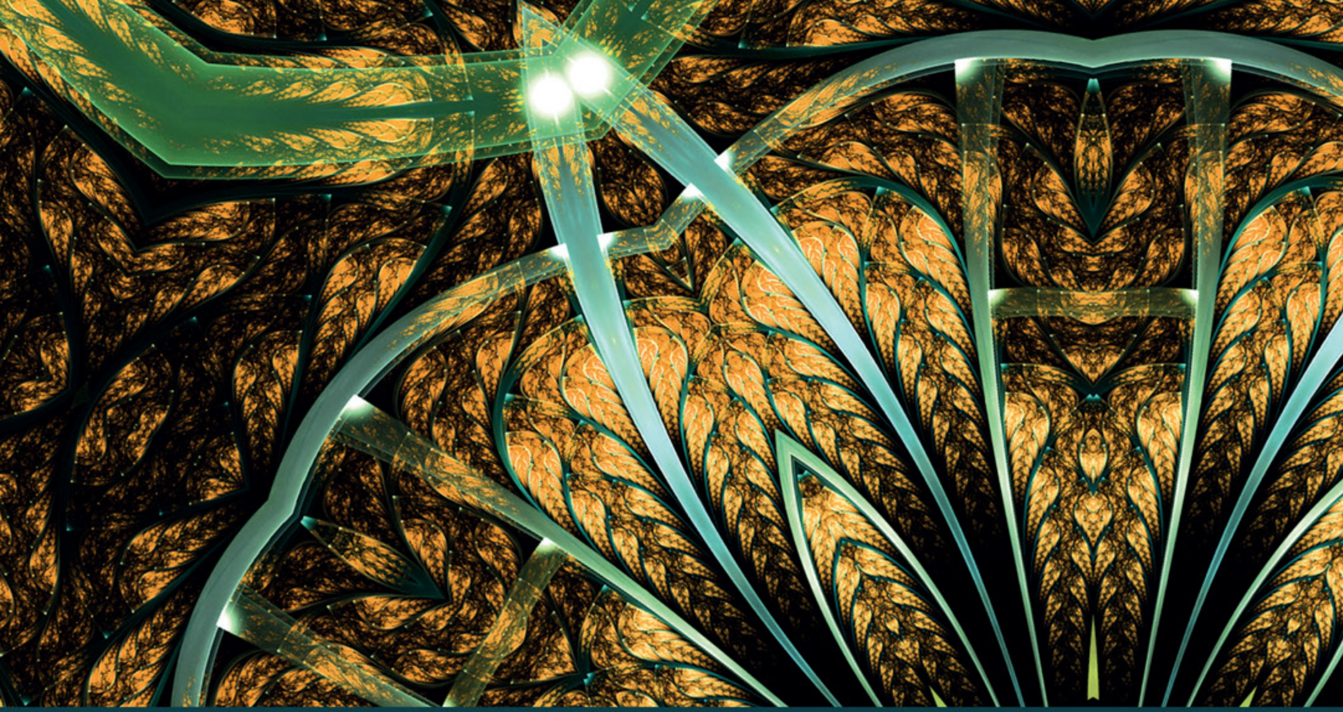

SUSTAINABLE AQUAFEEDS

TECHNOLOGICAL INNOVATION AND NOVEL INGREDIENTS

Edited by
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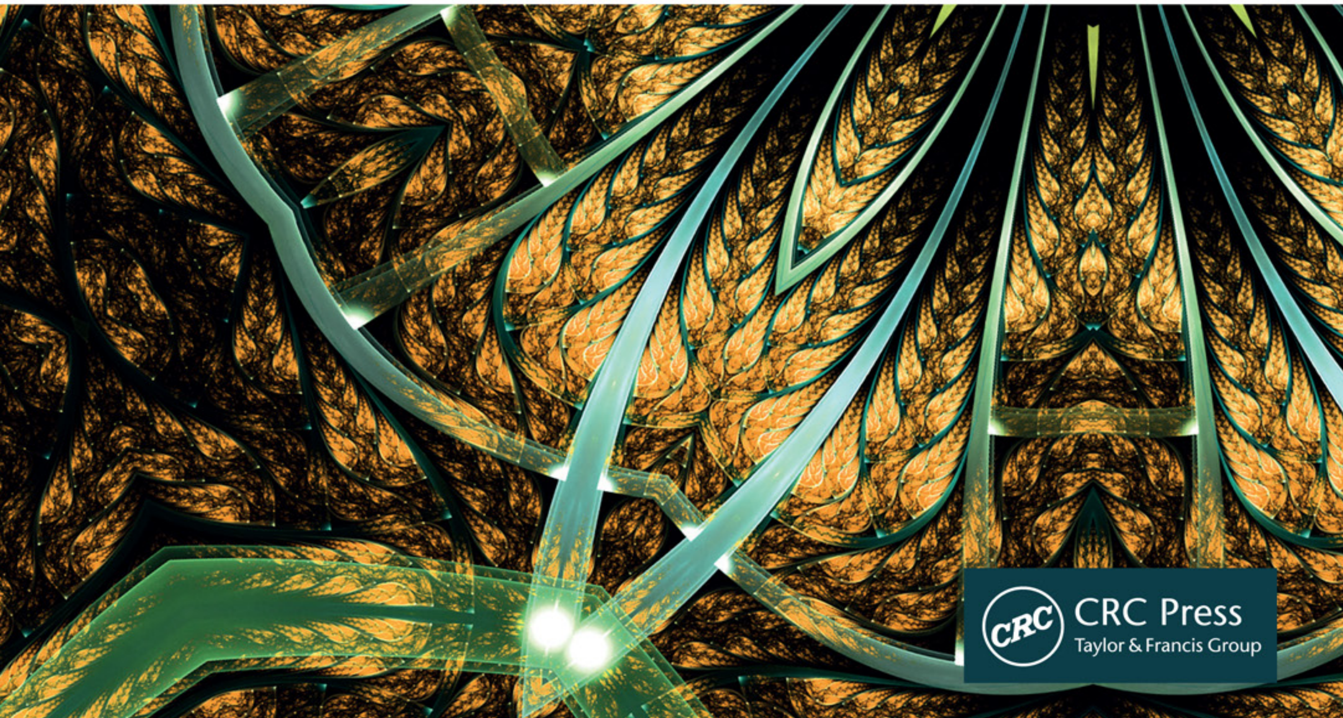

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Contents

8 Innovative Protein Sources in Aquafeeds

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INTRODUCTION

One of the most important goals in aquaculture nutrition research is to find a protein source with adequate nutritive properties to replace fish meal (FM) in aquafeed. In the past century, economic and environmental problems were foreseen for using this protein source, which would be caused by the deterioration and overexploitation of the marine environment, and by rising demand due to increasing aquaculture and its use to feed other livestock species (Sanchez Muros et al., 2014). The search for alternative sources recently resulted in a significant drop in fish in-fish out (FIFO) from 0.63 in 2000 to 0.22 in 2015 (http://www.iffo.net/fish-fish-out-fifo-ratios-conversion-wild-feed, consulted 7/11/2018), of which soya is the mostly widely used. Nevertheless, soya also involves environmental problems, such as the deforestation of areas with high biological value (Carvalho, 1999; Osava, 1999), considerable water use (Steinfeld et al., 2006), the utilization of pesticides and fertilizers (Carvalho, 1999), and transgenic varieties (Garcia and Altieri, 2005), which lead to significant environmental deterioration (Osava, 1999). Other sources have been checked and show different problems in relation to nutritive value, such as anti-nutritional factor presence, inadequate balance between essential amino acids/non-essential amino acids, amino acid bioavailability, which occurs in most vegetal origin sources, or inadequate fatty acid (FA) profile characteristics of animal sources. Price is another handicap, which should be competitive and include manufacturing and transporting, but affects the source as protein concentrates that must be submitted to transformation processes. Availability must also be considered, as many studies have been doing with local sources with good results but with low local production. Food safety is another factor to take into account. The protein source must be free of organic, inorganic, and biological toxins or pollutants, and this restriction limits the inclusion of animal meal in animal feed. Finally, alternative protein sources cannot compete with human food as with soya, which is important in animal-to-human feeding.

Then the innovative alternative protein concept for aquafeed must include not only the nutritional quality of the source but must also bear in mind availability, price, food safety, human competition, and sustainability.

This chapter studies the potential of some protein sources that have aroused much expectation because they are promising matches for the above-mentioned requirements to be considered an alternative protein source.

MARINE INVERTEBRATES

Marine invertebrates include different animal families that include crustacea, Mollusca, copepods, polychaetes, rotifers, and many other interesting species in aquaculture feeding.

Many of these species are suitable for aquaculture. In fact, lots of them are cultured for human or aquaculture feeding.

CRUSTACEA

The crustacea form part of the natural diet of wild fish, and some crustacea species are cultured as human food (i.e. shrimp) or fish feed (i.e. artemia). The culture technology is well-known for these two species and can be used as the basis for culturing other crustacea species.

Crustacea are rich in protein, with a lipid content high in EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (Chapelle, 1977) that depends on the species; for example, artemia possess a low DHA level (Vismara et al., 2003). Krill larvae are rich in EPA, DHA, and 16:0, while in the adult stages, FA 14:0, 16:0, and 18:1n-9 are dominant (Hagen et al., 2001). Nutritional condition, developmental mode (planktotrophy vs. lecithotrophy) and clade also affect proximate biochemical composition (Anger, 1998).

From the alternative protein sources point of view, this variability is positive because it allows the nutritive values of crustacea to be manipulated for aquaculture feed, clade choice, and to feed a rearing system to obtain adequate nutritional composition to replace FM.

Nowadays, artemia and copepods are cultivated to be used as feed for larvae fish, but there are other interesting species, such a krill.

Krill

Euphausia superba and *Euphausia pacifica* are two of the most abundant species on earth, with an estimated biomass of around 500 million tons. Gross postlarval production is estimated at 342–536 million tons/yr^{-1} (Atkinson et al., 2009). This is a vast quantity that allows it to be used without limitation. Nevertheless, krill is essential for supporting the primary production system with an estimated predator consumption of 128–470 Mt/yr^{-1}. These data reveal the need for the precautionary management of developing krill fisheries (Atkinson et al., 2009). The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) restricts harvesting to 1 percent of the total biomass (Burri and Nunes, 2016).

