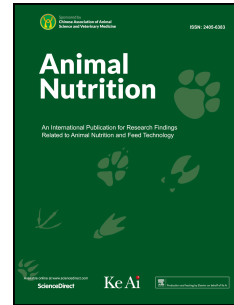


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Sustainable feed processing methods to enhance growth and digestibility in freshwater aquaculture: A review

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1 **Sustainable feed processing methods to enhance growth and**
2 **digestibility in freshwater aquaculture: A review**

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22

23 **Abstract**

24 Freshwater fish farming is one of the fastest-growing food sectors, contributing significantly
25 to global food security. Despite the impressive global growth of aquaculture, high operational
26 costs remain a major challenge, with feed production costs constituting the highest proportion
27 of expenses. To reduce reliance on expensive materials, alternative ingredients have been
28 explored, but issues such as reduced digestibility may arise. To address these challenges,
29 various processing methods have been developed, ranging from traditional to advanced
30 technologies. This review provides comprehensive insights into these methods, their
31 applications, and their impact on growth performance and nutrient digestibility, with a focus
32 on freshwater fish. Evidence from previous studies shows that alternative ingredients derived
33 from poultry by-products, fisheries by-products, plants, and insects have been processed using
34 techniques such as thermal treatment, extraction, bioconversion, nanoparticles, and pulsed
35 electric fields. These approaches have shown positive effects in improving the growth and
36 digestion of fish. Among these, fermentation methods stand out for their multiple benefits such
37 as converting low-value ingredients into high-value protein sources, providing pre-digested
38 nutrients, additional digestive enzymes, and probiotics. They have a lower environmental
39 impact, use less energy, produce minimal pollution, and are cost-effective. Processing methods
40 continue to evolve to ensure high product output, improved nutrient retention and functionality.
41 Selecting processes that minimize energy use, reduce environmental impact, and lower costs
42 while enhancing ingredient functionality is essential for achieving sustainability in aquaculture.

43 **Keywords:** Novel ingredients, Processing methods, Thermal treatment, Extraction,
44 Bioconversion, Nanoparticle, Fish feed

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46

47 1. Introduction

48 Aquaculture is a vital component of modern agriculture and one of the fastest-growing food
49 sectors aiding in global food security. It has seen remarkable growth in recent years, driven by
50 advancements in technology, improved farming practices, and high demand for sustainable
51 seafood. This industry primarily relies on freshwater-farmed fish such as Nile tilapia
52 (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), rainbow trout (*Oncorhynchus*
53 *mykiss*), and common carp (*Cyprinus carpio*) because of their adaptability and tolerance to
54 various environmental conditions, highlighting their crucial role in maintaining a stable aquatic
55 supply (Abdel-Warith et al. 2021; Baßmann et al. 2017; Crichigno and Cussac 2019;
56 Nedoluzhko et al. 2020). According to FAO (2022), these four species, Nile tilapia, African
57 catfish, common carp, and rainbow trout, were among the top 15 most farmed aquatic species
58 in the world, with global production totals of 4.4, 1.3, 4.2, and 7.4 million tonnes, respectively.
59 Despite the impressive increase in aquaculture production throughout the year, however the
60 operational costs of aquaculture present a challenge within the industry, with feed production
61 costs constituting the highest proportion of operational expenses. Feed costs account for at least
62 75% to 90% of the total production costs due to the high price of raw materials such as fish-
63 meal (FM) and fish oil (FO) which are considered unsustainable for their finite supply (Ahmad
64 et al. 2022). Reports indicate that a decline in global fish landings is reaching an alarming stage
65 which causes the increasing price of FM and FO (Seminara et al. 2024). Over 16 million tonnes
66 of fish from both fisheries and aquaculture were used for FM production. However, the supply
67 of FM continues to decline due to increasing demand, driven by the rapidly growing
68 aquaculture industry, as well as by pig and poultry farming and the pet food sector (FAO, 2022).
69 Meanwhile, other ingredients such as soybean meal (SBM), corn, wheat bran, rapeseed meal,
70 sunflower meal, cottonseed meal, and palm kernel meal, are also in high demand and compete

71 with poultry feed (Tacon et al. 2011). The heavy reliance on these ingredients has raised
72 sustainability concerns, highlighting the need to alternative resources for aquaculture feeding.

73 The utilization of plant, animal, and agricultural by-products presents a promising opportunity
74 to reduce reliance on finite marine resources and alleviate pressure on fish stocks. By
75 incorporating by-products into feed formulations, industries can reduce waste, decrease
76 negative environmental impact, and promote circular economy by diversifying feed sources
77 and reducing dependency on costly or unsustainable inputs. This practice helps reduce the
78 overexploitation of marine ecosystems while promoting sustainability in both aquaculture and
79 agriculture sectors. Substituting traditional ingredients with sustainable novel ingredient,
80 primarily from plant sources, can render digestibility issues and significant changes in nutrient
81 content, particularly when replacing FM (Hussain et al. 2024). Replacing FM with agriculture
82 and poultry by-products can lead to significant variation in protein content due to differences
83 in their amino acid compositions. In addition, the presence of digestibility inhibitors which are
84 naturally present in certain feed ingredients such as anti-nutritional factors (ANFs), microbial
85 loads, starch, fibers, and excessive moisture can significantly interfere with the digestion and
86 absorption of nutrients by fish (Francis et al., 2001). ANFs such as protease inhibitors, lectins,
87 phytates, tannins, and glucosinolates are commonly found in plant-based feed ingredients
88 which serve as a protective role for the plant, but when present in high concentrations, they
89 reduce the fish's capability in digesting feed made from these novel ingredients (Samtiya et al.
90 2020). However, if the diet is properly formulated, replacing FM with novel ingredients can
91 provide the proper nutrient and energy balance, enhancing the true potential of the alternative
92 ingredient (Glencross, 2020).

93 The processing of novel ingredients has long been practiced by human in developing aquafeed.
94 Ingredients such as plant seeds and grains are typically hulled to remove the outer husk, while
95 leafy materials, often from farm by-products or invasive plants, can be harvested and used

96 directly without removing husk as ingredients in fish feed (Emshaw et al., 2023; Naret, 2019).
97 Other animal by-products from poultry or fisheries are sorted and cleaned before processing
98 (Al-Zahiri and Al-Shawi, 2021). Nowadays, insect farming is widespread, and the use of
99 insects as protein sources has gained attention (Lu et al., 2020). All these raw ingredients,
100 including plant-based materials, agricultural by-products, fishery by-products, and insects are
101 traditionally processed through drying (thermal treatment), either by sun-drying or oven drying,
102 to reduce moisture content, then minced into powder for long-term storage before being used
103 to make pellets (Al-Zahiri and Al-Shawi 2021). Recently, advanced drying methods, such as
104 freeze-drying, spray-drying, and microwave drying, have emerged to replace conventional
105 drying techniques as these methods increase nutrient retention while reducing energy
106 consumption (Glencross et al. 2007; Thongprajukaew et al. 2015). Instead of drying, raw
107 materials from plants, fish viscera, or poultry viscera can also undergo fermentation, a process
108 in which microbes transform raw materials into value-added ingredients (Emshaw et al. 2023;
109 Santana et al. 2023). The nutrient-rich meals generated through fermentation have also found
110 successful applications as protein sources in fish feed. An interesting development in
111 aquaculture is the use of biofloc technology (BFT), which resembles fermentation but is
112 applied in tanks and ponds to improve water quality (Wei et al., 2024). At the end of the biofloc
113 cycle, the surplus biomass produced is rich in protein and can potentially be used as a feed
114 ingredient (Khatoon et al. 2016). Additionally, extraction techniques are commonly applied to
115 defat these materials, increasing their protein content while reducing lipid content (Kaiser et
116 al. 2021). This can be done through pressing or using solvents like hexane, followed by further
117 refinement through acid-base treatments and ultrafiltration, resulting in protein isolates that
118 contain more than 90% protein (Ehsani et al. 2024). These protein isolates are suitable for use
119 as protein sources in fish feed. Extraction techniques are also being used to create feed additives
120 through nanotechnology, where ingredients are broken down into minute particles and

121 incorporated into feed to improve digestibility (Abdel-Tawwab et al. 2022). Overall, these
122 processing methods including thermal treatment, fermentation, and extraction have been
123 developed as effective strategies to enhance the nutritional content and digestibility of novel
124 ingredients. As these ingredients contain antinutrients and harbor bacteria, processing these
125 components becomes a crucial step in improving nutritional values and digestibility (Olude et
126 al. 2016). Removing ANFs can indeed enhance nutrient availability, and it's typically
127 accomplished by either breaking down complex molecules into simpler forms or by removing
128 water-soluble ANFs through leaching (Ravoninjatovo et al. 2021). Also, processing methods
129 play a crucial role in breaking down cell walls, gelatinizing starches, and activating enzymes,
130 thus improving the overall nutritional quality of the food (Samtiya et al. 2020).

131 In terms of sustainability, a multi-dimensional indicator should be considered to guarantee the
132 sustainability of processing methods, which will include resources use (novel ingredients),
133 energy use, and food security (fish output) (Martyntenko and Vieira 2023). According to the
134 FAO (2018), about 1.3 billion metric tons of waste are produced annually from global food
135 production. However due to their protein contents and valuable bioactive compounds, these
136 food wastes hold significant potential as novel ingredients in fish diet. Selecting processing
137 methods that require less energy use, have minimal environmental impact incur low operating
138 costs while simultaneously increasing the functionality of the processed ingredient is important
139 to meeting sustainability criteria. This review aims to provide comprehensive insights into
140 processing techniques for protein-rich raw materials and production of additives from novel
141 ingredients, with a particular focus on the environmental impact and feasibility of these
142 methods. This review therefore seeks to enhance understanding of how processing methods
143 can aid in ingredient digestibility enhancement of freshwater-farmed fish. This review
144 addresses a critical gap in the literature by evaluating innovative techniques not only for their
145 scientific novelty but also for their real-world applicability. It serves as a foundation for future

146 applied research and the transfer of technology from the laboratory to practical aquaculture
147 settings. The insights presented are particularly valuable for researchers, feed formulators, and
148 aquaculture practitioners aiming to improve fish nutrition, reduce dependence on traditional
149 FM and FO, and support environmentally sustainable practices.

150 **2. Processing techniques for protein-rich raw materials**

151 *2.1 Thermal treatment*

152 Thermal treatment is a drying process that removes water from wet ingredients by applying
153 heat to induce a phase change of water from solid or liquid to a vapor phase through the
154 application of heat (Betoret et al. 2016). Traditionally, feed ingredients were dried under
155 sunlight. According to Noori et al. (2022), a sunny day with temperatures ranging from 30-36
156 °C, humidity between 15-30%, and light intensity of 500-1100 W/m² is sufficient to dry a 5-
157 mm layer of ground ingredients (food wastes such as rice, bread, meat, fruit peel, vegetable
158 peels, etc), reducing moisture content to below 8% while retaining high protein levels.
159 However, this method requires large spaces and labor and is highly dependent on weather
160 conditions. Therefore, more sophisticated machines are now used, including conventional oven
161 drying, convection oven drying (Salinas et al., 2018), vacuum drying (Menon et al., 2020), and
162 microwave drying (Graham et al. 2003). A convection oven is among the most widely used
163 agricultural dryers, where ingredients receive heat from airflow (Salinas et al. 2018). Vacuum
164 drying involves drying ingredients in a vacuum environment, which lowers the boiling point
165 of moisture, thus requiring lower temperatures to evaporate the moisture (Menon et al. 2020).
166 Microwave drying uses electromagnetic waves (commonly between 915 - 2450 MHz) that pass
167 through the ingredients, causing dipolar molecules (such as water, salt, and protein) to vibrate
168 and this internal friction raises the temperature and heats the entire ingredient uniformly (Calín-
169 Sánchez et al. 2020; Graham et al. 2003).

