	26348 ± 1329
Total budget	**9571 ± 595^{a}**	**-2065 ± 296^{b}**	**-1942± 1719^{b}**

Note: C: control without supplementary feed; CG: cereal grains; and TTC: thermally treated cereals Superscripts a, b represent outcomes from the non-parametric multiple comparison test (Kruskall-Wallis and multiple comparison test). Mean values in the same row with different superscript differ significantly ($P < 0.05$).

4. Discussion

Sustainable aquaculture depends upon environmentally-friendly and economically and socially viable culture systems (Bosma and Verdegem, 2011). As with all food-animal production systems, fish culture generates waste. Depending upon the nature of the fish culture system, part or all of that waste is discharged into surface water bodies. In that respect, growing fish in ponds has a number of advantages over other commonly used fish culture systems as excreta and other metabolic wastes are not discharged directly into the receiving waters as they are in some cage or raceway culture systems (Verdegem, 2013). When fish are grown in ponds, waste is held within the culture system for some time before it is released. During that time, natural processes can remove potential pollutants from the water ("self-purification"), including suspended sediment, P and N (Boyd, 1985; Cereghino et al., 2014). The amount of nutrient and organic matter ultimately discharged from these ponds, therefore, is substantially lower than the overall waste loading to the pond (Schwartz and Boyd, 1994; Všetičková et al., 2012). Overall, nutrient turnover in ponds is relatively quick, which means that the amount of nutrient remaining in the water is not significant. While there are certainly nutrient peaks following feeding (Boyd, 1990), there are no significant long-term effects on nutrient loading during the production period. As nutrients are first stored in phytoplankton, larger ponds tend to have a longer response time to environmental change and a higher buffering capacity. In the short term, therefore, different management practices tend to show no significant effect (Wezel et al., 2013)

Good water quality management is of extreme importance in aquaculture as stocking density, species combination, nutrient quality and quantity and the culture system can all have a strong influence (Diana et al., 1997). As a rule, pond management practice, as the source of nutrient, is generally assumed to have a strong impact on water quality. In our study, however, we found that supplementary feeding with cereals had no significant effect on increasing nutrient concentrations in the water (total P, total N, chlorophyll a, ammonium, P and N). Conversely, parameters related to water transparency showed significant differences (higher turbidity and suspended solids and lower Secchi depth) in the control pond, probably due to increased bottom feeding as carp increase their predation on benthic organisms. When ingesting feed (cereal), carp do not penetrate so deeply into the sediment. Bottom feeding results in the mixing and resuspension of bottom sediment (Bioturbation; see Scheffer, 1997; Kloskowski, 2011), changing conditions and transfer mechanisms at the water-sediment interface (Ritvo et al., 2004). These effects may be stronger in the presence of larger carp, which are capable of penetrating deeper into the substrate than smaller fish, and may be more intense in summer when carp take mainly benthic prey (Adámek and Maršálek, 2013). Previous studies (e.g. Hlaváč et al., 2015), have also observed no difference in water quality between carp ponds with and without supplementary feeding. Note, however, that the pond bottom in that study consisted of a poor sandy substrate, which may well have influenced the water quality measurements. Similar results were also obtained by Dulic et al. (2010) and Ciric et al. (2015), however, who demonstrated that different supplementary feeds (grain cereals or pelleted and extruded supplements) had no effect on water quality in carp ponds.

The different treatments applied in our study had no influence on the zooplankton community (i.e. no influence on fish grazing pressure); however, we did observe high temporal variation. Highest values were recorded in the control pond in 2013, probably due to previous pond management history in combination with spring weather conditions. The control pond had lower fish biomass at the end of the 2012 growing season, producing an average of 100–200 kg.ha^{-1} less than ponds with supplementary feeding. This resulted in an increased autumn inoculum of ephippia at the end of the growing season. When fish were stocked again in 2013, T was very low, which led to low feeding activity in the stocked fish. The increased inoculum in the sediment had good conditions for development, therefore, resulting in a high large-zooplankton biovolume in this pond during the first sampling of 2013. As T increased (at the end of May) and feeding activity increased, feeding pressure on zooplankton increased, resulting in a considerable decline in biovolume. This is in accordance with the results of Ning et al. (2010), who suggested that high biomass of planktivorous fish can potentially influence zooplankton resting-stage communities in riverine slack waters in addition to active zooplankton communities due to the inherent association between resting stages and active forms of zooplankton taxa.

Supplementary feeding with cereals (i.e. CG and TTC treatments) resulted in a decreased demand for macrozoobenthos, which was reflected in their increased density and biomass compared to the control (though the differences were usually not significant). The same was also true for chironomid density, with mean values increasing from 5.21 to 5.57 higher than the control in the CG pond and 1.81 to 7.79 times higher in the TTC pond. High variability between samples, however, meant that the differences were not significant. Macrozoobenthos density and biomass are known to fluctuate extensively in carp ponds, being lowest values observed between June and July (or August) due to intensive grazing pressure (Kloskowski, 2011) and emergence the dominant species, *Ch. plumosus.* While the overall share of Chironomus larvae in total density of carp pond macrozoobenthos may be relatively low, biomass is definitely determined by this one genus (Matěna, 1989).