Nutritional value: Krill is an excellent source of vitamins, minerals, essential amino acids, n-3 polyunsaturated FA, natural carotenoid pigments, nucleotides, and organic acids (Lee and Meyers, 1997; Everson, 2000). Krill products are known to be excellent feed attractants in the fish diet.

Whole krill is a high protein food whose protein content is estimated into fall within the 60–65% range (Nicol, 2000) and has a higher amino acid content than trout or salmon (Tou et al., 2007). Lipid content ranges from 12% to 50% on a dry weight basis. Differences are attributed to sampling occurring during different seasons (Saether et al., 1986).

The FA profile is low (26.1%) in both saturated fatty acids (SFAs) and (24.2%) monounsaturated fatty acids (MUFAs), but is high (48.5%) in polyunsaturated fatty acids (PUFAs). Palmitic acid (16:0) is the predominant SFA, oleic acid (18:1n-9) is the predominant MUFA, and PUFAs consist mainly of n-3 FA. Kolakowska et al. (1994) reported that n-3 PUFAs accounted for approximately 19% of the total FA in Antarctic krill caught in winter, while EPA and DHA were particularly abundant. Lipid content also varies with Northern krill species, which are particularly rich in lipids (182 g/kg^{-1}) (Suontama et al., 2007).

The main phospholipid in krill meal is phosphatidylcholine (Tou et al., 2007), which delivers omega-3 FA and choline. Choline is an essential vitamin that must be added to aquafeed (Gong et al., 2000). Phospholipids are also involved in cholesterol uptake and distribution, which is an essential nutrient in shrimp feed (Gong et al., 2000).

A characteristic of Arthropoda is the presence of an exoskeleton. Chitin forms part of the exoskeleton and consists in b-1,4-linked N-acetylglucosamine. It requires the action of enzymes chitinase

(EC 3.2.1.14) and chitobiase (EC 3.2.1.30) for *in vivo* degradation. The presence of chinase in fish digestive secretion has been discussed. Most examined fish seem to possess some chitin-degrading enzymes, such as chitinases and/or chitobiases, in their digestive tract (Danulat and Kausch, 1984; Lindsay, 1987; Lindsay and Gooday, 1985; Danulat, 1986; Rehbein et al., 1986; Kono et al., 1987; Sabapathy and Teo, 1993; Moe and Place, 1999; Gutowska et al., 2004).

Some data also indicate that chitinase activity in those fish feeding on chitin-rich prey is greater than in other fish (Gutowka et al., 2004; Karasuda et al., 2004; Fines and Holt, 2010) and that feeding chitin-rich diets increases enzyme activity (Danulat, 1986). However, current data are rather incomplete and, to some extent, contradictory. The current discussion is about whether the origin of chitinase activity is endogenous or due to digestive microbiota.

In the Atlantic salmon (*Salmo salar* L.), feed in which FM is replaced with krill meal seems to have no influence on the apparent digestibility coefficients (ADCs) of dry matter and protein, while chitin is not utilized to a great extent (Olsen et al., 2006). Nevertheless, the level of inclusion affects digestibility, while lipid digestion does not reduce at 60% krill FM replacement (Suontama et al., 2007). At a high Antarctic krill inclusion level (>80% of diet proteins), lipid digestibility lowers (Olsen et al., 2006).

ADVANTAGES AND CONSTRAINTS

One beneficial effect of chitin on the fish immune system has been described. The fish fed a diet supplemented with chitin displayed high total haemocyte counts (THCs) and marked prophenoloxidase and superoxide dismutase activities (Zhu et al., 2010; Gopalakannan and Arul, 2006). The chintin immuno-stimulating system effect depends on the administration channel (Esteban et al., 2000), the inclusion level (Esteban et al., 2001), or the size of chitin particles (Cuesta et al., 2003). Krill also affects adherent distal intestine microbiota and enterocytes, shown to be replete with numerous irregular vacuoles (Ringø et al., 2006).

The main inconvenience of krill meal is fluoride content at around 1,000–6,000 mg/kg. The European Union has set a maximum fluoride level in feed at 150 mg/kg dry feed (Council Directive, 1999) for its potential accumulation in organs, especially bone. Studies have related krill meal inclusion and fluoride accumulation in many fish species, such as Atlantic salmon *Salmo salar* (Julshamn et al., 2004), Atlantic cod *Gadus morhua*, and Atlantic halibut *Hippoglossus hippoglossus* (Moren et al., 2007). Fluoride accumulation provokes reduced growth. Indeed 30% krill FM replacement with meal reduces the growth of rainbow trout due to fluoride accumulation in vertebral bones (Yoshitomi et al., 2006). The same authors (Yoshitomi et al., 2007) obtained good results with no negative effects on growth, survival, or nutritive indices with 100% low-fluoride krill meal replacement. However, the fluoride effect depends on both salinity (Julshamn et al., 2004) and species.

Studies in replacing FM with krill meal generally report good results for Russian sturgeon at 30% replacement (Gong et al., 2016) and for gilthead sea bream at 9%, which enhances gilthead sea bream growth and reduces both lipid accumulation and hepatocyte damage (Saleh et al., 2018), as well as 60% substitution for Atlantic halibut (*Hyppoglossus hyppoglossus*) (Suontama et al., 2007) and 40% substitution in juvenile spotted halibut (*Verasper variegatus*) (Yan et al., 2018). The total replacement of FM with low-fluoride krill in the diet is successful with no defects in growth performances for *Oncorhynchus mykiss* (Yoshitomi et al., 2007) and with normal krill Atlantic salmon (*Salmo salar*) (Olsen et al., 2006).

From the environmental point of view, the main constraint lies in krill being a very abundant, but finite, source that supports the primary production system. Hence sustainable harvesting has been established at 1 percent of total biomass (Burri and Nunes, 2016).

Crabs

Dean et al. (1992) studied the inclusion of not only blue crab for the fingerling channel (*Ictalurus punciatus)* diet, but also the 10% inclusion of blue crab without carapace. These authors reported a similar weight gain and feed efficiency to the fish feed in an FM diet. Nevertheless, under their production conditions, the caged channel catfish fed the control diet or the Atlantic herring diet displayed greater daily gain and net production than those fed the crab diet.

Copepods

The utilization of copepods in larvae and juvenile feeding started a long time ago, but has not been used as a protein source alternative.

Cultured copepods have good nutritive composition; 6.9–22.5% DW (dry weight) of lipids with EPA and DHA of 8.3–24.6%, and 13.9–42.3%, respectively. Protein amounted to 32.7–53.6% (determined as protein-bound amino acids) with a stable fraction of indispensable amino acids (37.3–43.2% of PAA). Abundant astaxanthin has been detected in copepods (413–1422 µg/g DW), as have vitamin C (38–1232 µg/g DW), vitamin E (23–209 µg/g DW) thiamine (3.5–46.0 µg/g DW) and riboflavin (23.2–35.7 µg/g DW) (van der Meeren et al., 2008). The nutritive benefits in larvae nutrition of copepods have been well demonstrated (van der Meeren et al., 2008).

Nevertheless, their use as an alternative protein source of FM has not yet been checked. Nowadays, calanus (gen. *Calanus*) is considered a potential source of n-3 highly unsaturated FA (HUFA) (Olsen et al., 2004) that can help to reduce dependence on marine fish oils. The increased use of calanus oil has probably led to increased interest in calanus nutritive values or in nutritional characteristics of oil production waste.

CONCLUSIONS

Currently, the more promising crustacean as a protein source is krill because it is an abundant source with a high protein percentage. Nevertheless, more studies are needed to establish the chitin effect on digestibility and the immune system. Fluoride accumulation is well-studied and the use of low-fluoride meal reports good results in the studied fish species.

INSECTS

Introduction

If the environmental and economic sustainability of aquaculture are to be ensured, the contribution of FM as fish feed must be lower. Interest in insects is currently growing as they are one of the most promising protein sources for feed production (Gasco et al., 2020).

Interest in insects as feed has grown mainly in developed countries over the last decade. In the market scenario, the insect business continues to grow, with companies being founded worldwide, especially those that perform the mass breeding of the black soldier fly (*Hermetia illucens*, HI), whose world production was 14,000 tons (wet weight) in 2016, and was 7,000 – 8,000 tons in 2014/15 (Sogari et al., 2019). From a scientific-academic point of view, so many projects and publications have been produced in these years that it is extremely complex to synthesize all the knowledge being produced. However, to look in-depth at some aspects related to insects, such as animal feed, readers can consult different reviews (Barroso et al., 2014; Gasco et al., 2020; Gómez et al., 2019; Govorushko et al., 2019; Kenis et al., 2014; Makkar et al., 2014; Sánchez-Muros et al., 2014, among others).

Reasons for Using Insects as Feed

Insect meals appear to be safe, cheap, and sustainable. Compared to other feed or food sources, insect breeding offers several environmental benefits. Indicators that provide insight into the sustainability of insect production can be included: (1) greenhouse gas emissions are much lower than other animal production as insects only consume lignin (termites and cockroaches) and produce methane (Govorushko, 2019); (2) much less water and space are needed to reproduce and raise insects than with livestock (Tabassum et al., 2016); (3) insects offer higher feed conversion efficiencies, are poikilotherms, and do not invest energy to maintain body temperature (Oonincx et al., 2010); (4) insects can transform abundant low-cost organic waste into protein-rich animal biomass for use in animal nutrition (Ramos-Elorduy, 1999). A socio-economic advantage lies in insect breeding requiring low capital and technology investment, which could be developed by the most disadvantaged population in society (Govorushko et al., 2019).

In line with nutritional value, overall, insects are generally rich in proteins (30–68% on a dry matter (DM) basis), although less protein-rich than FM (Barroso et al., 2014), but have well-balanced amino acid profiles (Finke, 2015; Gasco et al., 2018; Koutsos et al., 2019). Insects have no anti-nutritional factors as regards vegetable ingredients (Spranghers et al., 2017).

USE OF INSECT MEALS IN AQUACULTURE

As insects are included in the diet of many fish species in their natural environments, we consider that their use as feed in aquaculture can be a very interesting option. The European Commission (Annexe II of Regulation 2017/893 of 24th May 2017) has recently authorized the use of insect-processed animal proteins that derive from seven species of insects farmed for aquaculture purposes. These species include two flies (HI; *Musca domestica*), two mealworms (*Tenebrio molitor*, TM; *Alphitobius diaperinus*), and three crickets (*Acheta domesticus*; *Gryllodes sigillatus*; *Gryllus assimilis*).

