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Jihočeská univerzita
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CONTENT

1. BACKGROUND

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

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

2. THE AIM OF THE METHODOLOGY

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

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

3. NOVELTY OF THE METHODOLOGY

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

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

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

4. AVAILABILITY AND DEVELOPMENT OF THE DATABASE

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

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

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

5. GENERAL FEED PROFILE AND NUTRIENT UTILIZATION IN CARP

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

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

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

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

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

6. DIGESTIBLE NUTRIENT SUPPLY FROM DIFFERENT FEED INGREDIENT CATEGORIES

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

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

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

7. FULFILLING OPTIMUM NUTRITION FOR CARPS

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

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

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

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

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

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

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

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

8. PROBLEMS AND PROSPECTS OF FISHMEAL-FISH OIL REPLACEMENT

Growth-retarding antinutritional factors in plant origin feedstuffs

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

Nutritional bottlenecks in plant origin feedstuff compared to fishmeal

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

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

Contemporary fishmeal replacements

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

Improving nutrient utilization from plant-based feedstuff

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

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

Advantages and disadvantages of fish oil replacement

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

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

9. APPLICATION OF THE CERTIFIED METHODOLOGY

Feed formulation tools and calculations involved

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

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

$$X_i = \frac{X_i \times B_i}{100}$$

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

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

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

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

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

Category/ Level	Software name®	Website	License
Single user versions/ Intermediate level	WinFeed	www.winfeed.com	Trial, Premium
	AFOS	https://animalfeedsoftware.com/	Trial, Premium
	FeedAccess (online only)	http://www.feedaccess.com/	Premium
Enterprise versions/ Advanced level	Bestmix	www.adifo.be	Premium
	Alix²	www.a-systems.fr	
	Brill	www.feedsys.com	
	Format	www.formatinternational.com	

Using the database for fishmeal replacement

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

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

10. SIGNIFICANCE AND TARGET AUDIENCE

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

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

A

INGREDIENTS	FORMULA %
Blood meal (poultry/ non-bovine)	15.2
Feather meal	0.15
Linseed oil	1.73
Meat Bone meal	2.14
Poultry meal	27.81
Wheat	51.01
Vit-Min Premix	1
DL-Methionine	0.14

NUTRIENTS	ANALYSIS	CRUDE
Dry Matter(%)	90.9	90.9
Crude Fibre(%)	3.7	3.7
Crude Ash(%)	4.7	4.7
Digestible Protein(%)	38.0	40
Digestible Lipid(%)	7.5	8.2
Digestible Energy(kcal/kg)	4524.6	4918
Dig Protein-Dig Energy ratio(mg/kcal)	77.9	
Digestible Arginine(%)	2.1	
Digestible Histidine(%)	1.1	
Digestible Isoleucine(%)	1.0	
Digestible Leucine(%)	2.9	
Digestible Lysine(%)	2.2	
Digestible Methionine(%)	0.7	
Digestible Phenylalanine(%)	1.7	
Digestible Threonine(%)	1.5	
Digestible Tryptophan(%)	0.3	
Digestible Valine(%)	2.0	
Digestible Phosphorus(%)	0.6	
Digestible Linoleic18:2n-6(%)	2.2	
Digestible Linolenic18:3n-3(%)	1.0	
Crude Phospholipids(%)	1.0	

Formula Cost /unit	0.24 USD/kg	**Or, 0.22 EUR/kg**	
Formula Cost /Bag	3.3 EUR	(Bag Size = 15 kg)	**Or, 90 CZK/bag**

B

INGREDIENTS	FORMULA %
Blood meal (poultry/ non-bovine)	15.79
Linseed oil	1.42
Meat Bone meal	3.7
Poultry meal	23.77
Rapeseed meal	6.16
Silk worm pupae/ meal worm	6.6
Wheat	41.5
Corn	0.07
Vit-Min Premix	1

NUTRIENTS	ANALYSIS	CRUDE
Dry Matter(%)	91.2	91.2
CrudeFibre(%)	4.2	4.2
Crude Ash(%)	5.9	5.9
Digestible Protein(%)	42.0	45
Digestible Lipid(%)	7.4	8
Digestible Energy(kcal/kg)	4558.8	4955.2
Dig Protein-Dig Energy ratio(mg/kcal)	86.6	
Digestible Arginine(%)	2.1	
Digestible Histidine(%)	1.2	
Digestible Isoleucine(%)	1.0	
Digestible Leucine(%)	2.9	
Digestible Lysine(%)	2.2	
Digestible Methionine(%)	0.7	
Digestible Phenylalanine(%)	1.7	
Digestible Threonine(%)	1.5	
Digestible Tryptophan(%)	0.4	
Digestible Valine(%)	2.0	
Digestible Phosphorus(%)	0.6	
Digestible Linoleic18:2n-6(%)	2.0	
Digestible Linolenic18:3n-3(%)	1.0	
Crude Phospholipids(%)	1.0	