170 Novel ingredients usually contain high moisture, which favors higher activity of
171 microorganisms such as bacteria and fungi, therefore it is crucial to dry these novel ingredients
172 to a certain moisture content (below 10%) to increase shelf life without relying on more
173 expensive techniques such as freezing and canning (Noori et al. 2022). During drying process,
174 novel ingredients can be spoiled or reduced in quality if subjected to inappropriate conditions.
175 Using high temperatures reduces the drying period however, it can damage ingredients and
176 consume more energy. Mild drying conditions will enhance quality of the ingredients but
177 lengthen the drying period (Betoret et al. 2016). At high drying temperatures, vitamins and
178 antioxidants may undergo degradation (Betoret et al. 2016). The use of high temperatures may
179 also cause the Maillard reaction, a chemical reaction between amino acids and reducing sugars,
180 leading to browning of the ingredients and reducing the availability of amino acids and proteins
181 (Kchaou et al. 2019). Meanwhile, using room temperature for drying may facilitate fungal
182 growth, such as *Aspergillus* spp., which thrive at temperatures ranging from 8-35 °C and can
183 cause ingredient spoilage, reduce quality, and contaminate feed, potentially leading to food
184 poisoning in animals. (Mannaa and Kim 2017). Hence, it is crucial to precisely control and
185 optimize drying process (such as temperature, air velocity, and drying time requirements)
186 without losing bioactive compounds while increasing the functionality of dried products with
187 the highest level of nutrient retention (Alam et al. 2023; Betoret et al. 2016). For example,
188 conventional oven drying at 60 °C for 24 h until a stable dry weight is achieved, is a common
189 practice aimed at preserving heat-sensitive nutrients such as vitamins, proteins, and amino
190 acids (Mondal et al. 2019). Sokac et al. (2022) reported that vacuum drying at 70 °C was the
191 most effective processing method for maintaining the stability of bioactive compounds,
192 particularly polyphenols, when compared to conventional oven drying or solar drying. Calín-
193 Sánchez et al. (2020) reviewed that the convection drying at temperatures between 50 -70 °C,
194 as well as microwave drying at 120 to 480 W are effective in preserving crucial bioactive

195 compounds, including phenolics and antioxidants such as flavonoids, carotenoids (β -carotene
196 and lycopene), vitamin C, and anthocyanins.

197 Optimizing processing conditions not only increases nutrient retention but also simultaneously
198 breaks down antinutrients. The presence of ANFs in fish feed (formulated with thermally
199 untreated ingredients) hinder the absorption and digestion of certain nutrients, thereby reducing
200 their bioavailability (Agiriga and Siwela 2018). In contrast, thermally processed ingredients
201 can improve nutrient digestion particularly protein by up to 95% compared to less than 40%
202 with thermal-untreated ingredients (Rehman and Shah 2005). Thermal treatment processing
203 methods, such as conventional oven and microwave drying, have been proven to minimize and
204 remove heat-sensitive antinutrients in novel feed ingredients (Samtiya et al. 2020). Heat
205 treatments result in starch hydrolysis, increasing the capability of starch digestion when fish
206 are fed plant-based alternative protein sources (Rehman and Shah 2005). Furthermore, thermal
207 processing aids breakdown of complex oligosaccharides such as raffinose, stachyose, and
208 verbascose, which can lead to gastrointestinal problems in fish (Ravoninjatovo et al. 2021).
209 Applying thermal heat under optimal conditions can also removes other ANFs, such as tannins
210 (up to 73%), phytates, and trypsin inhibitors (Ravoninjatovo et al. 2021; Samtiya et al. 2020).
211 Phytates reduce mineral bioavailability by forming complexes with metal ions, thereby
212 preventing mineral absorption. Meanwhile, tannins and trypsin inhibitors reduce protein
213 digestibility by forming irreversible tannin-protein complexes and by inhibiting trypsin activity
214 by acting upon the overlapping reactive sites of the enzymes, respectively (Samtiya et al. 2020).
215 Thermal treatment such as convection oven and steam drying stands out as one of the most
216 preferred processing methods in the fish feed industry for enhancing food quality and ensuring
217 nutritional integrity. Novel ingredients such as poultry by product, plants and insects which go
218 through thermal treatment have been proven to be effective in promoting the growth of tilapia
219 (*O. niloticus.*) catfish (*C. gariepinus*) and carp (*C. carpio*) and successfully being used as a

220 main protein source with concentration up to 50% (Table 1). However, the high energy
221 consumption (electricity) associated with prolonged or high-temperature treatments raises
222 environmental concerns, contributing to increased carbon emissions and depletion of non-
223 renewable resources, particularly coal. To address these concerns, optimizing the water
224 removal process from novel ingredients becomes crucial, aiming for better product quality with
225 minimal environmental impact and the lowest possible operating costs. Although convection
226 ovens and vacuum drying require high energy consumption, they still offer advantages over
227 conventional ovens in terms of efficiency and effectiveness (Kaveh et al. 2021). In advancing
228 thermal methods, Kaveh et al. (2021) suggested microwave method for drying novel
229 ingredients due to its lower energy consumption, higher efficiency (in terms of energy, drying,
230 and thermal), and lower emission of carbon dioxide. Calín-Sánchez et al. (2020) highlighted to
231 combine techniques, such as the vacuum-microwave drying, as a potential method for
232 achieving high nutritional retention in product while minimizing energy consumption. Despite
233 these advancements, convection and steam dryers remain are still among the most commonly
234 used drying methods in agricultural, possibly due to their lower initial equipment costs.
235 However, adopting more sophisticated techniques should priority when processing fish feed
236 ingredients to maintain product functionality (nutrient retention) for better fish digestibility and
237 growth. Although, sophisticated drying technologies may involve higher upfront investment,
238 they offer potential long-term benefits in terms of cost and sustainability.

239 ***2.2. Protein extraction: defatting, concentrates, and isolate***

240 The defatting process (via pressing or hexane extraction) plays a critical role in enhancing
241 protein levels by reducing lipid content in ingredients, which can otherwise impact feed quality
242 (Kaiser et al. 2021). This process yields defatted meal in the form of cakes or pellets, along
243 with extracted oil. It is commonly applied to for novel ingredients such as insect meal and
244 microalgae meal, both of which contain high lipid. Insect meal typically contains

245 approximately 20% lipid and 46% protein, respectively (Abdel-Latif et al. 2020), whereas
246 microalgae meal can exceed 50% protein and contain around 20% lipid (Roy and Pal 2014).
247 Without the defatting, fish (such as tilapia) fed with novel ingredients (such as black soldier
248 fly) showed lower digestibility of fat than the group that consumed defatted meal (Kishawy et
249 al. 2022). When defatted, both insect meal and microalgae meal had higher protein contents of
250 60% - 65% (Basto et al. 2021; Soto-Sierra et al. 2018). Besides, plant ingredients and grains,
251 such as soybeans, sunflower seeds, and corn, can undergo defatting to remove excess oil
252 (producing oil for the vegetable cooking oil industry) and improve their suitability for fish feed.
253 The resulting defatted meal contains higher protein and can be used directly as defatted meal,
254 further processed into protein concentrate, or even more refined into protein isolate (Loveday
255 2020). To obtain protein concentrate, the protein in the defatted material (in the form of flakes
256 or finely ground flour) is solubilized in an alkaline solution using NaOH at pH 7.5 - 9.0 to
257 remove non-protein components such as sugars, leaving behind the protein component in the
258 solution, followed by protein precipitation using acids like HCl at pH 4.5–5.0
259 (Nourmohammadi et al. 2023; Okafor et al. 2015). After precipitation, the protein is separated,
260 typically through centrifugation, washed, and dried using spray drying techniques to reduce
261 protein deterioration (Liadakis et al. 2022). To obtain protein isolates, purification processes
262 are carried out to achieve a higher purity level using advanced membrane technologies such as
263 ultrafiltration with 3, 5, or 10 kDa to remove small molecules and ions, thereby further
264 purifying the protein with improved nutritional and functional properties compared to
265 precipitation methods (Ehsani et al. 2024; Mondor et al. 2012). Such advanced membrane
266 technology offers mild operational conditions (low temperature and pressure), low solvent
267 consumption, and high protein purification (Ehsani et al. 2024). Simultaneously, ultrafiltration
268 is an effective method to reduce the quantities of some undesirable ANFs, such as phytic acid
269 and oligosaccharides, to low levels (Mondor et al. 2012; Chen et al. 2019). When insect meal

270 is used as a novel ingredient, removing chitin through protein purification is crucial, as high
271 chitin levels in the diet negatively affect the growth and nutrient digestibility of fish (Hasan et
272 al. 2023). Overall, defatted meal, protein concentrate, and protein isolate contain protein levels
273 of more than 50%, 70%, and 90%, respectively, as illustrated in Fig. 1. The degree of protein
274 purity enhances feed formulation at lower inclusion rates, thereby reducing the overall nutrient
275 load released into the aquatic environment. The application of defatted meal, protein
276 concentrate, and protein isolate have been reported in many commercial fish diets such as carp,
277 catfish, tilapia, and rainbow trout (*Oncorhynchus mykiss*) (Fig. 2 and 3). These have
278 successfully replaced FM by 70-100%, highlighting the potential of utilizing these protein
279 concentrates and isolates as promising ingredients in fish diets to reduce feed costs and achieve
280 aquaculture sustainability. The use of defatted meal, protein concentrate, and protein isolate
281 from novel ingredients has been reported to help prevent pollution problems associated with
282 the disposal of agricultural wastes, particularly from the vegetable cooking oil industry (Amer
283 et al. 2019).

284 **2.3. Bioconversion**

285 Bioconversion is processing method that involves microbial transformation of raw materials
286 into desired products or value-added ingredients. Examples of such processing methods include
287 solid state fermentation (SSF), the silage fermentation process and BFT. Their roles in
288 aquaculture illustrate the various ways in which microorganisms contribute to global waste
289 management. This waste is typically processed into a nutrient-rich meal, providing an
290 environmentally sustainable and cost-effective source of feed for the cultured aquatic species.
291 Nutrient-rich meals generated through these processes have found successful applications in
292 fish feed. Examples include meals derived from accumulated organic matter within the biofloc
293 particles (Das and Mandal 2021; Ekasari et al. 2019b) and SSF from various sources such as
294 lupin meal (Bowyer et al. 2020), pigeon pea (Alegbeleye et al. 2012), sesame seed meal (Olude

295 et al. 2016), date palm seed (Dawood et al. 2020a), shrimp head waste meal (Nwanna 2019),
296 wheat (Omar et al. 2021), poultry by-product (Dawood et al. 2020b) and fish silage (Soltan et
297 al. 2017). The successful applications of these methods in species such as carp, tilapia and
298 catfish, emphasize the importance of bioconversion in promoting sustainable practices within
299 the aquaculture feed industry.