This chapter does not intend to be an exhaustive review, as more and more studies address the partial substitution of FM for insect meals in fish. Among other experiments, insect meals have been successfully tested:

- With HI larvae meal in Atlantic salmon (*Salmo salar* L.) (Lock et al., 2016; Belghit et al., 2018), gilthead sea bream (*Sparus aurata*) (Fabrikov et al., 2020), Nile tilapia (*Oreochromis niloticus* L.) (Devic et al., 2018), Jian carp (*Cyprinus carpio* var. Jian) (Li et al., 2017; Zhou et al., 2018), rainbow trout (*Oncorhynchus mykiss* Walbaum) (Renna et al., 2017; Elia et al., 2018; Fabrikov et al., 2020), sea bass (*Dicentrarchus labrax* L.) (Abdel-Tawwab et al., 2020; Magalhães et al., 2017), tench (*Tinca tinca*) (Fabrikov et al., 2020) and tilapia (*Oreochromis* sp.) (Bondari and Sheppard, 1981)
- With TM larvae meal in African catfish (*Clarias gariepinus*) (Ng et al., 2001), gilthead sea bream (*Sparus aurata*) (Fabrikov et al., 2020), rainbow trout (*Oncorhynchus mykiss* Walbaum) (Chemello et al., 2020; Fabrikov et al., 2020), rockfish (*Sebastes schlegeli*) (Khosravi et al., 2018), sea trout (*Salmo trutta m. trutta*) (Hoffmann et al., 2020), tench (*Tinca tinca*) (Fabrikov et al., 2020), and tilapia (*Oreochromis niloticus)*(Sánchez-Muros et al., 2016)
- With cricket meal (*Gryllus bimaculatus*) in African catfish (*Clarias gariepinus*) (Taufek et al., 2018)

The most frequently used insects in aquaculture are TM and HI. As Kenis et al. (2014) point out, this could be due to the possibility of these species being mass-reared in small production units at both the community and industrial levels and can be fed waste or by-products.

Generally, when the degree of inclusion of insect meals was below 25% of diet (regardless of the degree of FM substitution), no negative effects on fish growth performance have been observed. However, when higher inclusion rates have been evaluated, the results were not as positive. In meagre (*Argyrosomus regius*), Guerreiro et al. (2020) found that nutritional indices linearly lowered with increasing dietary HI levels. High FM substitution levels (more than 50%) make production rates worse (Reyes et al., 2020) in sea bass and, even if inclusion is very high (100% FM substitution and up to 75% feed), rejection occurs in Siberian sturgeon (*Acipenser baerii* Brandt) (Caimi et al., 2020). With high FM replacement levels (60%) in Pacific white shrimp (*Litopenaeus vannamei*), not only is growth lower, but pathological changes appear in the hepatopancreas (Cao et al., 2012).

These results coincide with a recent meta-analysis by Hua (2020) on the effect of including insect meals on fish growth performance. This researcher concluded that moderate levels of insect meals can cause comparable growth performance to FM diets. However, when large proportions of insect meal are used, growth reduces, and the effect depends on the employed insect species. This author indicates that TM is better tolerated at high levels than HI, which usually leads to greater declining fish production rates.

The possible reasons for worse growth with higher insect meal inclusion levels are:

- Essential amino acid (EAA) deficiencies and EAA/non-essential amino acids (NEAA) imbalances are among the most important reasons behind these negative results (Cummins et al., 2017). Panini et al. (2017) found in shrimp that, if supplemented with methionine, 100% FM could be replaced with TM meal. Although insect meals are rich in EAA (lysine, methionine, leucine) (Caimi et al. 2020) compared to FM, insect meal is deficient in lysine and tryptophan and is limited in threonine and sulphur AA (Makkar et al., 2014; Sánchez-Muros et al., 2014). Barroso et al. (2014) compared the amino acids profile of different insect species with FM and found that the profile of amino acids was related to the taxonomic group. According to these authors, the order Diptera has the most similar amino acid profile to FM.
- Another limitation could lie in the cuticle (exoskeleton) of insects, as it contains chitin fibers, a polysaccharide of glucosamine and N-acetylglucosamine, both of which contain N atoms (Jonas-Levi and Martinez, 2017). As the N-factor for meat is 6.25, and based on the idea that proteins contain approximately 16% of nitrogen (Merrill and Watt, 1973), estimating the protein content (calculated as nitrogen × 6.25) could prove misleading in insect meal (Barker et al., 1998). Therefore, Janssen et al. (2017) considered that in order to estimate the protein content of whole larvae, a conversion factor of 4.76 should be used. However, Finke (2013) studied the nutrient content of several insect species and discovered that only a small amount of nitrogen was associated with chitin.
- It would appear that the crude protein digestibility of insects is affected by chitin content. Marono et al. (2015) found that the digestibility of crude protein from HI and TM correlated negatively with their fiber and chitin contents. Defatting insect meals also seems to affect digestibility. In an experiment with sea bass and feed with 20% FM substitution for several insect meals, Basto et al. (2020) revealed that the apparent digestibility coefficients of crude proteins were high in defatted TM (93%), intermediate in defatted HI (87%) and TM (89%), and moderate in HI (76%). Yet despite insects being defatted, in sturgeon Caimi et al. (2020) found that feeds with high inclusion HI rates showed lower apparent digestibility coefficients of crude protein compared to 100% FM feeds.
- We must also consider that the nutritional value of insects varies with age. Aniebo and Owen (2010) found that the fat content in *Musca domestica* larvae increased with age, and this was inversely related to protein content. It would seem that insects, with a complex metamorphosis, contain more fat and fiber (chitin) and less protein when they approach the pupal stage.
- Finally, the nutritional value of insect meals varies according to the processing followed during their manufacture. According to Hoffmann et al. (2020), the key to improving protein retention in the digestive system of insect meals may be the hydrolysis of their protein. Currently, data on the hydrolysis of insect material are limited, but these authors used diets with 20% TM in trout and found that hydrolyzed and unprocessed TM had similar effects. Another common treatment in insect processing is defatting, which can provide a meal that is easily used as an ingredient in aquaculture feed. With defatting, meals with higher percentages of crude protein that are more resistant to degradation can be obtained

> (Chemello et al., 2020). In addition, the high proportion of fat in larvae, which sometimes does not have a suitable FA profile for fish, can be reduced. Chemello et al. (2020) evaluated progressive FM substitution (up to 100%) with increasing concentrations of a partially defatted TM meal in the diet of rainbow trout. These authors observed no negative effect on fish growth. As a final example of the importance of processing methods on the nutritional value of insects, Kinyuru et al. (2010) devised a method of toasting and drying grasshopper samples, which affected their vitamin content and significantly decreased their protein digestibility.

It is also necessary to correctly evaluate whether fillet quality can be affected by insect meal inclusion. This quality is primarily affected by the fatty acid profile, which depends on the quality of fat in diet (Sanchez-Muros et al., 2014). Terrestrial insects contain mainly n-6 FA and small amounts of n-3 PUFA, which could represent a limit in animal nutrition (Barroso et al., 2014). As FM is increasingly replaced with insect meal in feed, there is generally a proportional reduction in n-3 PUFA in the n-3:n-6 ratio and in the unsaturation rate in fish fillets. This has been observed, among others, in blackspot sea bream (*Pagellus bogaraveo*) (Iaconisi et al., 2017), meagre (*Argyrosomus regius*) (Guerreiro et al., 2020), Pacific white shrimp (Panini et al., 2017), and rainbow trout (Belforti et al., 2015; Stadtlander et al., 2017). In some experiments, 100% FM has been replaced with insect meal with no significant differences in the FA profile, e.g. in Atlantic salmon (Bruni et al. 2020). However, this could be due to the additional fish oil present in insect-containing diets. The FA profile also significantly affects lipid digestibility (Hua and Bureau, 2009). To avoid these disadvantages, larvae can be defatted or their FA profile can be modified by the substrates used for their feeding. Different experiments (Barroso et al., 2017, 2019; Liland et al., 2017; St-Hilaire et al., 2007) have increased omega-3 fatty acid in larvae by including components rich in these FA (fishery waste or seaweed) in their diet (Figure 8.1)

Regarding fillet quality, Bruni et al. (2020) found that complete dietary FM substitution with HI meal did not impair the physicochemical quality of Atlantic salmon fillets. By taking into account sensory aspects, Bondari and Sheppard (1981) ran an experiment with channel catfish (*Ictulurus punctatus*) and blue tilapia (*Tilapiu urea*) fed a diet with HI (50% inclusion). They found that it did not affect their taste.

FIGURE 8.1 *Hermetia illucens* larvae rearing in fish discards (Courtesy of F Barroso, University of Almería, Spain).

In a sensory analysis in rainbow trout using two HI types (normal and fish offal-enriched) with an FM substitution degree up to 50%, an untrained consumer panel found no significant differences between the trout fed different diets (Sealey et al., 2011).

Critical Points to Be Solved in the Future

- *Legislation*: Some legislative barriers must be overcome. For example, the EU currently considers that insects, as food in aquaculture, can be fed only with the animal raw material listed in Regulation (EU) 2017/1017. Therefore, insects cannot be fed manure, waste, former foodstuffs containing meat, or waste fish or food from restaurants or catering establishments (Gasco et al., 2020). We believe that this legislation should become more flexible in forthcoming years, as it limits the potential of insects as sustainable food. One of the biggest advantages of insects is precisely that they can be raised with waste from catering establishments or the food industry as they do not compete with humans for this food resource. Furthermore, by breeding insect by-products, their production becomes more economical, and, as Van Huis (2015) points out, they can alleviate waste disposal. However, the microbiological content of insect meals must be controlled both during processing and storage to guarantee their hygienic and sanitary quality, as with any other raw material.
- *Market price*: although marked insect production growth is expected for the food and feed market in forthcoming years, it still remains on a small industrial scale. It is difficult to find the market price because demand is still limited, and companies adapt their price according to the size of orders (Gasco et al., 2020). Therefore, mass insect breeding is not yet sufficiently developed to obtain a competitive price in relation to other protein sources.
- *Heterogeneity*: Nutrient content varies widely between not only insect species, but even within the same insect species. Nutrient characteristics depend on life stage, environment, diet, processing or slaughter methods, etc. This limits their use in the feed industry, as they need to include an availability of raw material of homogeneous and stable quality.
- *Cultural acceptance*: Despite the production advantages of insect meal, its direct consumption clashes with cultural barriers in more developed countries. However, these barriers can be overcome with correct information on the sustainability of their production and with nutritional advantages over other foods. Several studies have shown that consumers have neophobia when faced with direct insect consumption (food) (Sogari et al., 2019), but will readily accept eating insect-fed animals (feed) (Verbeke et al., 2015; Mancuso et al., 2016; Ferrer Llagostera et al., 2019). Specifically, Mancuso et al. (2016) studied consumer acceptance of farmed fish fed insect meals and obtained very positive consumer attitudes because almost 90% were prepared to eat fish that were fed insects.