Formula Cost /unit	0.3 USD/kg	**Or, 0.27 EUR/kg**	
Formula Cost /Bag	4.05 EUR	(Bag Size = 15 kg)	**Or, 110 CZK/bag**

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

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

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

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

11. ECONOMIC ASPECTS

Valuation of ingredient databases

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

Economic aspects of fishmeal and fish oil replacement

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

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

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

Economic scenario analysis of different formulations

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

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

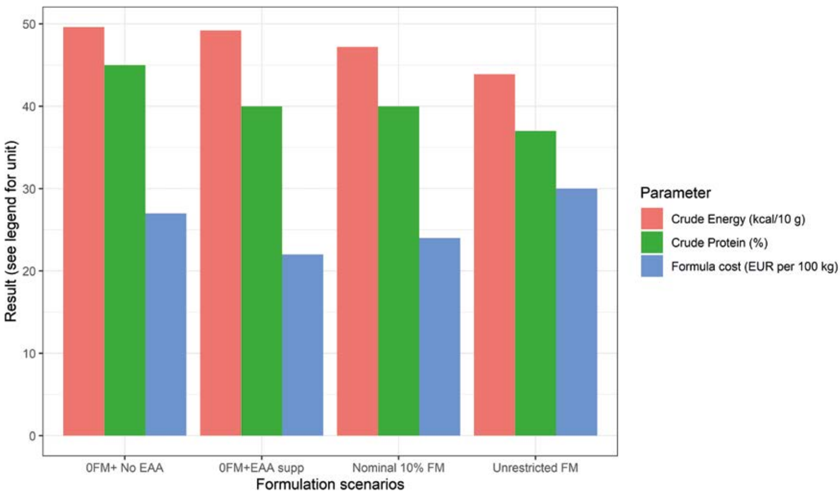

Fig. 4. *Scenario analysis of different formulations (0FM+No EAA = No fishmeal, no amino acids; 0FM+EAA supp = No fishmeal, amino acid supplementation; Nominal 10% FM = Nominal 10% inclusion of fishmeal, EAA supplementation; Unrestricted FM = unrestricted use of fishmeal).*

11.1. Implications at farm level

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

12. LIST OF PUBLICATIONS THAT PRECEDED THE METHODOLOGY

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

13. CZECH SUMMARY

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

Poslední čtyři desetiletí byl u kapra obecného prováděn výzkum vhodnosti různých složek krmiva, které mohou nahradit rybí moučku a rybí tuk. Účelem této metodiky je nahradit rybí moučku a rybí olej v krmivu pro kapry – a) informováním o rozsahu dostupných alternativních krmiv, b) shrnutím rozsahu stravitelnosti živin různých kategorií krmiv a optimálních požadavků na výživu kapra; c) demonstrací metodologie (t.j. rybí moučka, olejová náhrada) pomocí databáze složek krmiv (obsahující informace o stravitelných živinách a energii), d) představením technických možností, problémů a vyhlídek na nahrazení rybí moučky a rybího oleje pomocí softwaru pro komerční přípravu krmiv. Metodika představuje postup vytváření receptur krmných směsí pro kapra s využitím alternativních krmných ingrediencí, shromažďuje informace o jejich nutričních hodnotách, stravitelnosti a potenciálního dopadu na životní prostředí. Představuje 3 alternativní přístupy pro vytváření krmných směsí nahrazujících rybí moučku a olej a vysvětluje jejich limitace. Modelové formulace odvozené z databáze naznačují, že formulace bez rybí moučky (RM) mohou být o 10–27 % levnější než konvenční formulace na bázi RM. Krmiva bez RM, s nebo bez přídavku aminokyselin (náklady na recepturu 0,22–0,27 EUR.kg^{-1}) mají nižší náklady na recepturu než konvenční krmiva na bázi RM (náklady na recepturu 0,3 EUR.kg^{-1}).

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15. DEDICATION

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Internal reviewer
Samad Rahimnejad, Ph.D.
University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II, 38925 Vodňany, www.frov.jcu.cz

External reviewer
Jan Másílko, Ph.D.
Bavarian State Research Center for Agriculture, Institute for Fisheries, Department for Carp Farming, 91315 Hoechstadt a. d. Aisch, Germany

Reviewer for the state administration
Dipl.-Ing. Tereza Barteková, Ministry of Agriculture of the Czech Republic, Department of State Administration of Forests, Hunting and Fisheries, Těšnov 65/17, 110 00 Praha 1

Certificate of application of certified methodology no. 63473/2020-MZE-16232
Ministry of Agriculture of the Czech Republic, Department of State Administration of Forests, Hunting and Fisheries, Těšnov 65/17, 110 00 Praha 1

Affiliation
M.Sc. Koushik Roy – 50%
Assoc. Prof. Jan Mráz – 50%

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Institute of Aquaculture and Protection of Waters, Zátiší 728/II, 38925 Vodňany, www.frov.jcu.cz

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