300 In SSF, microorganisms such as fungi or bacteria are utilized to convert solid substrates, like
301 agricultural residues or food processing by-products, into desired products (such as protein-
302 enriched animal feed ingredients). Plants with high protein content, reaching up to 30%, that
303 are locally available, highly productive, classified as pest species or derived from farming
304 waste are used as alternative ingredients to replace costly ingredients, such as FM in fish feed
305 formulations. For processing, plants are harvested, dried to less than 10% moisture, ground,
306 and sieved with less than 2 mm to ensure homogeneity (Emshaw et al. 2023). The plant is then
307 fermented using either fungi (such as *Aspergillus* and *Saccharomyces*), bacteria (such as
308 *Lactobacillus* and *Lactococcus*), or a combination of different fungi and bacteria to enhance
309 fermentation results (Cao et al. 2021). To simplify the process, commercial instant dried yeast
310 (*Saccharomyces*) can be used solely to skip the bacteria cultivation stage (typically requires a
311 broth medium) and proceed directly to fermentation (Fujaya et al. 2023). From 10% to 15%
312 boiled molasses are added, and fermentation is carried out in a sealed container for a certain
313 period at the optimal temperature of fermentative microbes, usually ranging from 34-37 °C
314 (Cao et al. 2021; Velásquez et al. 2015). At the end, the fermented plant substrate is dried and
315 incorporated into fish feed reaching up to 70% (Emshaw et al. 2023). To date, SSF offers
316 several benefits, primarily in enhancing fish digestibility by providing pre-digested nutrients.
317 SSF degrades ANFs such as phenols, saponins, tannins, phytates, trypsin inhibitors, and
318 oxalates, which are common in plants (Hamidoghli et al. 2020; Helmiati et al. 2021). In fish,
319 SSF derived product stimulates the digestive activity of protease, amylase, cellulase (Cao et al.

2021: Helmiati et al. 2021), lipase and pectinase (Ashry et al. 2023), improves gut microbiota (Guo et al. 2023) and intestinal morphology (Yang et al. 2022) (Fig. 4).

More than 20 million tons of fish by-products from fish processing are produced per year worldwide (Toppe 2018). Body viscera consisting of intestine, liver, swim bladder, heads, bones, fins, and tails constitute more than 30% of a fish's total body weight and all these parts are not consumed by humans or animal and are disposed of as waste (Haider et al. 2015). However, such fish wastes contain a high protein levels, reaching up to 88% protein (similar to FM), and is rich in essential amino acids, essential fatty acids, collagen, vitamins, and minerals (Santana et al. 2023). As such, sustainable management is crucial to reduce fish waste disposal, including low-value fish, by converting them into fish silage, which can be incorporated as a protein source in fish feed (Raeesi et al. 2023). Fish waste can be fermented by adding lactic acid groups of microbes (such as *Lactobacillus*, *Lactococcus*, *Hansenula*, and *Saccharomyces*) and carbohydrate sources (such as molasses), producing a brown liquid product known as fish silage (Santana et al. 2023; Tazim et al. 2021). The purpose of using lactic acid bacteria is to reduce the pH of silage, thus inhibiting the proliferation of other pathogenic bacteria (Santana et al. 2023). More specifically, collected fish waste is washed, minced, added with 5% molasses and 5% bacteria, and stored in aerobic conditions for more than 30 d (Haider et al. 2017). At the end of this process, the fish waste is liquefied into brown silage, forming three layers, fish oil at the top, highly soluble proteins and minerals in the middle layer, and semi-soluble materials and bones at the bottom (Raeesi et al. 2023). Fish waste has been digested by fermentation microbes, providing pre-digested nutrients and additional digestive enzymes from microbes, aiding fish digestion when fed with fish silage (Fig. 5).

Biofloc technology (BFT) are aggregates of microorganisms, including bacteria, yeast, microalgae, and micro and macroinvertebrates, which are suspended in ponds and tanks

345 (Ekasari et al. 2019b). The main purpose of BFT is to enhance water quality, as these
346 microorganisms utilize nitrogenous waste and ammonia in the cultured tanks (Barzamini et al.
347 2021). The BFT is managed by controlling the C:N ratio up to 20-fold, usually using molasses
348 as a carbon source to ensure the efficacy of BFT in remediating the cultured tanks (Hapsari
349 2016; Mabroke et al. 2018). However, when BFT concentration reaches maximum capacity,
350 the surplus should be removed or discharged untreated at the end of the harvest cycle in the
351 aquaculture farm (Prabu et al. 2017). The surplus bioflocs can be harvested and used as a
352 valuable resource rather than waste. The surplus can be obtained from aquaculture farm
353 effluents via effluent sedimentation or filtration with a 10 µm pore size (Khatoon et al. 2016).
354 This surplus is considered microbial protein (sometimes known as microbial floc meal) that
355 could be reused as a raw ingredient in making fish feed since this biomass consists of high
356 protein content ranging between 24% - 50% and lipids ranging between 0.5% - 3.5% (Mabroke
357 et al. 2018). Microbial protein is either air-dried under sunlight, oven-dried, or freeze-dried
358 until the moisture content is less than 10%, then powdered into fine particles and ready to be
359 incorporated into fish feed (Ruby et al. 2017). Currently, using microbial floc meal to replace
360 up to 30% of FM appears promising for fish without any negative effects on growth and
361 digestibility (Abdewahab et al. 2023) (Fig. 6). Microbial protein comprising a diverge range of
362 single-cell proteins, it is highly nutritious as it aggregates proteins from various organisms. Its
363 small particle size may also enhance absorption in fish. The addition of several amino acids,
364 such as lysine and tryptophan, may further optimize the use of microbial floc meal in fish diets
365 (Prabu et al. 2017; Raghav et al. 2023).

366 **3. Processing techniques for making additives**

367 ***3.1. Extraction: Crude extracts and essential oils (EO)***

368 Extraction is a fundamental processing method used to isolate valuable compounds from novel
369 ingredients like plants and, occasionally, animal materials (Popova and Bankova 2023). After
370 drying of ingredients, extraction is carried out to isolate specific constituents such as bioactive
371 compounds and EO (Hassaan and Soltan 2016; Lee et al. 2017). Traditionally, these extracts
372 were used in their crude form, leading to lower concentrations of bioactive compounds
373 compared to chemically extracted counterparts. Later, the extracts were obtained by boiling the
374 macerated plants with water or other chemical solvents such as hexane, methanol, and ethanol
375 to dissolve and separate the target compounds from the raw material, thereby obtaining more
376 concentrated extracts (Dedi et al., 2016; Yousefi et al., 2021). Such processing method to
377 extract bioactive compounds from plants and herbs to be used as additives have been proven
378 to be effective in promoting intestinal health and digestive enzymes in catfish, carp, tilapia, and
379 trout, leading to improved growth (Fig. 7). With advancements in extraction techniques, there
380 has been a shift towards obtaining extracts by producing EO. This process can be carried out
381 using pressing, in which the raw material is subjected to high pressure, resulting in a residual
382 cake and the desired oil. Other common methods involve steam distillation of plant parts such
383 as leaves, bark, stalk, flowers, or rhizomes, with heat aiding in the rupture of the plant's cellular
384 structure while steam passes through the plant, evaporating the EO within the plant cells
385 (Chung et al., 2021; Junior et al., 2022). EO is a lipophilic mixture of organic compounds
386 (colorless and fragrance liquid) produced from secondary plant metabolism (Dawood et al.
387 2022). EO stands out for its richness in specific compounds, primarily containing terpene-
388 derived compounds and oxygenated molecules (such as alcohols, aldehydes, ketones, phenols,
389 acids, and esters) (Dawood et al. 2022). The distinct concentration of bioactive constituents in
390 EO makes them potent and targeted in their applications, surpassing the concentration achieved
391 through traditional plant extracts (Stevanović et al. 2018). When used in fish feed as additives,
392 EO offer several advantages such as improved effectiveness with only small quantities,

393 enhanced bioavailability, and digestibility (Fig. 8). The structural compound of EO are resistant
394 to gastric acid, increasing their bioavailability while at the same time EO enhance fish intestinal
395 permeability and promote the growth of beneficial microbiota to growth, thereby improving
396 higher nutrient absorption (Dawood et al. 2022). EO can also also regulate appetite control
397 genes in the hypothalamic pituitary glands of fish (Souza et al. 2019), indirectly enhancing feed
398 consumption in tilapia, carp and rainbow trout (Fig. 8.). Examples of EO used in aquafeeds
399 include ingredients such as black soldier fly (Li et al. 2017), black cumin (Öz et al. 2023), clove
400 basil (Abdel-Tawwab et al. 2018a), cinnamon (Junior et al. 2022), ginger (Chung et al. 2021),
401 lemongrass (Souza et al. 2020), parsley (Farag et al. 2022), oregano (Abdel-Latif et al. 2020),
402 rosemary (Medeiros et al. 2023), thyme (Ibrahim et al. 2022), and turmeric (Larenas-Uría
403 2016).

404 **3.2 Nanotechnology**

405 Researchers are increasingly exploring innovative approaches to extract, modify, and utilize
406 bioactive compounds through the application of nanoparticle technology, particularly to
407 address challenges in aquaculture such as enhancing nutrient utilization in fish farming.
408 Nanoparticles are tiny small particles (less than 100 nm in size) which gives them a
409 significantly larger surface area-to-volume ratio compared to their bulk counterparts (Abdel-
410 Tawwab et al., 2022). This unique characteristic allows nanoparticles to improve solubility,
411 facilitate easier penetration through cell membranes and tissues, and consequently increase
412 cellular uptake (Mabrouk et al., 2021). These mechanisms collectively enhance the bioactivity
413 of molecules and improve targeted delivery to specific tissues, such as the intestine, making
414 nanoparticles more effective and efficient than their conventional forms (Chris et al., 2017;
415 Rohani et al., 2017). Several nanoparticle-based applications have been developed to improve
416 nutrient digestibility in fish (Fig. 9). For instance, reducing plant extracts like curcumin to

417 nanoparticle size and incorporating them into fish feed has been shown to enhance digestion
418 (Abdel-Tawwab et al., 2022). Curcumin nanoparticles are typically produced by extracting
419 curcumin from raw turmeric rhizomes using Soxhlet extraction, followed by a physicochemical
420 fabrication method such as sonication.