CONCLUSIONS

In summary, we believe there is still plenty of work to be done.

Although insect meals can hardly replace FM satisfactorily, if we wish to promote the commercialization of insects as a valued raw material in aquaculture, we must assess not only their proximate composition, but also aspects like digestibility or anti-nutritional components, the effect of insect breeding methods, and feed production technologies (method of drying, processing methods, etc.) of meals on nutritional value. We must also determine the optimal levels of insects (or combinations of different insect types) to adapt to these needs according to the nutritional needs of each fish species.

YEAST

INTRODUCTION

Yeasts are potential sustainable ingredients in aquafeeds given their ability to convert low-value lignocellulosic biomass into high-value feed with limited dependence on land, water, and climate conditions (Øverland et al., 2013).

Yeast has been utilized as a nutritional supplement in animal feed for more than 70 years. In aquaculture, it has been well-studied as probiotic systems, immunostimulants, and live feed (Manoppo et al., 2011; Murthy et al., 2009; Gatesoupe, 2007; Gopalakannan and Arul, 2010; Jones et al., 2020). The use of yeast as an FM substitute in aquaculture systems has recently drawn considerable attention (Øverland et al., 2017; Montoya-Camacho et al., 2019; Guo et al., 2019). Yeast acts as a health promoter in fish given the presence of peptides, free nucleotides, and mannan oligosaccharide (Rawling et al., 2019).

NUTRITIVE VALUES

Yeast proximal composition varies depending on the used species. Table 8.1 shows the different crude protein (CP), crude fat (CF), and fiber (F) percentages in several yeast species employed in aquaculture feeds.

Although CP varies from one specie to another, it also varies within the same species. CP varies in yeast species in accordance with the substrate used to cultivate the yeast (Ritala et al., 2017). As yeasts are single-cell organisms, their CP content is strongly influenced by the nitrogen present in the genome, which represents 6–12% of total nitrogen (Halasz and Lasztity, 1991, 2017). The EAA profile of the main yeasts used in aquaculture (*Saccharomyces. cerevisiae*, *Candida utilis*, and *Kluyveromyces marxianus*) show similar values to FM, although methionine levels are lower than those in FM (Øverland and Skrede, 2017). Lipids represent a low percentage of the proximal composition of yeasts, but genetically modified *Yarrowia lipolytica* produces up to 20.3% of CF with 30% EPA of total lipids. Brown et al. (1996) analyzed different marine yeast FA, of which the main FA are palmitic acid (16:0), oleic acid (18:1n9), and linoleic acid (18:2n6). Yeast is also a source of minerals like phosphorus, calcium, sodium, zinc, iron, copper, manganese, and selenium (Chanda and Chakrabarti, 1996; Cheng et al., 2004).

USE OF YEAST MEALS IN AQUACULTURE

Hatlen et al. (2012) carried out an experiment with genetically modified *Y. lipolytica* to be included in the diet of *S. salar.* Although growth performance was not affected by yeast inclusion, digestibility

TABLE 8.1
Proximal Composition of Principal Yeast Used as Feed in Aquaculture

Specie	CP (%)	CF (%)	F (%)	Reference
Y. lipolytica	29.8	20.3	n.a.	Hatlen et al. (2012)
S. cerevisiae	32.0	4.0	10	Zerai et al. (2008)
S. cerevisiae	44.2	2.9	0.3	Pongpet et al. (2016), 2016)
C. Utilis	56.0	0.3	3.7	Øverland et al. (2013)
C. Utilis	39.0	2.1	n.a.	Hansen et al. (2018)
C. Utilis	41.0	n.a.	n.a.	Gamboa-Delgado et al. (2015, 2016)
K. marxianus	51.0	0.8	0.8	Øverland et al. (2013)
K. marxianus	42.0	1.3	n.a.	Ribeiro et al. (2014)
R. mucilaginosa	17.0	–	–	Chen et al. (2019)

decreased when yeast inclusion was higher. The experiment (Zerai et al., 2008) carried out with *S. cerevisiae* at different substitution levels (25%, 50%, 75%, 100%) in *Oreochromis niloticus* showed substitution-dependent growth performance, but it was possible to replace 50% FM without compromising growth. The inclusion of *K. marxianus* in the *O. niloticus* (Ribeiro et al., 2014) diet during different seasons resulted in reduced growth performance mainly in spring. However, lipid content was not altered by yeast diet, and CP was higher in the muscles from the fish fed yeast diets. *S. cerevisiae* inclusion in *Dicentrarchus labrax* led to increased growth performance for 30% yeast substitution, and CP rose in fish that were fed yeast diets (Oliva-Teles and Gonçalves, 2001). Øverland et al. (2013) substituted FM for 40% *S. cerevisiae* in *S. salar*, which negatively affected the fish growth parameters. Similar results have been reported for *Oncorhynchus mykiss* (Cheng et al., 2004), Atlantic salmon (Øverland et al., 2013), and Arctic char (*Salvelinus alpinus*) (Langeland et al., 2013).

This lower protein digestibility is due to tough cell walls and their negative effect (Øverland et al., 2013). In Arctic char, greater energy and amino acid digestibility were found for disrupted *S. cerevisiae* than for intact cells, with no significant differences in Eurasian perch (*Perca fluviatilis*) (Langeland et al., 2016).

Moreover, the digestibility of *S. cerevisiae* depends on fish species, which increased in gilthead sea bream depending on FM substitution levels (10% and 20% substitutions) (Salnur et al., 2009). In pacu (*Piaractus mesopotamicus*), lipid digestibility increased, while protein digestibility remained unaffected (Ozório et al., 2010). These differences in digestion efficiency among fish species can be related to different digestive enzyme activity levels (Langeland et al., 2013).

Digestibility can also be affected by yeast species. Substituting FM for *C. Utilis*, *K. marxianus*, or *S. cerevisiae* in *Salmo salar* feed led to a similar CP digestibility to FM for *C. utilis* and *K. Marxianus*, while low CP digestibility was observed for *S. cerevisiae* (Øverland et al., 2013).

Yeast is often used in aquaculture as a growth promoter and immunostimulant in functional feeds due to various bioactive components. Positive health effects are well-documented in several fish species, such as salmonids (Tukmechi et al., 2014; Refstie et al., 2010), *Ictalurus punctatus* (Welker et al., 2012), *Lateolabrax japonicas*, (Yu et al., 2014), hybrid *Morone saxatilis* (Li and Gatlin, 2003; Li and Gatlin, 2004), *Sparua aurata* (Rodríguez et al., 2003), hybrid tilapia (He et al., 2011), Cyprinus carpio (Gopalakannan and Arul, 2006, 2010), and *Labeo rohita* (Tewary and Patra, 2011). Yeast inclusion increases the total gut weight of fish, possibly due to the high content of nucleic acid converted into nucleotides and acting as a growth promoter of intestinal epithelial cells. Morphological studies on the distal intestine have indicated no adverse effect for *C. utilis* and even report diminishing the possible adverse effects of high inclusion levels of vegetal protein (Grammes et al., 2013; Hansen et al., 2018).

The manipulation and preparation of yeast cells are most important because these processes influence CP digestibility in fish. The tough cell walls of yeasts can inhibit access to the nutrients inside cells. This can be seen in the different digestibility observed during fish trials with several fish species (Nazzaro et al., 2021). The cell wall represents 26–32% of the cell's total dry weight. Nesseri et al. (2011) suggested that mechanical (high-pressure homogenization, wet milling, sonication) and enzymatic methods can be used to disrupt the cell wall. Enzymatic disruption has several advantages, given selectivity only in the cell wall, but this process is slow compared to mechanical methods. Asenjo and Dunnill (1981) conducted a study to combine the enzymatic and mechanical cell wall disruption.

Yeast extract is obtained by removing the cell wall material with higher protein contents than in whole or hydrolyzed yeast. However, the cell wall fraction is rich in bioactive and immunostimulant compounds like -glucan and mannan oligosaccharides, which are a very interesting feed ingredients for combining properties as a source of nutrients and bioactive components (Overland and Skrede, 2017) that can be lost in yeast extract.

CONCLUSIONS

As stated before, yeasts have been used in aquaculture for different purposes. They can be employed in dietary compounds of diet as health promoters to improve gut microbiota, to act as an immunosuppressor, and to promote growth. As aquaculture rapidly grows, the industry cannot meet the protein demand for aquaculture feeds. Hence yeasts have been tested in fish trials by partially replacing FM with similar growth rates to FM diets. Yeast can be an alternative protein source for aquaculture, but further studies are necessary to improve the production and processes to obtain yeasts with better chemical compositions and to improve digestibility.

BIOFLOCS

INTRODUCTION

Biofloc technology (BFT) has emerged as an alternative aquaculture system based on the limitation of water exchange and the culture of microorganisms for feeding purposes, whose origin is established in France in the 1970s, with several studies performed on various penaeid species (Dauda, 2020). However, it was not until the beginning of the 21st century when this technology became very important thanks to the numerous advantages that it provides from both the economic and environmental points of view (de Schryver et al., 2008; Khanjani and Sharifinia, 2020; Robles-Porchas et al., 2020). So initial studies confirmed the beneficial influence of BFT on water quality and growth performance of fish and crustacea, such as Nile tilapia (*Oreochromis niloticus*) and Pacific white shrimp (*Litopenaeus vannamei*), respectively (Azim and Little, 2008; Haveman et al., 2009).