In general, an increase in fish biomass leads to increased eutrophication due to higher algal growth and greater turbidity and nutrient concentration. Fish ponds are kept intentionally eutrophic in order to achieve economic levels of fish production. During recent years, supplementary fish feeding has come to represent a more important source of nutrient in the pond than addition of fertiliser. Compared to commercial pelleted feeds, cereals can provide valuable components of common carp nutrition (Urbánek et al., 2010; Másílko et al., 2014b). Furthermore, they are an easy and cheap source of digestible energy in the form of carbohydrate (especially starch) (Gatlin et al., 2007) and they are more resistant to nutrient leaching due to their strong hull (formed of insoluble, non-swelling materials such as cellulose). Such carbohydrate-rich grains could play an important role during self-purification processes in ponds. When feeding carp with cereals alone, the fraction of nutrient supply driving the bacterial-detrital food chain can rise to almost 95%, with only 5% directly utilised for fish growth (Olah, 1986). By adding carbohydrates to the pond, bacterial growth is stimulated and N is taken up through production of microbial protein (Avnimelech, 1999). Magondu et al. (2013), using maize flour as the carbohydrate source for manipulating the C/N ratio, improved water and sediment quality by reducing toxic inorganic N compounds (such as ammonia and nitrite), thereby improving nutrient utilisation efficiency and reducing nutrient discharge from the ponds.

Cereal digestibility can be increased by subjecting cereals to pre-treatment, especially thermal-based treatments such as roasting, cooking and expanding (Hlaváč et al., 2015). Másílko et al. (2014a) concluded that common carp were better able to utilise such technologically modified cereals than whole seeds. Heat treatment improves carbohydrate availability in starch rich grains, thereby increasing the digestible energy level through increased starch gelatinisation and release of oils within the grain matrix. This has been well documented for many terrestrial animal and fish-feeds thanks to advances in extrusion/roasting technologies in feed manufacture (Drew et al., 2007; Davies and Gouveia, 2010). Increased digestibility of dietary components such as protein and energy may well help to explain the results obtained in this study regarding supplementary feeding with TTC. Furthermore, Lamarra (1975) and Vanni (1996) have both shown that large carp are able to retain more P per unit weight than smaller fish, thus supporting the pond's nutrient budget. This is in accordance with our results, whereby larger carp fed with modified cereals also retained more nutrients per water surface area.

In intensive ponds, 11–27% N and 20–32% P of total food input is removed in harvested fish (Avnimelech and Lacher, 1979; Boyd, 1985). In semi-intensive ponds, these figures are reduced to 5–25% of N and 5–18% of P input (Acosta-Nassar et al., 1994; Green and Boyd, 1995). The fish stocking densities mentioned in these latter studies, however, were much higher (> 10 000 ind.ha^{-1}), even in semi-intensive ponds, compared to our experimental ponds (> 100 ind.ha^{-1}). What is more, the fish in these studies were fed using commercial dietary supplements with almost twice as much P and N. Central European carp ponds are known to trap nutrients and transform them through primary and subsequent production links within the pond food chain (Wezel et al., 2013). If such ponds are managed appropriately, they can even retain nutrients, as recorded by Knösche et al. (2000). This was also demonstrated in our study, with ponds retaining an average of 5.5 kg.ha^{-1} N and 1.9 kg.ha^{-1} P.

Fish play an important role as regards nutrient cycling in the freshwater food web. They are efficient at nutrient reclamation and translocation within ecosystems and indirectly influence the nutrient cycle via cascading trophic interactions (Vanni, 2002). Stoichiometric variations in fish tissue, therefore, could influence pond nutrient budgets. In fish, N is stored as protein in muscle tissue and nearly half of the P is located in bone and scales (DaCosta and Stern, 1956). The chemical content of a fish's body is species-specific (Sterner and George, 2000; Tanner et al., 2000) and varies greatly throughout the year depending on a range of factors such as fish length and mass (Torres and Vanni, 2007). Schreckenbach et al. (2001), for example, found that the wet body composition of common carp averaged 26.8% dry matter, 22.3 g.kg^{-1} N and 4.8 g.kg^{-1} P (note, however, the fish used in their study were of different ages (juveniles and adults) and from different habitats (lakes and ponds). Other studies, however, have also found similar results, with Hartman (2012) reporting 8.4 mg.kg^{-1} P and Rothschein et al. (1983) finding 32% dry matter and 7.9 g.kg^{-1} P in the body tissue of common carp. In our previous study (Hlaváč et al., 2014), we also observed average values for freshwater fish of 8.1 mg.kg^{-1} P and 29 mg.kg^{-1} N. Thus, our mean values for stocked and harvested common carp (Table 2) are generally in accordance with these results.