421 Metal-based nanoparticles also serve vital roles in aquaculture. Essential trace elements such
422 as copper, chromium, magnesium, manganese, and selenium (when delivered in nanoparticle
423 form) can enhance the growth of beneficial gut microbes, stimulate digestive enzyme secretion,
424 and increase nutrient absorption in the fish intestine (Seerengaraj et al., 2021). The synthesis
425 methods for these nanoparticles vary, for example, copper nanoparticles are produced from
426 metal salts via wet chemical reduction, while selenium nanoparticles are synthesized from
427 sodium selenite using biological agents such as bacteria and fungi (Omosanya et al., 2021).
428 Additionally, nanoencapsulation technologies, such as chitosan-based nanoparticle systems,
429 are being developed to enhance the stability, bioavailability, and targeted delivery of nutrients
430 like vitamins, minerals, and omega-3 fatty acids (Jiménez-Fernández et al., 2014; Misra et al.,
431 2023). These nanoencapsulated systems ensure prolonged residence time in the gastrointestinal
432 tract and targeted release at the site of absorption, thus improving nutrient uptake (Fajardo et
433 al., 2022). Chitosan nanoparticles are typically synthesized via ion-induced gelation using
434 chitosan extracted from crustacean exoskeletons (Omosanya et al., 2021). The process involves
435 mixing a chitosan solution with a bioactive compound and tripolyphosphate, followed by
436 lyophilization to form chitosan-bioactive nanoparticles (Singh et al., 2021).

437 Another promising nanotechnology-based approach to enhance the bioavailability of bioactive
438 compounds in the fish intestine is the use of nanoemulsions. Nanoemulsions are colloidal
439 dispersions consisting of two immiscible fluids, where one fluid is dispersed in the form of
440 small droplets within the other (McClements et al., 2021). These droplets typically range in

441 size from 100 nm to 500 nm (Li et al., 2020). This technique is particularly useful for delivering
442 bioactive compounds that would otherwise be rapidly eliminated from the body without
443 encapsulation (Inal et al., 2022). Nanoemulsions serve as effective delivery systems due to their
444 high stability (they resist gravitational separation and droplet aggregation). This is especially
445 advantageous for the delivery of functional oils, such as FO, which are prone to degradation or
446 separation (Li et al., 2020). The formation and stabilization of nanoemulsions require
447 emulsifiers (or surfactants), which are amphiphilic molecules containing both hydrophilic and
448 hydrophobic groups (McClements et al., 2021). The hydrophobic (fatty acid) tail binds to the
449 oil phase, while the hydrophilic head faces the water phase. This dual interaction reduces
450 interfacial tension between oil and water, thereby enhancing the stability of the nanoemulsion
451 (Li et al., 2020). A wide range of plant-based emulsifiers can be used for nanoemulsion
452 formation. These include proteins (e.g., soy and pea protein), polysaccharides (e.g., gum arabic
453 and modified starch), phospholipids (e.g., soy or sunflower lecithin), and natural surfactants
454 like quillaja saponin (McClements et al., 2021). Nanoemulsions are typically produced using
455 high-energy methods, which apply intense disruptive forces to break down the oil and water
456 phases into fine droplets (McClements et al., 2021). Common techniques include
457 microfluidization, high-pressure homogenization, and sonication. Among these, high-pressure
458 homogenization is one of the most widely used methods due to its effectiveness in producing
459 highly stable nanoemulsions (Li et al., 2020). After nanoemulsion synthesis, the size and
460 morphology of the droplets are typically characterized using techniques such as dynamic light
461 scattering (DLS) (Abdel-Tawwab et al., 2018b) or scanning electron microscopy (SEM) (Khan
462 et al., 2020), before application in fish trials.

463 The cost of nanoparticle production depends on several factors, including raw materials,
464 synthesis methods, and the equipment used. As shown in Fig. 9, various nanoparticles (such as
465 metal ions, chitosan, and turmeric) have been shown to improve specific growth rate (SGR),

466 feed conversion ratio (FCR) and protein efficiency ratio (PER) in species such as African
467 catfish, common carp, Nile tilapia, and rainbow trout. According to market estimates based on
468 online platforms, the price ranges per kilogram for metal ion nanoparticles are as follows,
469 copper (\$580 - \$900), chromium (\$100-500), magnesium (\$15-30), manganese (\$150),
470 selenium (\$160,000-200,000), and zinc (\$6.50-125). Turmeric and chitosan nanoparticles are
471 sold at approximately \$45-180 and \$10-50 per kg, respectively. While incorporating these
472 metal ions, chitosan, and turmeric nanoparticles as essential elements or feed additives may
473 enhance fish digestion and performance, it can also increase the overall cost of feed
474 formulation. However, due to their higher bioavailability compared to bulk minerals (achieving
475 absorption rates of up to 90%), smaller doses are sufficient and at the same time, intermittent
476 supplementation (e.g., twice per week) can be an effective strategy to reduce costs without
477 compromising efficacy. Conducting small-scale trials in aquaculture settings is recommended
478 before scaling up production. The use of nanoparticles may also accelerate fish growth,
479 potentially shortening production cycles and increasing profitability, despite the higher feed
480 costs associated with nanoparticle incorporation.

481 **4. Emerging non-thermal technologies (pulse electric field, PEF)**

482 Conventional techniques such as thermal heat and traditional extraction face limitations
483 including the degradation of thermolabile bioactive compounds, low extraction yields, and the
484 use of chemical solvents, which can lead to loss of functionality and bioavailability
485 (Athanasiadis et al. 2023). Plants with complex structures contain a membrane envelope that
486 controls the movement of intracellular components, preventing their extraction from intact cells
487 during the extraction process and thus reducing the extractability of specific compounds
488 (Ranjha et al. 2021). To address these issues, novel processing methods such as PEF have
489 gained attention in recent years and have shown great potential in preserving heat-sensitive
490 substances while improving extraction rates of bioactive compounds from plants (Mpakos et

491 al. 2024). PEF is a non-thermal pre-treatment technique (before conducting the extraction
492 process) where plants are placed between two metal electrodes in a chamber and subjected to
493 repetitive short-duration electrical pulses ($0.5-40 \text{ kV cm}^{-1}$), requiring low energy input (1-20
494 kJ/kg) (Carpentieri et al. 2022; Kronbauer et al. 2023). This electroporation process induces
495 porosity or permeabilization of the plant cytoplasmic membrane, facilitating the penetration of
496 solvents into the cells and enhancing the extraction of intracellular components such as
497 proteins, lipids, or other bioactive compounds (Carullo et al. 2020). This higher purification of
498 the extract without the plant cell wall may reduce indigestible complex carbohydrates,
499 specifically cellulose, thereby improving the capability of fish to digest feed made from plant
500 extract. The use of PEF has been shown to increase extraction yields of bioactive compounds
501 from plants by 18%-97%, and of EOs from seeds and fruits by 48% - 85.5%, compared to
502 traditional extraction techniques (Ranjha et al., 2021). For now, the use of PEF in the
503 production of fish feed is relatively limited, usually used in the food and beverages industries.
504 High initial costs due to the need of specialized equipment (units manufactured for industrial-
505 scale applications are available at prices ranging from \$45,000-2,000,000), restrict its
506 application in aquaculture feed manufacturing (Aoude et al. 2021; Kovacic et al., 2021).
507 However, there may be some potential applications of PEF technology in this field due to its
508 advantages in intensifying plant extracts and enhancing the digestibility of plant extracts.

509 **5. Economic and environmental impact**

510 The use of thermal heat in fish feed production is unavoidable today and continues to be
511 globally adopted. This is achieved through the most prevalent method for drying ingredients,
512 such as conventional oven drying and convection oven drying, despite their potentially
513 significant greenhouse gas emissions and higher cost compared to sunlight drying (Krishnan et
514 al. 2022). The primary challenge of the thermal heat process lies in producing a high-quality
515 end product while minimizing greenhouse gas emissions. Using more sophisticated techniques

516 should be a primary concern in processing fish feed ingredients to maintain product
517 functionality (nutrient retention) for better fish digestibility and growth. Sophisticated
518 machines may have a high initial cost but could provide long-term benefits in terms of cost and
519 environmental impact. The most recommended thermal treatment is microwave drying or
520 integrating technique such as vacuum-microwave due to its lower possible energy consumption
521 and lower emission of carbon dioxide (Calín-Sánchez et al. 2020; Kaveh et al. 2021). However,
522 both microwave drying and vacuum drying involve higher initial equipment costs, which the
523 prices reaching up to \$15,000 per equipment (for laboratory, and may be higher for industrial
524 application), as well as additional maintenance expenses.

525 Regarding the extraction technique, primary concepts have been outlined by Chawla et al.
526 (2024) in achieving green extraction methods, including (1) enhancing processes or procedures,
527 (2) using non-specialized equipment, and (3) discovering alternatives or minimizing the use of
528 solvents. The most common extraction process involves heating raw materials using alcohol-
529 based solvents (such as ethanol and methanol) and distilling them through the Soxhlet method
530 (Jitan et al., 2018). In advancing extraction procedures towards eco-friendly practices, several
531 techniques have been recently combined and utilized, such as microwave-assisted extraction,
532 pressurized liquid extraction, and enzyme-assisted extraction to increase the extraction amount
533 and quality while reducing the use of toxic organic solvents (Savic and Gajic 2023). For solvent
534 use, alcohol-based extraction, mainly using ethanol, has traditionally been used to extract
535 bioactive compounds from raw materials due to its effectiveness in extracting a wide range of
536 compounds such as phenolics, flavonoids, alkaloids, and EOs from plant materials.
537 Alternatively, water can be considered as a solvent due to its eco-friendliness, universal solvent
538 capabilities, suitability for recovering thermally labile phytochemicals, and widespread
539 availability (Usman et al. 2023). However, in achieving ecologically friendly extraction and
540 ensuring cost effectiveness, raw materials used are the most important consideration. Discarded

541 organic materials from farming or industrial waste, such as fruit peels, vegetable scraps, and
542 expired products, represent substantial resources for sustainable extraction at no cost. However
543 many current extraction techniques still generate waste and managing this wastes remains
544 crucial to ensuring the overall sustainability of the process. This differs from defatted
545 ingredients, where the extracted oil is used in commercial industry, and the defatted residue
546 can be incorporated into fish feed as a primary protein source.

547 Fermentation of locally available plants, pest plants, plantation waste, or fish waste is
548 relatively easy to carry out. This low-cost technology uses only microbes to convert these
549 wastes into valuable feed ingredient. Fermented wastes have been shown to be high-quality
550 feed ingredients due to of their balanced nutrient profile, higher protein content, presence of
551 pre-digested nutrients with balanced essential amino acids and fatty acids, absence of ANFs,
552 and richness in bioactive compounds, factors that collectively enhance digestibility in fish. The
553 bioconversion of such materials, including industrial and aquatic wastes and pest plants, not
554 only reduces environmental pollution but also results in the production of value-added
555 products. In the case of BFT, microbial aggregation in the culture system remediates the water,
556 optimizing water use to minimal or zero water exchange in fish rearing (Khanjani et al. 2024).
557 BFT has been reported to reduce costs by up to 30%, and improve farming profitability
558 supporting intensive fish production (i.e., high stocking densities within smaller area)
559 (Khanjani et al. 2024). However, proper disposal of effluent and sludge from BFT is necessary.
560 Using this effluent as microbial floc meal in fish diets results in no untreated effluent being
561 released into the environment. Since feed represents approximately 50% of the total production
562 costs in aquaculture (Tazim et al. 2021), using biofloc meal as a protein source a partial
563 replacement for expensive FM can significantly reduce feeding expenses as biofloc meal
564 contains high protein and lipid. Therefore, the use of biofloc meal not only offers economic
565 benefits but also significantly reduces environmental impact.