BFT is a zero-water exchange system. It improves the feed conversion ratio of reared species due to the production of microbial protein, which becomes an important food source that, in the long term, can efficiently decrease dietary protein content, even in intensive and super-intensive cultures (Walker et al., 2020) (Figure 8.2). Moreover, BFT can be a source of compounds with different bioactivities to help to improve the health status of cultured species by enhancing their immune system and antioxidant status (Haridas et al., 2017; Aguilera-Rivera et al., 2019). BFT has been successfully applied to the culture of many crustacea, but is more limited in fish species (Robles-Porchas et al., 2020). Notwithstanding, BFT is not free of disadvantages, such as the slightly higher initial cost for modernizing aquaculture facilities, limited species available to be cultivated in this system, or the

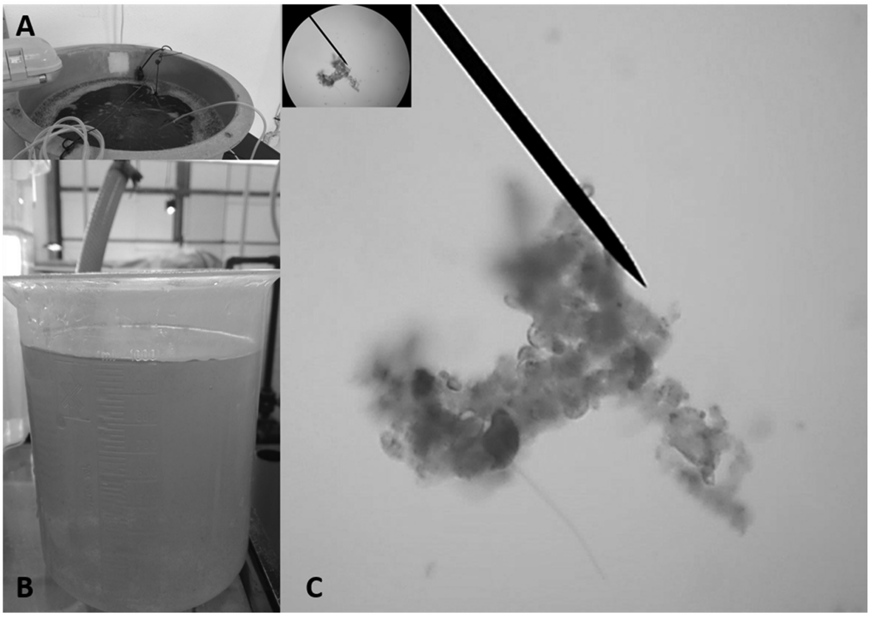

FIGURE 8.2 Experimental tank with BFT system (A), beaker with water sample containing bioflocs in suspension (B) and morphology of bioflocs under the microscope (C) (Courtesy of iMare Natural S.L. (2020)).

high dependence of constant aeration. However, its outstanding advantages, such as being mid and long-term cost-effective and its enviro-friendly technology, make BFT a firm option for a sustainable aquaculture future (Crab et al., 2012; Khanjani and Sharifinia, 2020).

BIOFLOC DEVELOPMENT AND COMPOSITION

BFT is based on a culture system of microorganisms that recycle waste nitrogen to proliferate (Dauda, 2020). The massive accumulation of these microorganisms, combined with other components, makes up amorphous structures of variable sizes called bioflocs, which range from several micrometres to millimetres (de Schryver et al., 2008). The start of biofloc formation takes place in the first week of culture, whereas its maturation, denoted by a change in culture color from green to brown and constant composition, can last between weeks and months depending on the factors conditioning this process (Ahmad et al., 2017; Martínez-Córdova et al., 2018; Robles-Porchas et al., 2020).

Bioflocs are heterogeneous aggregates that are composed of 30–40% inorganic material and 60–70% organic material (Chu and Lee, 2004). Among the organic components, chemoautotrophic and heterotrophic bacteria and cyanobacteria stand out for their abundance, mainly including phyla Proteobacteria, Bacteroidetes, and Actinobacteria, among others (Robles-Porchas et al., 2020; Zhao et al., 2012). Additionally, other microorganisms adhere to the organic matrix to form these aggregates, including viruses, microalgae, yeasts, and fungi, as well as invertebrates like rotifers, protozoa, amoebas, copepods, cladocera, ostracods, annelids, and nematodes (Ahmad et al., 2017; Ju et al., 2008; Martínez-Córdova et al., 2018). Other components that make up bioflocs include feces remains, uneaten food, dead cells, organic polymers, colloids, salts, and trace minerals (Azim and Little, 2008).

However, the final composition of the organisms present in bioflocs is conditioned by several parameters, among which the C:N ratio and type of carbon source are highlighted as the most important factors that can modify the relative content and type of different microorganisms (Liu et al., 2018a; Kim et al., 2021; Minabi et al., 2020; Tinh et al., 2021). Furthermore, as reviewed extensively by Dauda (2020), other aspects, like salinity levels, temperature, pH, dissolved oxygen, mixing intensity, light or cultured species are also key in biofloc composition. Controlling all the factors that determine the final biofloc composition, mainly regarding its microbial community, is indispensable as this fact conditions its nutritional value (Ahmad et al., 2017). Although biochemical composition is fixed, other aspects, like biofloc particle size or digestibility, define the nutritional value for a given species (Khanjani and Sharifinia, 2020).

NUTRITIVE VALUE

In nutritional terms, widely variable protein levels ranging from 7.7% to 50%, and lipid levels between less than 0.1 and 9.9 (both on a DM basis), characterize bioflocs (Table 8.2). Regarding essential nutrients, different studies have indicated that bioflocs can also contain significant quantities of omega-3 and omega-6 FA, and several EAA. For essential FA, docosahexaenoic acid (DHA), EPA, linolenic acid (ALA), arachidonic acid (ARA) and linoleic acid (LA) have been found (Castro et al., 2021). The most representative group of EAA in bioflocs comprise arginine, isoleucine, leucine, phenylalanine, threonine, and valine, although other amino acids, like histidine, tryptophan, and methionine have been observed, although in limited amounts (Castro et al., 2021). In the same way, bioflocs can also include non-essential amino acids (i.e. aspartate, glutamate, serine, glycine, alanine, proline), minerals (i.e. calcium, phosphorous, sodium, potassium, magnesium, zinc, iron), vitamins (i.e. thiamine B1, riboflavin B2, niacin B3, vitamin B12, vitamin E) and other bioactive compounds like phytosterols, carotenoids, or chlorophylls (Castro et al., 2021; Wei et al., 2016).

Given the nutritional value of bioflocs, several studies have demonstrated that they can be used as an effective food source which, among others, enhances growth performance and feed utilization

TABLE 8.2
Nutritional Composition of Bioflocs

Authors	Protein Range (%)	Lipid Range (%)	Ash (%)
Azim and Little (2008)	50.0	2.5	7.0
Ju et al. (2008)	30.5–36.9	1.9–5.9	14.8–38.9
Kuhn et al. (2010)	38.8–40.5	< 0.1–1.1	11.8–24.7
Xu and Pan (2014a)	21.3–32.1	1.6–2.8	43.4–61.4
Dantas et al. (2016)	24.7	0.4	36.6
Wei et al. (2016)	31.5–41.2	4.2–8.5	12.4–15.2
Da Silva et al. (2018)	14.2–21.3		53.2–60.1
Ekasari et al. (2019)	18.1–33.8	2.4–9.9	3.4–9.4
Mabroke et al. (2019)	24.5	5.0	16.0
Castro et al. (2021)	7.7–18.1	0.9–2.1	53.5–62.4

of fish and crustacea, and this improvement is achieved in different ways. Hence bioflocs can be simultaneously utilized with artificial diet in aquaculture production by being a natural food that is directly ingested from the culture water that supplements diets (Tierney and Ray, 2018). In the same way, biofloc intake can save dietary protein as protein content can be reduced in diets without negatively affecting growth (Ebrahimi et al., 2020), while the use of alternative protein sources, like plant or insect meals, to replace FM improves when BFT is applied (Jatobá et al., 2017; Tubin et al., 2020). Finally, bioflocs can also be included directly as a dietary ingredient to replace FM in both fish and crustacean species (Ekasari et al., 2019; Khatoon et al., 2016).

USE OF BIOFLOCS IN AQUACULTURE

Bioflocs as a Natural Complementary Feeding Source

In situ bioflocs are an important nutrient source as they are available 24 h/day as a fresh and constantly renewed food. Tacon et al. (2002) ran a feeding trial in Pacific white shrimp to investigate the effects of indoor running water culture system *vs.* an outdoor zero-water exchange culture system. They observed that this system achieved the best growth performance and feed utilization. Final body weight (FBW) was nearly 3-fold heavier than for those animals cultivated in the indoor running water system. These results were attributed to the possibility of obtaining additional nutrients from the organisms present in outdoor "green water". Following the line of these initial studies performed in Pacific white shrimp, other authors have observed the same positive effects of BFT on the culture of this species. Khanjani et al. (2015) obtained feed conversion ratio (FCR) values that ranged between 1.52 and 1.29 in a clear water system (CW) *vs.* BFT, respectively, and better animal growth performance. Subsequent studies that focused on the effect of carbon sources or probiotic supplementation on Pacific white shrimp performance have demonstrated that, once again, any of the tested BFT systems were better for growth and food utilization than the control groups without bioflocs (Khanjani et al., 2017; Liu et al., 2018b).

In other crustacean species, Cardona et al. (2015) studied the effect of CW, BFT, and BFT without external feed on blue shrimp (*Litopenaeus stylirostris*) culture. They observed that growth was about 4.4-fold better in BFT than in CW, plus survival improved. Moreover, the natural food consumed by the shrimps reared in BFT contributed to shrimp growth and increased the activity of digestive enzymes alpha-amylase and trypsin, which demonstrate this species' effective use of bioflocs (Cardona et al., 2015). In tiger shrimp (*Penaeus monodon*), Arnold et al. (2009) studied the effect of culture density (2,500 and 5,000 shrimp m^{-3}) and the addition, or not, of artificial substrates that could increase biofloc production. The results showed that, with the addition of an artificial substrate, BFT significantly enhanced animal growth performance regardless of stocking density, but especially impacted those animals cultivated at 5,000 shrimp m^{-3}. At this density, the final tiger shrimp weight doubled (from 0.2 to 0.4 g) with the artificial substrate, mainly due to higher nutritional source availability but also due to the improved water quality (lower concentrations of total ammonia nitrogen and nitrite) obtained with this treatment. Moreover, at this high density, the FCR significantly improved and obtained lower values (from 1.49 to 2.5) with added substrate compared to the treatment with no artificial substrate (Arnold et al., 2009). Similar results have been reported for the Pacific white shrimp cultured in CW and BFT with the addition of artificial substrates (de Morais et al., 2020; Olier et al., 2020). Olier et al. (2020) determined that other than the addition of a vertical substrate improving the growth performance and feed utilization of Pacific white shrimp, it can also save dietary protein without negatively affecting production and the chemical aspects of those shrimp reared in BFT.