Schafer et al. (1995) and Nwanna et al. (2010) have both shown that P concentration in the fish's body, including bone and scales, is highly correlated with P level in the diet. P content has also been positively related to bone ash content in different salmonid species (see Watanabe et al., 1980). These findings also correspond with our results, as fish feeding on natural (i.e. unsupplemented) food had lower body P content than fish receiving supplementary food. Natural food is relatively poor in P and N, with an "average" benthic invertebrate being composed of 1.8 g.kg^{-1} P and 17 g.kg^{-1} N wet mass (Penczak, 1985; Schindler and Eby, 1997). Ventura (2006) and Mitra et al. (2007) reported zooplankton (e.g. freshwater cladocerans and copepods) as having 0.24–1.3 g.kg^{-1} P and 9–11 g.kg^{-1} N wet mass. Thus, P content in invertebrate food is lower than that in both raw cereal (3.27 g.kg^{-1}) and TTC (3.37 g.kg^{-1}). Heat treatment also increased the dry matter content (Davies and Gouveia, 2010) in the cereal, which also increased the content of P and N.

Other factors, such as the trophic state of the water, also appear to be important factors influencing body nutrient composition in fish (Boros et al., 2012), as does the fish's physical condition. After overwintering, freshwater fish are in poor condition and their flesh has a higher water content (and consequently a lower proportion of dry matter). Fishes are thought to gain water when they are in energy deficit, and replace the water with fat as energy budgets exceed that needed for protein synthesis or reproductive growth (Hartman and Margraf, 2008). Schreckenbach et al. (2001), however, found no significant correlation between dry matter and P ($r^2 = 0.46$) or N ($r^2 = 0.0005$) in fresh fish body mass. Well-fed fish tend not to build skeletal matter but rather increase lipid storage, and this increase in mass may result in dilution of P (Vanni and Layne, 1997). Conversely, a lower food supply decreases body fat composition (Shimeno et al., 1997), causing an increase in the proportion of bony material and scales (rich in P). Once again, however, no significant correlation was found between condition factor and P and N content in roach (*Rutilus rutilus* L.) (Boros et al., 2012). In our study, P and N content were highest in harvested fish (compared to stocked fish), which is in accordance with the previous studies on this subject (Deegan, 1986; Torres and Vanni, 2007), both of which found variation in P and N content in the fish's body from early to later growth stages.

5. Conclusion

Pond farming of common carp is of great importance globally. However, changes in management practice aimed increasing fish yield, such as the addition of supplementary feed, have often resulted in eutrophication and impairment of waters downstream. In recent years, there have been increasing efforts to minimise waste discharge from aquacultural facilities by improving diet formulation and processing. The current challenge in aquaculture is to raise productivity while maintaining environmental sustainability, meaning that much of any production increase must be realised through low-cost technologies. Our results suggest that this could be achieved by using inexpensive thermally treated cereals. These not only improved carp growth and nutrient budget but also increased the biomass of carp harvested and removed more nutrients per hectare of water surface. This is a win-win situation for pond farmers, with a 10% lower feed conversion ratio, increased profits and a lower environmental impact. Supplementary diets based on thermally treated cereals, therefore, are important for the sustainable management of pond farming and will be of great use to those seeking more efficient use of nutrients and improved water quality.

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CHAPTER 5

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLODGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING STUDY
CURRICULUM VITAE

GENERAL DISCUSSION

Reduction of waste discharge into the environment

Management of waste discharged from fish farms is one of the major concerns for further development of aquaculture (Naylor et al., 2000; Mente et al., 2006). Global recognition of the need to minimise the negative environmental effects of various methods of production has been recognised through emphasis on the ecological use and management of the natural resources that ensure a sustainable aquaculture industry (Chamberlain and Rosenthal, 1995; O'Bryen and Lee, 2003; Satoh et al., 2003). The reduction of aquatic waste through use of environmentally-friendly fish feed is a major area of aquacultural research (Lall, 1991; Sugiura et al., 2000; Jahan et al., 2001). Minimising phosphorus waste is considered a key factor in the environmental sustainability of freshwater aquacultural operations as excess phosphorous can stimulate eutrophication and cause environmental degradation (Hua and Burea, 2010). Limited reports are available on the successful reduction of fish-feed related aquatic waste; however, some authors (e.g. Cho et al., 1994; Cho and Bureau, 1997) suggest that output of phosphorus and nitrogen from salmonid farms could be reduced through improved feed formulation and feeding systems. Watanabe et al. (1989) developed a new type of dry pellet for yellowtail (*Seriola quinqueradiata*) that was less nitrogen polluting than conventional feed. In other experiments, low-protein high-energy carp dietary supplements had been developed that effectively reduced total nitrogen loading in water effluent (Watanabe et al. 1987a; Watanabe et al. 1987b). As Jahan et al. (2003) reported, reduction of the nitrogen load in post-production waters is possible by applying, i) components with highly digestible protein, and ii) a diet with a balanced energy to protein content. Several studies have also indicated that phosphorus digestibility and retention is greater for fish fed diets supplemented with alternative protein sources derived from plants, as compared with traditional fish meal-based diets (Ketola and Harland, 1993; Hernandez et al., 2004; Brinker and Reiter, 2011).