566 The most expensive component of the aquaculture industry is feed thus, it is crucial to enhance
567 nutrient absorption via nanoparticle application (Korni et al. 2023). Nanoparticle application
568 makes bioactive compounds more soluble, easily penetrates cell membranes and tissues, and
569 thus increases cellular uptake. The usage of metal nanoparticles such as copper, chromium,
570 magnesium, manganese, and selenium improves beneficial gastrointestinal microbes, which
571 secrete more digestive enzymes and lead to higher nutrient absorption in the intestine
572 (Seerengaraj et al. 2021). Likewise, nanoencapsulation techniques, such as chitosan-based
573 nanoencapsulation and nanoemulsion, enhance the stability, bioavailability, and targeted
574 delivery of any additives, ensuring such feed additives have a longer residence time in the
575 gastrointestinal tract and are delivered to the target organ (Jiménez-Fernández et al. 2014;
576 Misra et al. 2023). Due to the high absorption of nutrients through nanoparticle technology, the
577 required dose of bioactive compound is reduced while promoting fish digestion, which in turn
578 reduces fish feces that can be harmful to the surrounding environment (McClements et al.
579 2021). For scaling up production, high-pressure homogenization and sonication are currently
580 the most common and effective methods for producing nanoparticles compared to traditional
581 methods, which use plenty of reagents and chemicals (Seerengaraj et al. 2021). These
582 nanoparticles are considered affordable for large-scale production of nano elements at both
583 industrial and commercial levels. They meet the conditions for ensuring sustainable
584 aquaculture by reducing chemical release into the surroundings.

585 The application of PEF requires relatively low energy while enhancing the extractability of
586 valuable intracellular compounds, making it a ‘green’ processing technique. PEF is considered
587 both environmental friendly and cost-effective due to its reduced energy requirements per unit
588 of processed product (Athanasiadis et al. 2023). PEF has been used to recycle biomass waste
589 generated from agricultural industries, converting it into valuable bioresources such as feed
590 additives that enhance fish digestibility. For instance, the juice processing industry generates a

591 significant amount of fruit peels, which can constitute up to 60% of the total fruit weight.
592 Improper disposal of these by-products contributes to greenhouse gas emissions and
593 environmental pollution. However, these wastes contain valuable bioactive compounds like
594 polyphenols, which can aid in fish digestion by promoting gut microbiota. Beyond bioactive
595 compound extraction, PEF is also effective for protein recovery. By optimizing key processing
596 parameters, such as electric field intensity, energy output, temperature, extraction ratio, and
597 duration protein yields up to 60% can be achieved, potentially allowing partial replacement of
598 FM in fish feed (Kronbauer et al. 2023). Table 2 presents a comparative analysis of various
599 processing methods, highlighting their respective advantages, drawbacks, economic viability,
600 and environmental impact, as assessed by authors.

601 **6. Conclusion**

602 The findings emphasize the importance of incorporating sustainable ingredients and enhancing
603 their nutritional value to meet the dietary requirements of commercial fish species through
604 different processing methods. This review also considers the practicality, cost-effectiveness,
605 and sustainability of processing methods. The development of novel ingredients, their
606 processing methods, practicality, and sustainability) are crucial to lay a strong foundation for
607 the future success of freshwater fish farming, increasing output productivity and meeting
608 current demands for human consumption. Most previous studies have primarily focused on
609 using waste as novel feed ingredients. These include by-products from fisheries, poultry, and
610 agriculture, which are processed using various techniques to produce either protein sources or
611 functional feed additives aimed at enhancing fish digestion. Consequently, fermentation can
612 convert low-value or waste materials into high-value protein sources while providing pre-
613 digested nutrients, additional digestive enzymes and probiotics. Additionally, BFT offers the
614 advantage of reducing dependency on external feed inputs. Both fermentation and BFT have a
615 lower environmental footprint, use less energy due to their operation at ambient temperatures,

616 produce zero pollution, and are cost-effective. However, very few studies have been conducted
617 to explore the potential of using biofloc meal to partially replace FM, and this gap should be
618 addressed in the future by expanding research in this area. In contrast, thermal treatments
619 consume large amounts of energy and contribute to higher greenhouse gas emissions, while
620 extraction methods also have environmental impacts due to the use of solvents and energy for
621 processing. Nanoparticle and PEF technologies, while innovative, are still emerging and can
622 be energy-intensive or involve higher initial costs. In summary, processing methods are
623 continually evolving to ensure high product output in terms of product yield, whereby
624 improving nutrient retention and functionality. Bioconversion methods, are considered the
625 most sustainable for fish feed production due to their efficient use of resources, reduced energy
626 consumption, and lower environmental impact.

627 **Author contributions**

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631 validation. **Khairul Adha A. Rahim:** Validation. **Mohd Nor Azra:** Validation. **Hongyu Ma:**
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633

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639 **Data Availability Statement**

640 This manuscript has no associated data.

641 **Declaration of competing interest**

642 We declare that we have no financial and personal relationships with other people or
643 organizations that can inappropriately influence our work, and there is no professional or other
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1633 **Table 1** Novel ingredients using thermal heat method and their effect on growth and digestion of several commercially important freshwater fish species

Fish species	Thermal heat methods	Novel ingredient	Result	Reference
African catfish, <i>Clarias gariepinus</i>	Sun-drying (NA temperature) and conventional oven drying (NA temperature)	Maggot flies, <i>Musca domestica</i>	Fish fed oven-dried maggot (at 33.5%) improved SGR, FCR, PER and had higher protein content compared to fish fed sun-dried maggot	Fasakin et al. 2003
	Convection oven drying (150 - 180 °C, 15 - 30 min) and autoclaving (150 °C, 15 min)	Pea seed, <i>Pisum sativum</i>	Fish fed convection oven-dried pea seed (at 30%) had significantly increased SGR, FI, FER and PER compared to fish fed autoclaved pea seed	Davies and Gouveia 2008
Common carp, <i>Cyprinus carpio</i>	Sun drying (NA temperature) and conventional oven drying (50 °C)	Fish offal	Fish fed sun dried offal (at 24%) improved SGR than fish fed oven-dried offal	Al-Zahiri and Al-Shawi 2021
	Convection oven drying (230 °C, 45 min) and autoclaving (121 °C, 15 psi, 30 min)	Pea seed, <i>P. sativum</i>	Fish fed with convection oven-dried pea (at 40%) improved SGR, FI, FCR, PER, protein content and lipid compared to fish fed autoclaved pea	Davies and Gouveia 2010
Nile Tilapia, <i>O. niloticus</i>	Air dried	Garlic, <i>Allium sativum</i>	Fish fed air-dried garlic (concentration at 0.3%) improved FCR and PER compared to fish fed fresh garlic	Abdel-Hakim et al. 2010
	Conventional oven drying (105 °C, 24 h) and freeze drying (3 days)	Biofloc meal	Fish fed oven-dried biofloc (at 4%) showed no significant differences in SGR, FCR, FER, and PER compared to those fed freeze-dried biofloc.	Binalshikh-Abubkr and Hanafiah 2021
	Autoclave (121 °C, 15 psi, 10 - 20 min) and conventional oven drying (204°C, 10-20 min)	Lima bean, <i>Phaseolus lunatus</i>	Fish fed oven-dried lima bean (at 50%) had improved ADC of protein and lipid compared to fish fed autoclaved bean	Adeparusi and Jimoh 2002
	Microwave drying (800 W, 4 min)	Palm kernel, <i>Elaise guinensis</i>	Fish fed microwaved palm kernel (at 0.3%) had improved SGR, FCR, protein and lipid content compared to fish fed fresh palm kernel	Thongprajukaew et al. 2015
Rainbow trout, <i>Oncorhynchus mykiss</i>	Freeze-drying, spray-drying, conventional,	Lupin, <i>Lupin angustifolius</i>	No significant differences in SGR, FCR, and FI were observed between fish fed spray-dried lupin (at 30%) and those fed freeze-dried lupin	Glencross et al. 2007

and oven drying (150
°C, 12 h)

1634 NA = not available, ADC = apparent digestibility coefficient; FCR = feed conversion ration; FER = feed efficiency ratio; FI = feed intake; PER = protein efficiency
1635 ratio; SGR = specific growth rate.

1636 ¹The protein content mentioned in the results refers to the proximate composition of the fish body

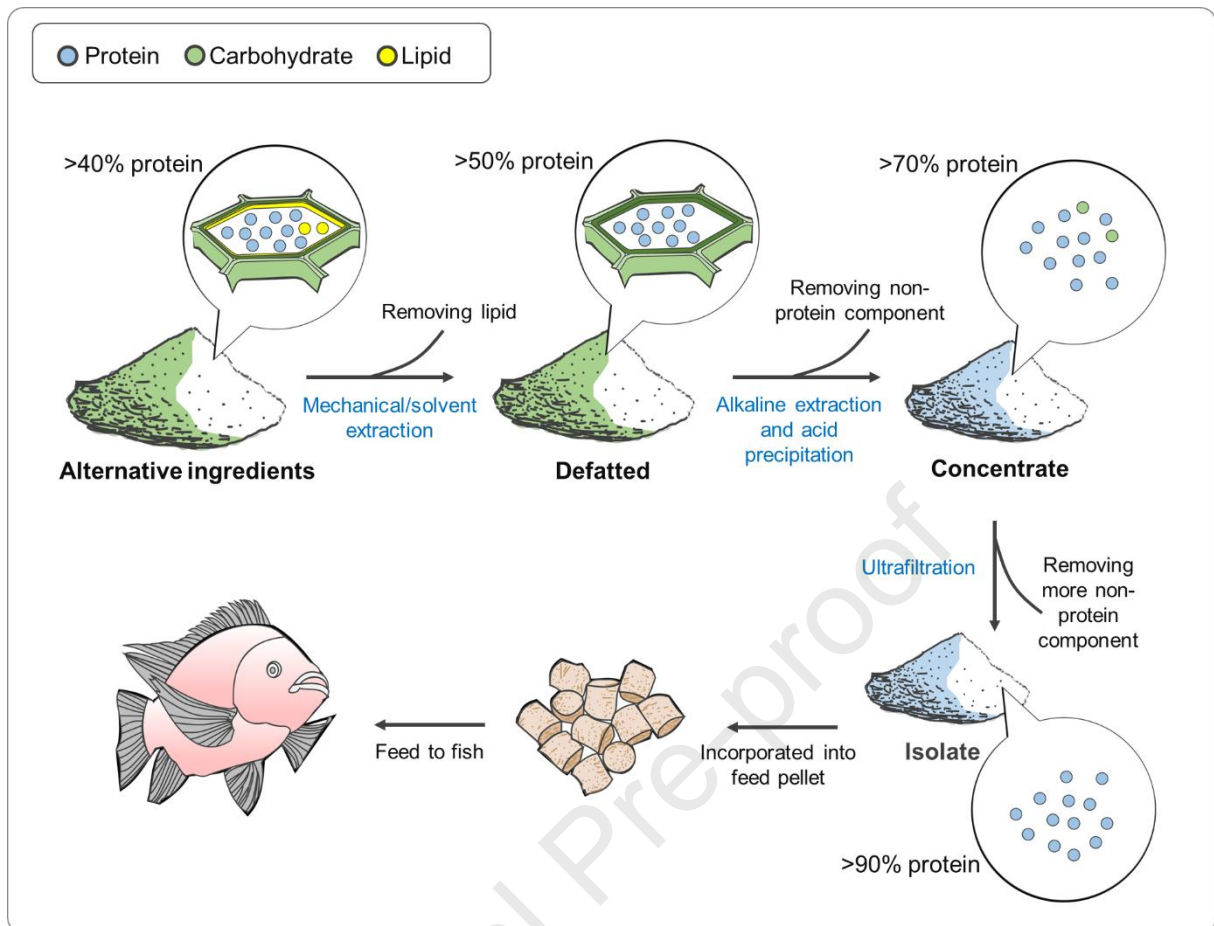
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1637 **Table 2** Comparison of processing methods in several aspects including advantages, drawbacks, economic and environmental impacts.