In fish species, BFT has also drawn considerable attention, with initial studies carried out in Nile tilapia and are being extended to other species. Ekasari et al. (2015) observed that Nile tilapia larvae did not improve significantly in growth performance when they were cultivated in a BFT system, which indicates that nutritional requirements were met independently of the culture system, while additional biofloc consumption did not contribute significantly to fish growth. Although the BFT effect was not shown on animal growth, such growth seemed more uniform in the BFT group compared to the control, and larvae survival of BFT (90–98%) was significantly higher than in the control group (67–75%) (Ekasari et al., 2015). These authors challenged fish to pathogenic infection with either bacterium *Streptococcus agalactiae* or salinity stress tests. They observed that the BFT group presented significantly higher survival than the control group in both challenges.

In other fish species, such as rohu (*Labeo rohita*) and Jayanti rohu (genetically improved *Labeo rohita*), it has been demonstrated that BFT enhances growth performance and feed utilization compared to the control groups (Kamilya et al., 2017; Vadhel et al., 2020). The growth performance of Jayanti rohu reared in BFT increased by around 60% (specific growth rate (SGR) from 0.54 to 0.84, and weight gain rose from 38.5 to 65.9%), and FCR improved 40%, which went from 1.56 to 0.91, whereas survival also increased from 90% to 100% (Vadhel et al., 2020). For Jayanti rohu, values also significantly improved, and were better in the genetically improved rohu with increases of around 90% (SGR from 0.37 to 0.38 and weight gain from 25.29% to 50.93%) for growth parameters and 50% for FCR (from 2.37 to 1.17) when animals were maintained in BFT. In both cases, the authors indicated that the enhanced parameters and welfare determined by diverse parameters could be attributed to the better quality and maintenance of the water in the BFT system (Vadhel et al., 2020). In golden carp (*Carassius auratus*) juveniles, Yu et al. (2020) tested five C:N ratios in BFT systems and found that the bioflocs produced higher C:N ratios (20:1 and 25:1), significantly enhanced weight gain, SGR, and protein efficiency ratio (PER) than the control group because of better water quality and the increase in the parameters related to liver protease, lipase and amylase enzymes, and different immunological and antioxidant factors.

Notwithstanding, although almost all the studies conducted in crustacean and fish species have demonstrated the positive effect of BFT compared to CW in different situations (Arnold et al., 2009; de Morais et al., 2020; Khanjani et al., 2015, 2017; Khanjani and Sharifinia, 2020; Olier et al., 2020; Vadhel et al., 2020; Yu et al., 2020), others authors have found no effect or only negative effects (Esparza-Leal et al., 2015; Ray et al., 2017; Tierney and Ray, 2018). Esparza-Leal et al. (2015) found that Pacific white shrimp (0.009 initial body weight, IBW) grew better in a CW system than in a BFT system, independently of culture density (from 1,500 to 9,000 orgs m^{-3}). However, the same authors indicated that the obtained results could be due to a problem with the alkalinity of the water employed in the BFT system, which was lower than that recommended for the species and could negatively influence good biofloc development (Esparza-Leal et al., 2015). Similar results have also been observed in shrimps of 0.42 g of the same species stocked in all the tanks at 250 m^{-3} and grown in CW *vs.* BFT, with the best results obtained for growth and FCR in CW systems (Ray et al., 2017). In fish, Fleckenstein et al. (2018) determined the effect of CW, BFT, or a hybrid treatment on tilapia with an IBW 0.17 g, and observed that the hybrid treatment was the best system to obtain the best growth performance, although no differences appeared between BFT and CW, and the FCR values worsened when the BFT system was employed. These authors indicated that, as in other cases, inferior water quality conditions could be responsible for diminished performance in BFT (Fleckenstein et al., 2018). These studies revealed the importance of carrying out effective BFT system management to positively contribute to production. Controlling the high diversity of the parameters that condition not only biofloc formation but also water quality can improve or worsen performance based on such management.

Dietary Protein Sparing Effect of Biofloc Technology

Regarding the use of FM in the diet, biofloc studies in aquatic species have clearly demonstrated the possibility of reducing protein content in the diet with no detriment to production and/or improving results related to growth performance and feed utilization when alternative meals are used. Thus, Pacific white shrimp is the most widely studied species in all its development stages. In 10-day-old postlarvae (≈1 mg), Correia et al. (2014) did not find any differences in survival (82% *vs.* 84%) and protein efficiency ratios (3.89 *vs.* 3.28) when shrimp were fed two different dietary protein levels (30 and 40% CP, respectively) in a BFT system. These authors concluded that substituting high protein (40%) for low protein (30%) feed in the nursery phase of Pacific white shrimp in a BFT system is a possible sustainable alternative to shrimp production with cheaper (lower protein) feed and reduced environmental impact, besides obtaining improved water quality. In the juveniles of this shrimp species, with an IBW of 1.5 g, Panigrahi et al. (2019) demonstrated that protein content in feed can be reduced from 40% in a CW system to 24% in a BFT system without affecting animal production and welfare. Among BFT treatments (24%, 32%, and 40% of dietary protein level), 32% was the lowest protein level in feed to give the best results. Hence the improvement in the CW system with 40% protein ranged between 32.6 and 52.6% for productivity, 22% and 27.6% for average body weight (ABW), 8.7% and 19.6% for survival, 10% and 31% for the FCR, and 32% and 83% for the PER when Pacific white shrimp were reared in BFT with different protein levels (Panigrahi et al., 2019). Similar results for this species, but with heavier IBW (5.3–6.5 g), have been obtained in other studies, which indicated that protein levels can lower to 25% or 30% when bioflocs are used as a nutritional supplement, with similar growth performance, feed utilization, and survival to those shrimps fed diets containing protein levels that come close to those in commercial diets (Jatobá et al., 2014; Panigrahi et al., 2020; Xu and Pan, 2014b).

Likewise, optimum results have been reported for other shrimp species, such as tiger shrimp and Indian white shrimp (*Penaeus indicus*). Megahed et al. (2018) carried out a study with low dietary protein levels (20%, 22%, 24%) and a 35% commercial level in Indian white shrimp (IBW 0.52 g). They observed no significant differences in final weight and the SGR and FCR between the control and low protein diet groups. However, the shrimps that were fed a low protein diet (86.6%) compared to the control (66.6%) had better survival results. In a later study, Panigrahi et al. (2020) evaluated BFT systems with different dietary protein levels (2%, 30%, 35%) against a control group without biofloc in the same shrimp species (IBW 0.75 g). These authors reported a significant improvement in all the growth and survival indices of the groups reared in a biofloc system, with the best results observed for those shrimp feds 30% protein compared to the other biofloc groups and controls. With tiger shrimp, Kumar et al. (2017) conducted a study to evaluate the effect of two dietary protein levels (32% and 40%) and two different carbon sources (rice flour and molasses), but without carbohydrates (control). Their results indicated better animal performance in all the experimental groups compared to the control, with optimum growth values and immune responses with the rice flour addition. This revealed adding rice flour at the 32% protein level could replace 40% protein feed.

Studies performed with alternative meals to replace FM in diet with Pacific white shrimp have reported optimum results when applying BFT systems. Jatobá et al. (2017) tested different soya protein concentrate levels (0%, 33%, 66%, and 100% FM replacement) in shrimps (IBW 4 g) reared in a super-intensive biofloc system. They found that growth performance was not negatively affected up to 33% replacement. In a similar study, Tesser et al. (2019) observed that FM and fish oil can be substituted for up to 75% soya protein concentrate and soya bean oil without negatively affecting Pacific white shrimp development (IBW 2.93 g) when reared in a BFT system.

Other protein sources, such as spent brewer's yeast, have been tested to replace FM in the diet for giant freshwater shrimp (*Macrobrachium rosenbergii*) with an IBW of 6.7 g when reared in a BFT *vs.* a CW system (Nguyen et al., 2019). Thus, experimental diets with 35% protein were formulated with 0%, 20%, 40%, and 60% FM replacement for spent brewer's yeast. The results revealed that neither growth nor survival was affected when increasing brewer's yeast levels in any system. In general, FCR was better in BFT than in CW, and the BFT group fed 60% spent brewer's yeast presented the best growth results (Nguyen et al., 2019).

Additionally, BFT can be improved by introducing other components into the system. In Pacific white shrimp with an IBW of 0.23 g, Brito et al. (2018) studied the effect of lowering dietary protein from 40% to 30% in a biofloc monoculture or a biofloc integrated system by using seaweed *Gracilaria birdiae*. Their results revealed that the integrated system improved the growth performance of shrimps by increasing weight gain by 21% and 5% in the shrimps fed 30% and 40% dietary protein, respectively. Moreover, growth was similar when comparing treatments with 40% protein, independently of the BFT system with 32% in the integrated BFT system. This study indicates that dietary protein content can be lowered without affecting zootechnical parameters when seaweed is employed as a supplemental food in an integrated BFT system (Brito et al. 2018).

In crustacea, dietary protein levels can also be lowered in fish species cultivated with BFT. In tilapia, one of the most widely studied fish species, da Silva et al. (2018) evaluated the effect of different protein contents (between 17% and 33%) on the diet to find that 10 g and 60 g of IBW protein can be lowered to 28% and 22%, respectively, when rearing tilapia juveniles in BFT. Likewise, Hisano et al. (2020) observed that different dietary protein levels (36%, 32%, and 28% CP), which were all supplied in diets from plant sources, did not influence growth performance, FCR, or health status (IBW 6 g) of tilapia reared in BFT systems. Therefore, a reduction up to 28% of dietary protein in this fish species would be profitable from the production cost and environmental impact points of view.