The aquacultural research community and the aquafeeds industry have long recognised and anticipated issues impacting the sustainability of fish meal in aquafeeds (Barrows and Hardy, 2001) and have been researching and developing aquafeeds that use alternative protein ingredients, particularly plant-derived proteins (Gatlin et al., 2007). Significant progress has been made towards the development of aquafeeds containing plant-based proteins. Recent literature indicates that growth performance in rainbow trout (*Oncorhynchus mykiss*) fed all-plant protein diets without fish meal were comparable with those of trout fed traditional fish-meal diets (Kaushik et al., 1995; Gaylord et al., 2007; Barrows et al., 2007). In addition, important information has been gleaned from studies evaluating feed palatability (Stickney et al., 1996), digestibility (Storebakken et al., 1998; Drew et al., 2005; Gaylord et al., 2008), nutritional content (Apines et al., 2003), anti-nutrients (Francis et al., 2001) and flesh quality (Bjerkeng et al., 1997; Brinker and Reiter, 2011), resulting in substantial improvements in grain-based diets for salmonids.

The majority of carp production in Central Europe, however, is based on semi-intensive fish ponds. Under semi-intensive rearing conditions, carp have access to natural food (zooplankton and zoobenthos) and the fish diet is supplemented with carbohydrate feed in the form of grain (wheat, rye, triticale, etc.) to fulfill their energy requirements. Close to 70% of phosphorus in cereals, however, is in the form of phytate, which fish cannot digest as they lack phytase for hydrolysis (Cho and Bureau, 2001; Nwanna et al., 2008a; Kumar et al., 2012), meaning that most phytate-phosphorous is excreted into the water, adding to eutrophication and algal blooms (Baruah et al., 2004).

Recently, pre-treatment of cereals has provided new possibilities for improving production efficiency as a means of solving this problem. The main aim of such technologies is to increase the nutritional value, palatability, acceptability and, especially, digestibility of fish feed (Másílko, 2014a). As reported by Przybyl and Mazurkiewicz (2004), Urbánek et al. (2010) and Másílko et al. (2014b), cereals provide valuable components of common carp nutrition. Compared to commercial pelleted feeds, cereals represent an easy and cheap source of digestible energy in the form of carbohydrates, and especially starch (Gatlin et al., 2007). If the cereal is unprocessed, its digestibility for carp is about 70%; however, this can be increased to 90% by heat treatment (Przybyl and Mazurkiewicz, 2004). Using heat treatment, starch rich grains improve carbohydrate availability, thereby increasing the digestible energy levels through increased starch gelatinisation and release of oils within the grain matrix (Burel et al., 2000; Ratnayake et al., 2002; Davies and Gouveia, 2010). Extrusion/roasting technology has become the primary technique used for fish feed production, mainly because of the higher physical (e.g. water stability, leaching) and nutritional quality of the feed (Hilton et al., 1981; Sorensen, 2012). The physical quality of feed used in aquaculture is also of great importance as it minimises breakage and formation of undersized particles during handling and transportation (Aas et al., 2011). Physical quality varies considerably among commercial diets (Chen et al., 1999), however, as different feed producers use different ingredients (Glencross et al., 2009; Sorensen et al., 2009) and processing conditions (Sorensen et al., 2009, 2011). The feeding habits of benthivorous fish (such as carp) also play an important role as regards ingestion of feed sinking to the bottom. In searching for spilled feed, benthivorous fish release large quantities of nutrients into the water column, thus enhancing phytoplankton production (Adámek and Maršálek, 2013). This may be especially important in aquacultural carp ponds (Avnimelech et al., 1999), and particularly in those with older carp that receive supplementary food from the onset of the growing season (Kloskowski, 2011). This is in accordance with our review (Hlaváč et al., 2014; paper I), where we conclude that mechanical and thermal treatment of feed cereal prior to its application can also help to decrease the amount of poorly or undigested feed and potentially improve water quality and nutrient budget in carp ponds. Our field experiments (papers II and III) assessed the potential of modified cereals in pond aquaculture, with special emphasis on decreasing the feed conversion ratio and supporting nutrient uptake by the fish.

Water quality in ponds

Generally speaking, water quality is a constant concern in fish culture. When its quality is low, fish may show impaired productive performance and increased mortality, leading to lower production and profit (Baccarin and Camargo, 2005). In many cases, a lack of basic knowledge on water quality means that producers themselves contribute to this decrease in quality (Bosma and Verdegem, 2011). In fish culture, it is common to use poor quality feed and inadequate strategies, such as the use of a high volume of feed without considering the loading capacity of the culture system. This leads to an excessive accumulation of organic residue from feed remains and fish excrement, causing a reduction in the level of dissolved oxygen and increasing the concentration of toxic substances (Tovar et al., 2000). In order to minimise the impact of these problems, producers should apply a basic protocol that assures safety; respects the maximum fish load in a given area; uses improved feed management techniques; and minimises waste by using good quality, highly digestible feed (Rosenthal, 1994), thereby lowering concentrations of nitrogen and phosphorus while maintaining its nutritional value (Boyd, 1999).