Processing method	Advantage	Drawback	Economy	Environmental impact
Thermal treatment	<ul style="list-style-type: none"> Remove moisture, increasing shelf life Breakdown ANFs 	<ul style="list-style-type: none"> Nutritional degradation at high temperatures Higher energy cost (oven drying and convection oven drying) 	<ul style="list-style-type: none"> Simple method, cost effective (sunlight drying, conventional oven drying and convection oven drying) 	<ul style="list-style-type: none"> High energy consumption (electricity) Greenhouse gas emission (conventional oven drying and convection oven drying) Using a microwave and vacuum drying may reduce energy consumption and gas emissions
Extraction	<ul style="list-style-type: none"> Extraction improves the concentration of bioactive compounds compared using raw herbs, thus improving effectiveness with only small quantities, enhancing bioavailability Using defatted meals and protein isolates offers higher protein content up to 90% Remove ANFs 	<ul style="list-style-type: none"> Involving a complicated processing method in refining protein content to yield protein isolate 	<ul style="list-style-type: none"> Defatted meal, protein concentrate and protein isolate can potentially replace a larger portion of FM, thus reducing feeding cost. 	<ul style="list-style-type: none"> Conventional extraction involving a high amount of solvent with high energy consumption (heating) Utilizing defatted extracts contributes to zero waste, especially in the vegetable cooking oil industry For producing protein isolate, advanced membrane technology offers mild operational conditions (low temperature and pressure), low solvent consumption, and high protein purification
Bioconversion	<ul style="list-style-type: none"> Nourishing protein content by conversion of novel ingredients into microbial protein Providing pre-digested nutrient Presence of bacteria promoting gut microbiota and the digestion of fish Degrade ANFs 	<ul style="list-style-type: none"> Time consuming during the fermentation process 	<ul style="list-style-type: none"> Simple method and cost effective 	<ul style="list-style-type: none"> In SSF, utilizing microbes to convert agricultural waste and invasive pest plants contributes to a zero-waste system In BFT, using microbial floc meal derived from surplus or effluents also contributes to a zero-waste system

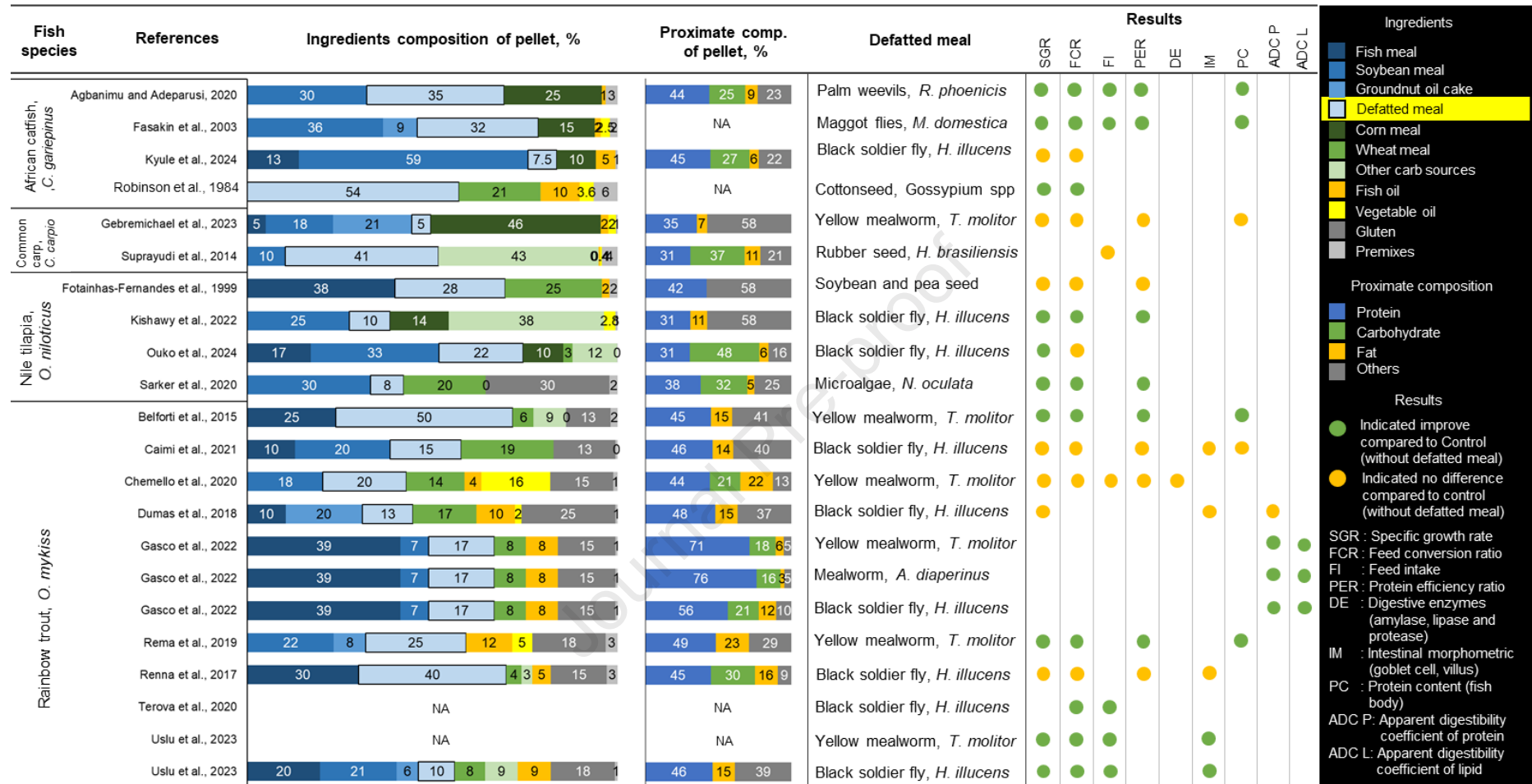
Nanoparticle	<ul style="list-style-type: none"> • Nanoparticles application enhances solubility and cellular uptake • Degrade ANFs • Nanoencapsulation and nanoemulsion enhance stability, bioavailability and targeted delivery 	<ul style="list-style-type: none"> • Nanoparticles are suitable only for enhancing the effectiveness of certain compounds but may not be suitable for providing an alternative to FM or soybean meal as a protein replacement. 	<ul style="list-style-type: none"> • Initial costs might be higher due to the need for specialized equipment and materials. 	<ul style="list-style-type: none"> • A very small amount of extracts are needed as the functional properties of the bioactive compound increase
PEF	<ul style="list-style-type: none"> • Improving the extraction rates of bioactive compounds due to the perforation of the cell membrane 	<ul style="list-style-type: none"> • The technique requires precise control of pulse parameters, including voltage, duration, and frequency. 	<ul style="list-style-type: none"> • Initial costs might be higher due to the need for specialized equipment • Higher extract yield may reduce cost 	<ul style="list-style-type: none"> • Intensifying the extract yield with reducing solvent use during the extraction process

1638 ANFs = antinutritional factors; BFT = biofloc technology; FM = fish meal; PEF = pulse electric field; SSF= solid state fermentation



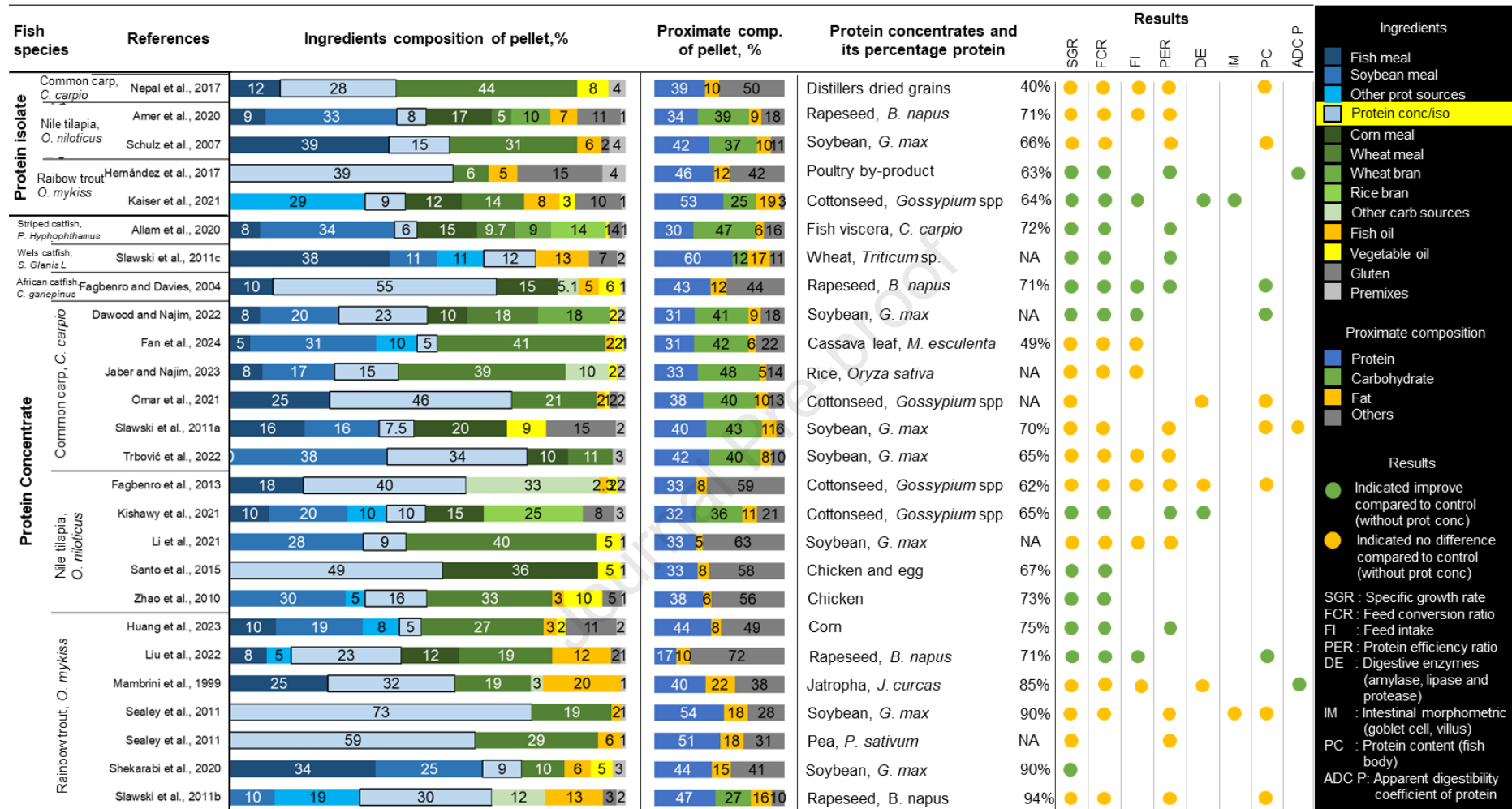
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1640 **Fig. 1** Lipid and protein extraction process in novel ingredients. Novel ingredients here use plant sources as
 1641 an example, but could be other ingredients.