In a later study, Klanian et al. (2020) investigated the influence of two different protein levels, which were also lowered during the experiment. The Nile tilapia fingerlings (IBW 2 g), cultivated at two stock densities (40 and 80 fish m-3) using BFT and CW technologies, were fed commercial pellets containing 45% (high protein group) or 35% (low protein group) protein for the initial 7 weeks and 35% (high protein group) or 25% (low protein group) protein for the final 9 weeks. Their results showed that BFT microbial proteins compensated the dietary protein restriction without harming survival, growth, feed utilization, or fish health status. Moreover, the BFT results were better when compared to CW systems when low protein was used in the diet, independently of stock density (Klanian et al., 2020).

In the fingerlings of Genetically Improved Farmed Tilapia (GIFT) (IBW 0.99 g), Sgnaulin et al. (2020) studied the combination of different dietary digestible protein levels (22%, 26%, 30%) and digestible energy (3,000, 3,150, 3,300 kcal/kg^{-1}) levels on the growth performance of this fish reared in BFT systems. These authors found that neither FCR nor survival was affected by several treatments, and weight gain was no different between 26% and 30%, but was lower at 22% compared to 30% digestible protein. These results suggest that the optimum combination of digestible protein and energy levels for GIFT fingerlings reared in biofloc systems was 26% and 3,000 kcal/kg^{-1} (Sgnaulin et al., 2020). Another study with hybrid tilapia (*Oreochromis aureus x O. niloticus*) has suggested that diets formulated by supplementing the first four limiting amino acids (Lys, Met, Thr, Ile) could include low digestible protein levels from 32.3% to 27.7% without adversely affecting productivity (Green et al., 2019).

Similar results to those observed for tilapia have also been obtained for several carp species. In gibel carp (*Carassius auratus gibelio*; IBW 250.8 g), three protein levels in the diet (25.7%, 30.1%, 35.3%) were tested in a BFT system against a control without bioflocs (Li et al., 2018). The results demonstrated that a low protein diet was recommended for the gibel carp culture in BFT systems compared to a high protein diet, as growth was similar for both 25.7% and 30.1% BFT compared to a 35.3% CW system, and the FCR was better in the 30.1% diet than in the 35.3% one in BFT (Li et al., 2018). In common carp (*Cyprinus carpio*) juveniles (IBW 30.5 g), Ebrahimi et al. (2020) evaluated the effect of two dietary protein levels (20% and 30%) with different carbon sources for biofloc culture (sugarcane molasses, rice bran, and their combination, plus a control) on several parameters, including fish performance. Their results indicated that 30% protein in diet and the use of rice bran increased weight gain, the SGR, and survival, and improved the FCR, compared to the control and the group with only molasses. Once again, it demonstrated that bioflocs spare dietary protein and are closely related to BFT system management (Ebrahimi et al., 2020).

By contemplating the use of alternative meals to substitute FM in the diet similarly to Pacific white shrimp, Nhi et al. (2018) tested in Nile tilapia the use of spent brewer's yeast at different substitution levels (0%, 30%, 600%, 100%). Compared to the CW system, tilapias (IBW 29 g) reared in BFT obtained higher growth performance indices and better FCR, PER, and survival. The use of brewer's yeast did not affect tilapia growth, feed utilization, or survival at any of the tested substitution levels. Thus, these authors concluded that brewer's yeast is a potential substitute for FM in tilapia diet, especially when fish are reared by BFT. Another recent alternative to FM is to use insect meals in diet. Tubin et al. (2020) utilized mealworm meal (*Tenebrio molitor*) at the 0% (control), 5%, 10%, 15%, and 20% inclusion levels to feed Nile tilapia (IBW 2.1 g) reared in a biofloc system. The overall results indicated that it is possible to include this meal up to 10% in diet without negatively affecting production.

Bioflocs as a Feedstuff Ingredient in Dietary Formulations

It should be taken into account that, in order to apply BFT, aquatic species must possess several abilities and characteristics as morphologically specialized structures that allow them to feed with bioflocs (Walker et al., 2020). As numerous available studies have shown, Pacific white shrimp and tilapia are the most successfully cultivated species in BFT systems, but its application to other aquatic species, including Indian white shrimp, blue shrimp, tiger shrimp, giant freshwater shrimp, rohu, and several carp species has been demonstrated (Arnold et al., 2009; Cardona et al., 2015; Ebrahimi et al., 2020; Kamilya et al., 2017; Kumar et al., 2017; Megahed et al., 2018; Nguyen et al., 2019; Panigrahi et al., 2020; Vadhel et al., 2020). However, many other species, mainly fish species, are unable to use this technology. In such cases and/or in order to improve biofloc use efficiency, an alternative application is their direct inclusion in diet as a feed ingredient. For this purpose, bioflocs can be produced in reactors specially designed for this purpose, or collected directly by the BFT system when produced in excess (Walker et al., 2020). After their processing, bioflocs can be used as an alternative protein source to FM replacement (Walker et al., 2020).

A study by Kuhn et al. (2010) used bioflocs obtained in not only sequencing batch reactors with sucrose supplementation, but also in a membrane biological reactor without carbon supplementation. After treatment, the dried bioflocs were used to feed Pacific white shrimp postlarvae (< 1 mg). The experiment consisted in a control diet (without bioflocs) and diets were supplemented with 10%, 15%, 21%, and 30% bioflocs from both production systems by replacing FM and/or soya bean protein. The results showed that despite survival (92.9 to 100%) or harvest biomass (536 – 574 g m^{-2}) not being affected, growth performance was better in the diet with biofloc inclusion and confirmed that the external bioflocs included in diet could replace FM and soya bean protein. In other studies by Valle et al. (2015) and Dantas et al. (2016), performed with the same shrimp species but with a postlarvae 2 mg IBW, different FM replacement levels, along with a combination of biofloc meal and fish protein hydrolysate at the 1:1 ratio, or with only biofloc meal, were respectively evaluated. After 42 days, the studies showed that FM replacement with biofloc meal was possible and even improved optimum growth performance and feed utilization compared to the control diet. Valle et al. (2015) established the optimal FM replacement level with fish protein hydrolysate and biofloc to be between 15.2% and 16.5%, but also indicated that diets should be supplemented with methionine when biofloc meal was used. Dantas et al. (2016) observed the best weight gain, SGR, and PER results when employing 30% biofloc meal with no differences in survival, which exceeded 91% in all treatments. Other authors have observed similar positive results for the same shrimp species (Ju et al., 2008; Shao et al., 2017; Khanjani et al., 2020). Therefore, according to the results of all these studies, biofloc meal can clearly be used as an alternative protein source to replace FM in the Pacific white shrimp diets.

In fish species, Prabhu et al. (2018) conducted a trial in which biofloc meal was supplemented at the 0%, 20%, 30%, and 40% inclusion levels in GIFT tilapia diet (2 g IBW). Their results showed that with up to 20% biofloc meal inclusion, both growth performance and feed utilization improved *vs*. the control diet. In tilapia with 12.1 g IBW, biofloc meal was evaluated to replace soya bean meal at three replacement levels (0%, 25%, and 50%) to obtain up to 25% soya bean meal substitution that had no negative effects on tilapia growth performance (Mabroke et al., 2019). With African catfish (*Clarias gariepinus*), Ekasari et al. (2019) studied the effect of biofloc meal as a feed ingredient on juveniles (1.4 g IBW). They included 0%, 5%, 10%, and 20% of biofloc meal by replacing soya bean meal, and concluded that the dietary 10% and 20% biofloc meal inclusion levels induced better results for feed intake, PER, FCR, and weight gain, besides improving animals' health status. Finally, in other aquatic species like sea cucumber (*Apostichopus japonicus*), biofloc meal has been used to replace macroalgae *Sargasswn thunbergii*. The results showed that the optimum dietary replacement level was between 27.74% and 30.75%, which improved the SGR, FCR, and PER (Chen et al., 2018).

CONCLUSIONS

In conclusion, BFT and the use of biofloc meal seem an eco-friendly and cost-effective alternative for developing sustainable aquaculture given the possibility of reducing dietary protein content and decreasing the dependence on mainly FM but also other alternative plant-protein sources that are beginning to generate environmental problems, such as soya bean meal. They all accomplish optimum growth performance and feed utilization of species besides offering the beneficial effects that bioflocs have on health status. Notwithstanding, in order to successfully apply this technology in aquaculture, it is essential to pay special attention to the management and composition of biofloc production because, if they are not properly performed and do not adapt to each species' requirements, the opposite effect to that sought could come into play. Today, continuing to study and improve the use of bioflocs in aquaculture still remains a challenge.

ALGAE

Introduction

Algae constitute a group of organisms that is present in fresh or seawater which, despite having different phylogenetic origins, share autotrophic and photosynthetic characters. In algae, we find multicellular groups that have developed tissues and acquired a macroscopic character (macroalgae) but which, unlike plants, have simple reproductive structures. Although they lack roots, stems, leaves and vascular tissues, these algae have a structure known as a thallus. Some algae have a microscopic character (unicellular or filamentous microalgae) with a wide variety, of which some 30,000 species have been studied. However, only some 100 genera have been cultivated on the laboratory scale, and studies have focused on about 20 for their potential beneficial use, while fewer than 10 have been produced in an industrial context (Mobin and Alam, 2017; Gaignard et al., 2019).

In this section of the chapter, an updated description is provided of how both microalgae and macroalgae increasingly play an important role as an alternative to animal protein sources in aquaculture feed.

MICROALGAE

Microalgae are generally classified as four groups: Rhodophyta (red algae), Chlorophyta (green algae), and Chromophyta (all other algae) and are eukaryotes, and prokaryotes Cynaobacteria (blue-green algae). In the last few years, the industrial use of microalgae has increased in the areas of food production (Figure 8.3), pharmaceuticals, and biofuels. This is because algal biomass is a rich source of nutrients, such as proteins, n-3 FA, and carbohydrates, as well as vitamins, minerals, and other bioactive compounds, like antioxidants. The fact that many species can be used as protein sources has not only promoted their use for human nutrition but also for animal nutrition, including aquaculture feeds (Becker et al., 2007; Mobin and Alam, 2017; Raja et al., 2018).