Water quality is strongly influenced by pond management, whether through the combination and density of culture species or the quality and quantity of nutrient input (Milstein, 1993; Diana et al., 1997). In general, common carp reduce water quality through their feeding activities by physically disturbing sediment and recycling nutrients. These activities can increase chlorophyll *a* concentrations, turbidity and water column nutrient concentrations, while decreasing macrophyte (Zambrano et al., 1999; Parkos et al., 2003) and aquatic invertebrate levels (Weber and Brown, 2009). The effects of common carp on aquatic ecosystems appear to be related, in part, to individual body size (Driver et al., 2005; Weber and Brown, 2009).

Fish culture, as in all food animal husbandry, produces waste, as fish are sloppy feeders, with around 1–30% of available food remaining uneaten (Van Der Meer et al., 1997; Verdegem et al., 1999). Nevertheless, the high amounts of organic matter resulting from primary production and, to a lesser extent from feeding, do not necessarily constitute a source of pollution. In a static water pond, most organic matter is mineralised *in situ*. During that time, natural processes ("self-purification") remove potential pollutants from the water, including nitrogen and phosphorus (Steidl et al., 2008; Cereghino et al., 2014), and the amount of nutrient and organic matter ultimately discharged from ponds is, therefore, often substantially lower than the overall waste loading to the pond (Schwartz and Boyd, 1994; Všetičková et al., 2012). Nutrient turnover in ponds is relatively quick, such that significant amounts of nutrient tend not to remain dissolved in the water. Large ponds with long hydraulic retention times have a longer response time to environmental change, as nutrients are first stored in the phytoplankton. For this reason, no significant short-term effects of management practice might be observed (Wezel et al., 2013). This is in agreement with our studies (papers II and III), where application of supplementary feed had no significant effect on most environmental variables. Conversely, bioturbation had a greater effect on nutrient concentration than nutrient input by cereals (paper II) and conditions influencing water transparency were significantly different (higher turbidity and suspended solids and lower Secchi depth) in control ponds with no supplementary feeding compared with ponds in which thermally-treated cereals were applied (paper III). This effect of carp bioturbation on water quality has been well documented in previous studies (Matsuzaki et al., 2007; Adámek and Maršálek, 2013).

Nutrient budgets

Nutrient discharges from fish farms can be determined retrospectively, relatively simply and with high degree of accuracy, from records of fish production and feed conversion ratios combined with chemical analysis of feed and fish (Einen et al., 1995). Prospective predictions of inputs (via feed) and outputs (via production and discharges) of given chemical elements on both a daily basis and over longer periods of time represent a valuable management tool for farmers and for regulatory and planning authorities. Chemical budgets are developed to precisely account for the fate of nutrients entering aquatic ecosystems and to assess the relative importance of different nutrient sources. Fertilisers and feeds generally represent the largest inputs of nitrogen and phosphorus to fish ponds (Green and Boyd, 1995), though ponds also receive nutrients from rainfall, surface runoff and regulated inflow. The principal fate of uneaten artificial feed in ponds is supplying organic nutrients that drive primary production and bacterial decomposition. Stable isotope studies have indicated that most fish production in ponds is based on natural food items, even in pellet-fed ponds, where 50–80% of fish production is based on natural food (Schroeder, 1983). Pond aquaculture, therefore, can benefit from improved strategies for natural food production and utilisation, which will also lead to less pollution. In ponds receiving protein-rich pellets, it has been shown that only 11–35% of the nitrogen and 13–36% of phosphorous supplied is retained as fish biomass

(Avnimelech and Lacher, 1979; Boyd, 1985; Acosta-Nassar et al., 1994). A large fraction of the unused nitrogen and phosphorous accumulated in the system, affects water quality in the overlaying water column. Most of the nutrients accumulated in the sediment are, in absolute terms, 100-1000 times higher than those in the water column (Bíró, 1995). Note, however, that the fish stocking densities mentioned in these studies were much higher (> 10 000 ind.ha^{-1}) than in our experimental ponds (> 100 ind.ha^{-1}) and that the fish were fed with protein-rich pellets with an almost two-times greater content of phosphorus and nitrogen. In general, Central European carp ponds are not considered as detrimental to the environment but rather as mechanisms for improving water quality as they are known to trap nutrients and transform them through primary and subsequent production links within the pond food chain (Wezel et al., 2013) and subsequent removal at fish harvest. If these ponds are managed appropriately, they are even able to retain nutrients, as shown by Olah et al. (1994), Knösche et al. (2000) and Gál et al., (2003). Conversaly, in our study in experimental storage ponds (paper II), we noted an unexplained source of phosphorus, possibly arising from sediment, bioturbation, insects, dust, fallout and/or rainfall (Cole et al., 1990; Boyd, 1995; Newman, 1995; Kopáček et al., 1997; Holas et al., 1999). Even in closed recirculation systems, around 15–25% of the nutrient balance remains unexplained (Verdegem, 2007). Despite this, the use of thermally-treated cereals improved carp growth performance and resulted in lower nutrient concentrations in effluent water due to improved digestibility (paper II), thus, supporting the pond's nutrient budget (paper III).