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Fig. 2 Optimum concentration of defatted meal inserted in fish pellet of freshwater fish including catfish, carp, tilapia and trout. For pellet ingredients, other protein sources including poultry meal, pea protein, krill meal and squid meal, other carbohydrate including wheat bran, rice bran, distiller's dried grains with soluble (DDGS), cassava and sunflower meal, gluten either consisting wheat or corn, premixes consisting vitamin and mineral. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash and fiber. NA = not available.



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Fig. 3 Optimum concentration of protein concentrates and protein isolates inserted in fish pellet of freshwater fish including catfish, carp, tilapia and trout. For pellet ingredients, other protein sources including distiller's dried grains with soluble (DDGS), crustacean, black soldier fly, pea isolate and blood meal, other carbohydrate source including cassava, barley and potato, gluten either consisting of wheat or corn, premixes consisting of vitamins and minerals. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash and fiber. NA = not available.



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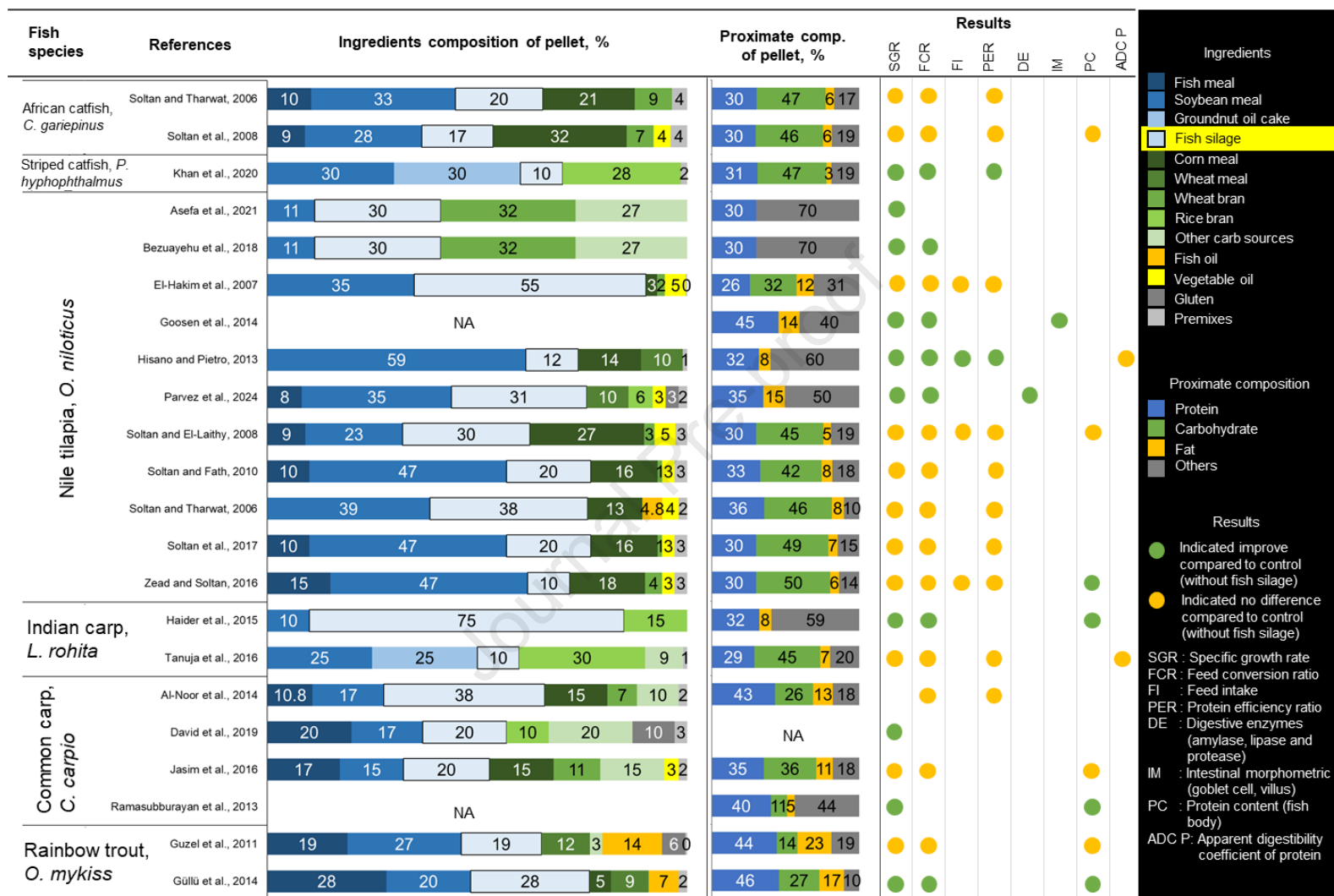
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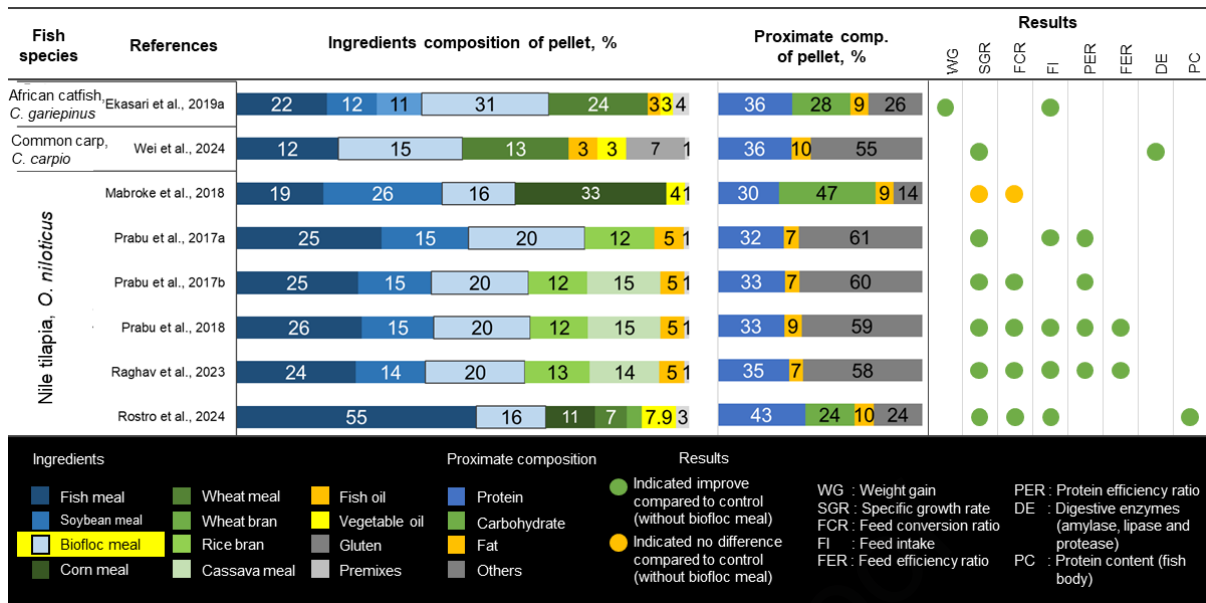
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Fig. 4 Optimum concentration of fermented substrate inserted in fish pellet of freshwater fish including catfish, carp, tilapia and trout. For pellet ingredients, other protein sources including poultry meal, mustard oil cake, groundnut oil cake, cottonseed meal, rapeseed meal and blood meal, other carbohydrate sources including cassava, tapioca, rice and distiller's dried grains with solubles (DDGS), gluten either consisting wheat or corn, premixes consisting of vitamins and minerals. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash, and fiber. NA = not available.