Fish are important pools of nutrients and can exert a major influence on the dynamics, distribution and ratios of limiting nutrients in freshwater ecosystems (Vanni, 2002). Nutrient cycling by fish can supply nitrogen and phosphorus at rates comparable to major nutrient sources and can support a substantial proportion of the nutrient demands of primary producers (Brabrand et al., 1990; Persson, 1997; Vanni et al., 2002). Few data exist, however, on the range of nutrient variation in fish bodies, and its taxonomic, allometric or ecological correlates (Sterner and George, 2000; Tanner et al., 2000; Vanni et al., 2002). Increased data on fish body nutrient content and ratios are expected to improve model output, therefore, and help clarify the role of fishes in nutrient cycles and budgets (Sterner and George, 2000, Dantas and Attayde, 2007). Fish body chemical content is species-specific (Sterner and George 2000; Tanner et al. 2000; Griffiths, 2006) and varies greatly throughout the year depending on many factors, such as fish length and mass (Dantas and Attayde, 2007; Torres and Vanni 2007). In our study (paper III), we found that the content of phosphorus and nitrogen was higher in harvested four-year-old carp compared to stocked three-year-old fish. This is in accordance with data known from the literature (Shearer, 1984; Deegan, 1986; Torres and Vanni, 2007), which has shown that phosphorous and nitrogen varies in a fish's body from early to later stages of growth. It is also well known that mineral concentrations over the whole fish body are highly correlated with the phosphorous level in their diet (Nwanna et al., 2008b; Nwanna et al., 2010). These findings correspond with our results (paper III), as fish fed with natural food had a lower content of phosphorus in the whole body compared with supplementary fed fish.

Natural food in pond management

Carp are omnivorous fish, feeding mainly on benthic macroinvertebrates and zooplankton (Spataru et al., 1983; Rahman et al., 2006). Carp diet consists mainly of zooplankton during the first year of life (Anto-Pardo and Adámek, 2015) with an abrupt shift to benthivorous feeding behaviour in > 2+ fish (Kloskowski, 2011; Adámek et al., 2003; Rahman et al., 2010).

As common carp grow, the proportion of zooplankton ingested decreases and the proportion of benthic macroinvertebrates increases (Rahman et al., 2009). Aquatic insects, mainly benthic larvae of chironomids, form the main dietary component of older carp (Anton-Pardo et al., 2014), along with large-bodied zooplankton such as *Daphnia* as the branchial sieve retains organisms larger than 0.25 mm (Sibbing et al., 1986).

Adult common carp occasionally reduce the abundance of large-bodied zooplankton indirectly through increased turbidity (high turbidity inhibits phytoplankton development and its ingestion by filter-feeders). In contrast, smaller planktivorous carp commonly reduce large-bodied zooplankton density through predation (Chumchal et al., 2005; Weber and Brown, 2009; Carey and Wahl, 2010). In addition, zooplankton abundance may be increased by nutrient input through excretion of supplementary feed providing nutrients for phytoplankton growth (Moriarty, 1997; Milstein et al., 2002). It is not clear, however, whether this is stronger than the effects of fish predation (Schindler, 1992; Khan et al., 2003). In studies presented here (papers II and III), the addition of nutrients through supplementary feeding may have stimulated algal growth, though this response may have been masked by high zooplankton grazing pressure or through increased turbidity, which inhibits zooplankton ingestion of phytoplankton in ponds. This is probably the reason why we did not find any difference in zooplankton biomass between ponds, with and without supplementary feeding.

Carp grazing pressure on bottom invertebrates may have significantly effects on the density of benthic macroinvertebrates (Parkos et al., 2003; Matsuzaki et al., 2007). These effects may be stronger in the presence of larger carp, which are capable of penetrating deeper into the substrate than smaller fish, and may be more intense in summer when carp take mainly benthic prey. Moreover, carp prefer artificial feed over natural food and, when it is available, grazing on zooplankton and benthic macroinvertebrates decreases (Rahman et al., 2010). This may help to explain the results obtained in our study (paper III), where supplementary feeding with cereals resulted in a decreased demand for macrozoobenthos, resulting in a clearly higher density and biomass compared to the control (though the differences were not significant in the majority of evaluations).

Conclusion

Feeding modified cereals results in a decrease in feed conversion rate and better nutrient uptake by fish, and hence a lower release of phosphorus to the surrounding and recipient water. Such supplements should prove beneficial, not only as a source of nutrient for fish but also indirectly as a management tool for maintaining good quality of fishpond water. Processed cereals are attractive because of their economic availability and they represent a valuable management tool for farmers who need to maintain acceptable water quality. Use of thermally and pressure-treated cereals may provide a framework for the sustainable management of carp ponds, resulting in improved phosphorus budget over the entire pond system.