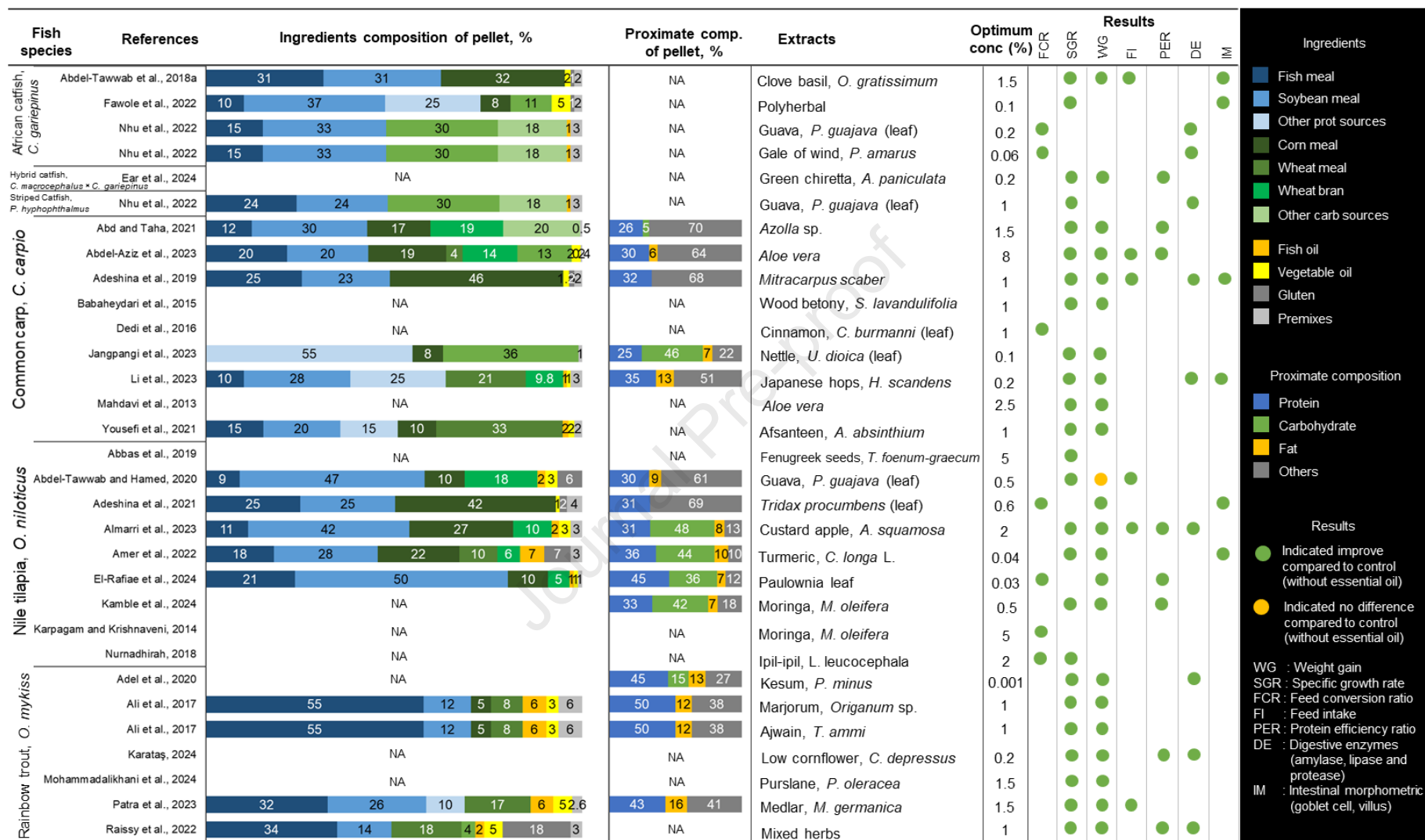
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Fig. 5 Optimum concentration of fish silage inserted in fish pellet of freshwater fish including catfish, carp, tilapia and trout. Fish silage commonly using fish by-products (more specifically fish head and viscera). For pellet ingredients, other carbohydrate sources including tapioca, barley, coconut husk and sunflower seed, gluten either consisting of wheat or corn, premixes consisting of vitamins and minerals. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash and fiber. NA = not available.



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Fig. 6 Optimum concentration of biofloc meal inserted in fish pellet of freshwater fish including catfish, carp and tilapia. For pellet ingredients, vegetable oil commonly used soybean oil, gluten either consisting of wheat or corn, premixes consisting vitamin and mineral. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash and fiber.



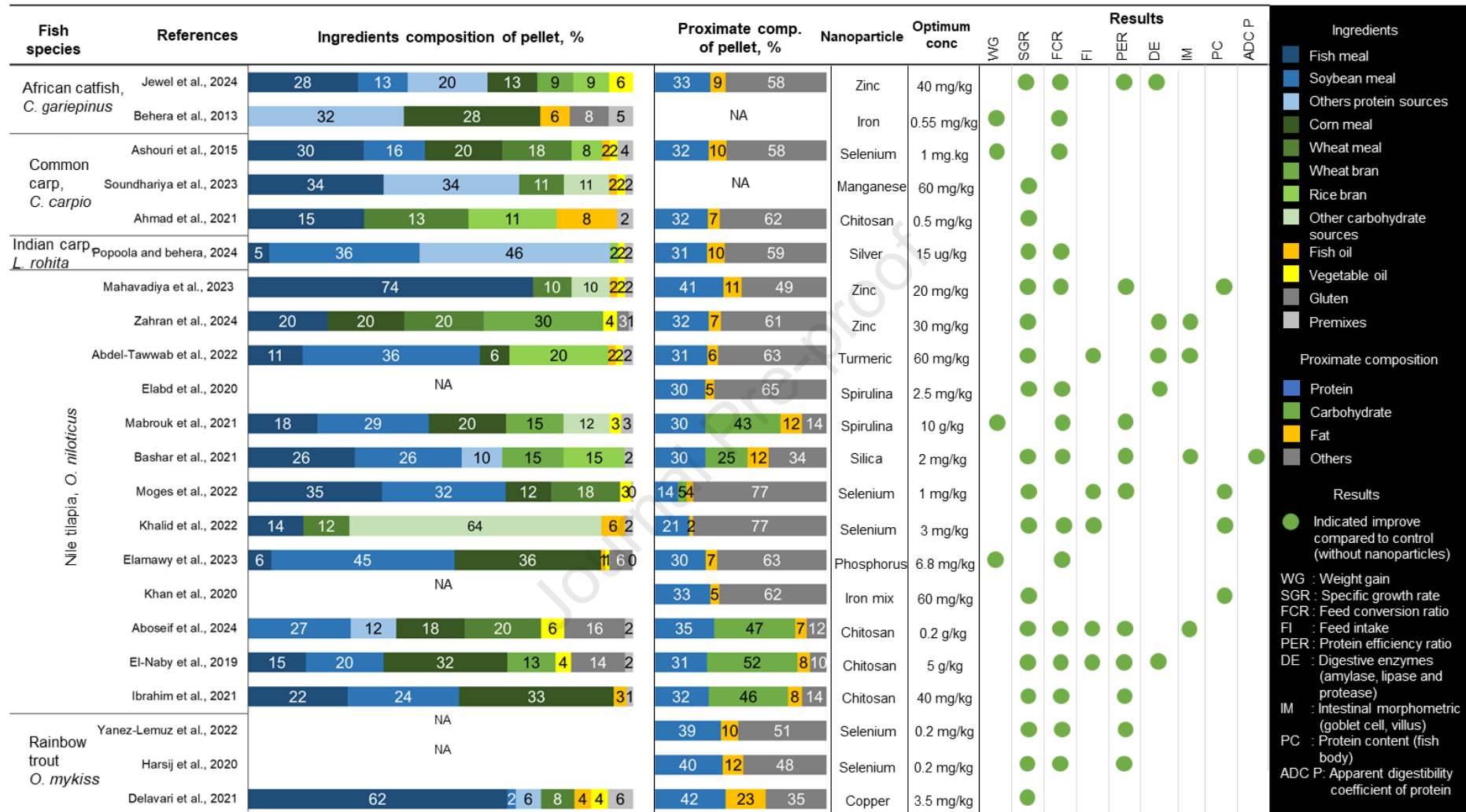
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Fig. 7 Optimum concentration of extracts inserted in fish pellet of freshwater fish including catfish, carp, tilapia and trout. For pellet ingredients, other of protein sources including poultry meal, mustard oil cake and rapeseed meal, other of carbohydrate sources including cassava and barley, gluten either consisting wheat or corn, premixes consisting of vitamins and minerals. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash and fiber. NA = not available.

Fish species	References	Ingredients composition of pellet, %	Proximate comp. of pellet, %	Essential oil	Optimum conc (%)	Results						
						WG	SGR	FCR	PER	DE	IM	PC
Bathing silver catfish, <i>R. quelen</i>	Carneiro et al., 2021	30 44 19 6 6 9 1	38 7 55	Oregano, <i>O. vulgare</i> L.	0.1	●						●
	Junior et al., 2022	30 35 15 12 3 3	46 54	Cinnamon, <i>C. cassia</i>	0.05	●						
Common carp, <i>C. carpio</i>	Abdel-Latif et al., 2020	9 40 21 19 3 3	29 52 7 12	Oregano, <i>O. vulgare</i> L.	2		●					
	Acar et al., 2021	23 37 19 12 5 4	34 8 58	Bitter orange, <i>C. aurantium</i>	0.25	●					●	
	Ghafariarsani et al., 2022	NA	NA	Thyme, <i>T. vulgaris</i>	2	●	●		●			
	Kesbiç et al., 2020	27 30 11 22 6 4	36 10 55	Monterey cypress, <i>C. macrocarpa</i>	0.5	●	●		●			
	Medeiros et al., 2023	NA	NA	Rosemary, <i>L. sidoides</i>	0.06	●	●			●		
	Yousefi et al., 2023	15 21 20 9 32 7 70.5	37 6 57	Pennyroyal, <i>M. pulegium</i>	0.025	●	●			●		
	Aguiar et al., 2023	57 27 8 0.5	32 68	Peppermint, <i>M. piperita</i>	0.05	●	●		●			
Nile tilapia, <i>O. niloticus</i>	Estaiano de Rezende et al., 2021	NA	NA	Thyme, red thyme and rosemary	0.12	●	●				●	
	Farag et al., 2022	15 20 34 11 3 2	32 52 5 11	Parsley, <i>P. crispum</i>	0.02				●	●		
	Ibrahim et al., 2022	27 29 28 9 3 2	35 43 5 17	Thyme, & black cumin, <i>N. sativa</i>	0.02	●	●					
	Mehrim et al., 2024	NA	30 48 22	Caraway, <i>C. carvi</i>	0.1			●		●		
	Shourbela et al., 2021	NA	NA	Oregano, <i>O. vulgare</i> L.	0.2	●	●					
	Souza et al., 2020	10 52 26 10 0.5	NA	Lemongrass, <i>C. flexuosus</i>	0.2	●	●					
Rainbow trout, <i>O. mykiss</i>	Gaballah et al., 2021	46 28 4 8 100.4	45 31 15 8	Date kernel, <i>P. dactylifera</i> L.	0.02	●	●					
	Hafsan et al., 2022	NA	48 19 33	Zarringiah, <i>D. kotschy</i>	0.02	●	●		●			
	Hasan et al., 2024	NA	NA	Oregano, <i>O. vulgare</i> L.	0.001	●		●	●			
	Larenas-Uría, 2016	NA	NA	Turmeric, <i>C. longa</i>	0.2	●						
	Naserabad et al., 2024	NA	NA	Purple rain, <i>A. jerdianum</i>	1		●	●	●			
	Şahin et al., 2017	NA	NA	Rosemary, <i>R. officinalis</i>	1		●					
	Sönmez et al., 2015	NA	NA	Thyme & sage, <i>Salvia</i> sp.	0.5		●	●				
	Yousefi et al., 2022	31 21 17 4 3 10 0.5	43 16 41	Thyme, <i>T. vulgaris</i>	2		●	●	●			
Zargar et al., 2019	NA	45 25 30	Thyme, <i>T. vulgaris</i>	0.05		●	●					

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Fig. 8 Optimum concentration of essential oil inserted in fish pellet of freshwater fish including catfish, carp, tilapia and trout. For pellet ingredients, gluten either consisting of wheat or corn, premixes consisting of vitamins and minerals. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash and fiber. NA = not available.



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Fig. 9 Optimum concentration of nanoparticles inserted in fish pellet of freshwater fish including catfish, carp, tilapia and trout. For pellet ingredients, other protein sources consisting of poultry meal, mustard oil cake, groundnut oil cake, and casein, other carbohydrates sources including canola meal and alfalfa meal, vegetable oil commonly used soybean oil, gluten either consisting of wheat or corn, premixes consisting of vitamins and minerals. For proximate composition of the pellet, others consisting of carbohydrate (only for labelling without green colour), ash and fiber. NA = not available.