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ENGLISH SUMMARY

The effect of supplementary feeding with treated feed mixtures in carp ponds upon discharged water quality

David Hlaváč

Minimisation of the environmental impact is a key factor in insuring the long-term sustainability of the aquacultural industry. Numerous studies have examined nutritional strategies as a means of reducing waste production and minimising the environmental impact of aquacultural waste. To achieve this goal, it is the challenge to the aquacultural industry to develop "environmentally friendly" feeds, feed management and feed production methods to reduce pollution. This applies mainly to pond farming of common carp, which plays an important role in global aquaculture.

The objective of my first study was to evaluate the possibilities of using modified cereals in pond aquaculture. It focused on issues that contribute to a better understanding of the interconnection between fish production, feed quality and applied feeding technologies with respect to water quality and nutrient budget. We concluded that pollution from carp fish ponds can be significantly reduced by using highly digestible feed and appropriate feeding strategies and feed processing. The addition of thermal and mechanical treatment prior to their application in carp ponds further contributes to decreasing loading by undigested or poorly digested supplementary feed and improves both water quality and nutrient budget in rearing ponds.

In the second study, I describe the impact of different feed cereal treatments (i.e. wheat, thermally-treated wheat, and thermally-treated and pressed wheat) on pond water quality and nutrient budget as regards the rearing of marketable size common carp. Supplementary feeding in the experimental ponds had no influence on water quality; with the exception of dissolved oxygen concentration, which was lower in thermally-treated and pressed wheat treatments than the control. No significant differences were observed among experimental ponds regarding zooplankton abundance. Use of treated cereals (especially thermally-treated and pressed wheat) resulted in a reduction in phosphorus loading. This was caused by better utilisation of the processed cereals by common carp.

In the third study, I describe the influence of supplementary feeding with modified cereals on environmental and biotic variables and nutrient budget. We applied cereals in four experimental ponds over two consecutive years: two replicates with thermally-treated cereals, one replicate with raw cereal and one with no supplementary feeding, the represented a control. Bioturbation by fish had a greater effect on nutrient concentration than input of nutrient from cereals. Further, parameters influencing water transparency were significantly ($P < 0.05$) different (higher turbidity and suspended solids and lower Secchi depth) in control ponds without supplementary feeding, compared with ponds in which thermally-treated cereal was used. No significant difference was noted in zooplankton density between the experimental ponds. Supplementary feeding of carp with cereals resulted in a decreased consumption of macrozoobenthos, whose density and biomass were obviously higher compared with the control, despite a lack of significance in the majority of evaluations. The use of thermally-treated cereals in carp farming management, not only improves carp growth but also the pond's nutrient budget, as the higher biomass of harvested carp removed more nutrients. The end result is a win-win situation, with 10% lower feed conversion ratio, higher profits and reduced potential environmental impact relative to improved feeding strategies.

CZECH SUMMARY

Vliv přikrmování upravenými krmnými komponenty v kaprových rybnících na kvalitu vody v recipientech

David Hlaváč

Minimalizace environmentálních dopadů akvakultury se jeví jako klíčový faktor pro zajištění dlouhodobé udržitelnosti tohoto odvětví. Četné studie vyhodnotily nutriční strategie jako jeden z hlavních prostředků ke snížení produkce odpadních látek a minimalizaci vlivu chovu ryb na okolní recipienty. K dosažení tohoto cíle je zapotřebí rozvíjet používání krmiv šetrných k životnímu prostředí, zefektivnit management krmení a v neposlední řadě také zdokonalit výrobní metody krmiv za účelem snížení znečištění především v rybníkářství, které zaujímá významné postavení v globální akvakultuře.

Cílem první studie bylo zhodnotit možnosti využití modifikovaných obilovin v rybniční akvakultuře a zaměřit se na otázky, které přispějí k lepšímu pochopení vztahů mezi produkcí ryb, používanými krmnými technologiemi a úpravou a jakostí krmiv s ohledem na faktory ovlivňující kvalitu vody a bilanci živin. Došli jsme k závěru, že znečištění z rybníkářství lze výrazně snížit použitím lépe stravitelného krmiva, vhodné krmné strategie a uplatněním nových technologických úprav krmiv. V souladu s těmito závěry lze očekávat, že tepelné a mechanické zpracování krmných obilovin před jejich použitím v kaprových rybnících může přispět ke snížení zatížení prostředí rybníka o nestrávené nebo špatně strávené doplňkové krmivo a mohlo by představovat nástroj pro zlepšení kvality vody a také bilance živin v chovných rybnících.

Ve druhé studii byl ověřován potencionální vliv přikrmování tržního kapra při použití odlišně upravených obilovin (pšenice, tepelně upravená pšenice a tepelně upravená a mačkaná pšenice) na kvalitu vody a bilanci živin. Vstup živin přikrmováním v experimentálních sádkách neovlivnil kvalitu vody, s výjimkou rozpuštěného kyslíku, který byl nižší u tepelně upravené a mačkané pšenice v porovnání s kontrolou. Dále nebyly pozorovány žádné významné rozdíly mezi experimentálními rybníky týkající se abundance zooplanktonu. Upravené obiloviny (zejména tepelně upravená a mačkaná pšenice) vykazovaly snížené uvolňování fosforu do recipientů. Tento účinek byl způsoben vyšší stravitelností upravených obilovin pro kapra obecného.

Vliv přikrmování upravenými obilovinami na environmentální proměnné a bilanci živin je popsán ve třetí studii. Ve dvou po sobě jdoucích vegetačních sezonách byly aplikovány obiloviny do třech experimentálních rybníků: tepelně upravené obiloviny ve dvojím opakování a neupravené obiloviny. Účinnost takto podávaných krmiv byla porovnávána s hodnotami z kontrolních podmínek bez přikrmování, kde obsádka kapra stejné hustoty byla chována pouze na přirozené potravě. Bioturbace ryb měla větší vliv na parametry kvality vody než přísun živin z obilovin, kdy proměnné ovlivňující průhlednost vody byly statisticky ($P < 0,05$) odlišné (vyšší hodnoty zákalu a nerozpuštěných látek a nižší průhlednost vody) v kontrolních rybnících bez přikrmování oproti rybníkům, kde byly použity tepelně upravené obiloviny. Žádné významné rozdíly nebyly pozorovány v hustotě zooplanktonu mezi experimentálními rybníky. Přikrmování obilovinami všeobecně vedlo ke snížení zájmu kapra o makrozoobentos, kdy hustota a biomasa byla v přikrmovaných rybních evidentně vyšší ve srovnání s kontrolou, ačkoliv rozdíly byly nevýznamné. Použití tepelně upravených obilovin zlepšilo nejen růst kapra, ale zefektivnilo i bilanci živin, protože vyšší biomasa vyloveného kapra „odčerpala" více živin na hektaru vodní plochy. Konečným výsledekem byla tzv. „win-win" situace s o 10 % nižším krmným koeficientem, vyšším ziskem a zlepšením ukazatelů možných dopadů na životní prostředí ve vztahu ke krmné strategii.

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LIST OF PUBLICATIONS

Peer-reviewed journals with IF

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TRAINING AND SUPERVISION PLAN DURING STUDY

Name	David Hlaváč
Research department	2009–2013 – Laboratory of Pond Aquaculture and Protection of Waters of FFPW since 2014 – Laboratory of Applied Hydrobiology of FFPW
Supervisor	Assoc. Prof. Dr. Zdeněk Adámek, CSc.
Period	3rd October 2011 until 15th September 2015
Ph.D. courses	**Year**
Applied hydrobiology	2012
Basic of scientific communication	2012
Pond aquaculture	2012
Biostatistics	2013
English language	2014
Scientific seminars at FFPW	**Year**
	2012 2013 2014 2014
International conferences	**Year**
The effect of supplementary feeding in carp ponds upon discharged water quality: A review. In: AQUA 2012: Freshwater farmers day, Prague, Czech Republic, 1st – 5th September, 2012. (Oral presentation)	2012
The effect of supplementary feeding in carp ponds upon discharged water quality. A review. In: Domestication in Finfish Aquaculture, Olsztyn-Mragowo, Poland, 126. (Poster presentation)	2012
The effect of supplementary feeding of common carp (*Cyprinus carpio* L.) with modified cereals on water quality and nutrient balance. In: Diversification in Inland Finfish Aquaculture II (DIFA II), Vodňany, Czech Republic, 24th – 26th September, 2013, p. 63. (Poster presentation)	2013
Foreign stays during Ph.D. study at FFPW	**Year**
Bavarian State Institute of Fisheries, Sub-station for Carp Pond Culture, Germany (3 months)	2012

CURRICULUM VITAE

PERSONAL INFORMATION

Surname: Hlaváč
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EDUCATION

2002–2006 Fish Farming High School in Vodňany
2006–2009 B.Sc. – University of South Bohemia in České Budějovice, Faculty of Agriculture, České Budějovice, Czech Republic,

Specialisation – Fisheries
2009–2011 M.Sc. – University of South Bohemia in České Budějovice Faculty of Fisheries and Protection of Waters

PROFESSIONAL EXPERIENCE

2011–2013 Ph.D. student at the University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Institute of Aquaculture in České Budějovice, Laboratory of Pond Aquaculture and Protection of Waters, Czech Republic

2014 – present Ph.D. student at the University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Institute of Aquaculture and Protection of Waters in České Budějovice, Laboratory of Laboratory of Applied Hydrobiology, Czech Republic

Ph.D. COURSES

Applied hydrobiology, Basic of scientific communication, Pond aquaculture, Biostatistics, English language

SPECIALISATION

Pond aquaculture and hydrobiology

KNOWLEDGE OF LANGUAGES

English (B2 level – Cambridge FCE certificate), German (basic)

FOREIGN STAYS DURING Ph.D. STUDY AT FACULTY OF FISHERIES AND PROTECTION OF WATERS

October – December 2012: Dr. Martin Oberle, Bavarian State Institute of Fisheries, Sub-station for Carp Pond Culture, Germany