The importance of employing microalgae arises from the high growth rate and their ability to accumulate nutrients of interest under not very complex culture conditions. A suitable nitrogen, phosphorus, and carbon dioxide concentration in medium, as well as pH, temperature, and light conditions, are specific to cultivated genera or species. These aspects are key for achieving adequate yields and the suitable use of the molecules of interest provided in microalgae cultures (Khan et al., 2018; Suparmaniam et al., 2019; De Morais et al., 2020).

In order to make algal biomass production economically viable, high-value by-product extraction is paramount. Obtaining microalgae extracts for commercial purposes requires separating organisms from the culture medium by means of filtration, centrifugation, sedimentation, flocculation, etc. Later, treatment will differ depending on the ultimate purpose. For instance, microalgae can be processed into algae paste (microalgae cells dispersed in liquid media) or may go through drying processes to obtain powders or freeze-dried cubes. This latter format is most useful for aquaculture feed purposes (Raja et al., 2018). In order to extract cellular components of interest, a physical, chemical, enzymatic, or mechanical treatment is required to rupture the cell wall. Finally, a selective extraction of the molecules of interest is carried out, regardless of whether they are proteins, lipids, carbohydrates, antioxidants, etc. (De Morais et al., 2020).

Microalgae and Aquaculture

Traditionally, microalgae use has been associated with one of the initial steps in aquaculture: zooplankton culture for later hatchery and nursery feeds. Their microscopic size renders them ideal for feeding rotifers, copepods, and brine shrimp (*Artemia* sp.) which, in turn, is employed to feed juvenile finfish and shellfish, including crustacea. Cultivated microalgae are also employed as direct

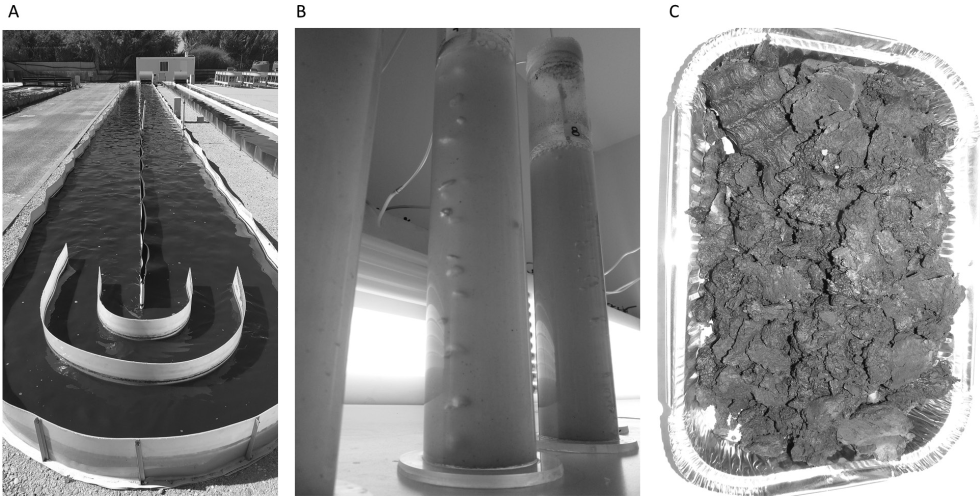

FIGURE 8.3 A and B, different technology systems for microalgae culture. C, Biomass of microalgae (Courtesy of F.G. Acien University of Almeria, Spain).

feeds for marine bivalve molluscs (oysters, clams, scallops), and the larvae of some marine gastropods (abalone and conch), sea urchins, shrimps, and some marine fish (Raja et al., 2018; Dineshbabu et al., 2019).

In recent years, the incorporation of microalgae into the market as formulated feed ingredients has gained relevance. Thus, certain types of microalgae are very promising for the industry because they present components with a high added value. *Chlorella* spp., *Pavlova lutheri*, *Haemotococcus pluvialis*, and *Phaeodactylum tricornutum* are particularly useful as algal meal. Others are reported as a source of n-3 FA (*Nitzschia* spp., *Phaeodactylum tricornutum*, and *Schizochytrium limacinum*) or pigments for fish, such as salmonids or crustacea (*Dunaliella* spp., *Haemotococcus pluvialis*) (Yarnold et al., 2019). Hence the variety of commercial microalgae preparations are intended to feed molluscs, crustacea, zooplankton, etc., covering nutritional requirements throughout their life cycle (Dineshbabu et al., 2019). It is important to bear in mind that the main biochemical components in algal cells not only varies according to species, but also to culture conditions, growth phase, and physiological status and that culture condition is critical to obtaining certain nutrients (Tibbetts et al., 2015; Madeira et al., 2017).

The use of microalgae as a source of high-quality proteins for single-cell proteins (SCPs) emerged some years ago and has been revealed as an alternative to classic protein sources of animal origin and even as an equivalent to convectional plants, while producing feed for aquaculture (Becker, 2007). In addition, greenhouse gas emissions from aquaculture have increased in recent years, while a drop in FM and fish oil production would be relevant to improve sustainable production (Hasan and Soto, 2017).

Regarding protein levels, not all microalgae are suitable for this purpose as the proximal analysis of different genera reveals a wide variability in their protein percentages (Table 8.3). The protein microalgae biomass can generally reach values between 40% and 70%. This is interesting because they are not only higher in protein than many plant sources, such as soya bean (38%), rice (approx. 10%), pea (2.8%), or even animal sources like milk (4%) or eggs (13%), but because they also present a suitable amino acid profile due to the relevance of EAA that are absent in many traditional plants (Torres-Tiji et al., 2020) (Table 8.3).

Many microalgae genera possess such high protein levels that they can sometimes represent up to half of the biomass weight (Table 8.3). Among the most remarkable ones in chlorophytes, we find *Dunaliella*, *Chlamydomonas*, *Nannocloropsis*, and *Chlorella*, and *Arthrospira phylum* in cyanobacteria (blue-green algae), commercially known as *Spirulina*. Cyanobacteria can reach 40–70% protein due to phycobiliprotein pigments. Some chorophytes show values above 50% (Niccolai et al., 2019), such as *Dunaliella*, with protein values over 80% in dry weight compared to other green microalgae like *Chlamydomonas* (48%) or the blue-green alga *Arthrospira* (46–70%) (Dineshbabu et al., 2019; De Morais et al., 2020).

However, despite the fact that some genera may present very high protein values, this is no guarantee for their suitability as a protein source because assessing its quality is essential. *Arthospira* (*Spirulina*) is one of the filamentous cyanobacteria whose culture accounts for almost one third of the world microalgae production. It has been recognized by the World Health Organization (WHO) for its high protein content and EAA profile, which make this genus, together with *Chlorella* (Table 8.3), suitable for animal feed (Mitra and Mishra, 2019; Niccolai et al., 2019; Lafarga et al., 2020). Finally, it is worth mentioning the role of certain red microalgae, such as *Porphyridium*, which, in addition to high-value compounds like pigments or FA, have recently gained relevance for their high-quality protein content and suitable EAA profile compared to conventional vegetable sources (Gaignard et al., 2019).

Another interesting aspect when considering microalgae as a protein source is the possible overestimation of protein content. In most cases, protein determination is based on nitrogen concentration, which can come from other constituents, like nucleic acids, amines, glucosamides, and nitrogen-containing cell wall materials. An overestimated protein content between 6% and 12% has been reported for *Scenedesmus*, *Arthrospira*, and *Dunaliella* (Becker, 2007).

TABLE 8.3
Protein Content and Amino Acids Profile of Soybean and Different Microalgae with Applications in Aquaculture

		Chlorophyta						*Cyanobacteria*			*Rhodophyta*	*Others*	
Amino acids (mg/100 mg protein)	***Soybean***	***Chlorella vulgaris***	***Chlorella protothecoides***	***Dunaliella bardawil***	***Scenedesmus obliquus***	***Chlamydomonas reinhardtii***	***Haematoccocus* sp.**	***Arthrospira maxima***	***Arthrospira platensis***	***Aphanizonemon* sp.**	***Porphyridium aerugineum***	***Nannochloropsis granulata***	***Euglena gracilis***
Alanine	5.0	7.6	6.2	7.3	9.0	8.8	11.8	6.8	9.5	4.7	8.4	7.1	15.8
Arginine	7.4	6.0	13.4	7.3	7.1	7.2	nd	6.5	7.3	3.8	8.6	7.4	3.4
Aspartic acid	1.3	8.4	7.1	10.4	8.4	9.7	11.8	11.6	11.8	4.7	15.0	11.4	7.1
Cysteine	1.9	1.4	1.6	1.2	0.6	nd	nd	0.4	0.9	0.2	2.2	1.6	0.2
Glutamic acid	19.0	10.7	10.3	12.7	10.7	11.3	13.7	12.6	10.3	7.8	15.6	14.1	9.5
Glycine	4.5	5.5	5.5	5.5	7.1	5.7	6.3	4.8	5.7	2.9	7.0	7.5	7.0
Histidine	2.6	1.9	3.0	1.8	2.1	2.3	5.8	1.8	2.2	0.9	1.9	2.3	2.2
Isoleucine	5.3	3.8	3.7	4.2	3.6	4.4	4.9	6.0	6.7	2.9	7.1	5.6	0.2
Leucine	7.7	8.3	5.6	11.0	7.3	9.8	6.4	8.0	9.8	5.2	11.9	11.0	3.7
Lysine	6.4	6.8	4.9	7.0	5.6	6.6	4.1	4.6	4.8	3.5	8.0	8.2	4.9
Methionine	1.3	2.2	2.1	2.3	1.5	2.7	nd	1.4	2.5	0.7	3.7	3.5	0.0
Phenylalanine	5.0	4.9	5.5	5.8	4.8	5.6	4.9	4.9	5.3	2.5	6.3	6.2	0.9
Proline	5.3	4.5	5.6	3.3	3.9	5.6	nd	3.9	4.2	2.9	5.0	11.2	0.0
Serine	5.8	3.7	5.1	5.0	3.8	4.3	5.9	4.2	5.1	2.9	7.0	5.6	10.6
Threonine	4.6	4.4	4.9	5.4	5.1	5.1	5.6	4.6	6.2	3.3	5.8	5.4	4.5
Tryptophan	1.4	1.5	0.5	0.7	0.3	2.8	nd	1.4	0.3	0.7	3.3	2.8	1.7
Tyrosine	3.7	3.4	4.7	3.7	3.2	4.3	2.7	3.9	5.3	nd	5.8	4.2	0.7
Valine	5.3	5.4	5.2	5.8	6.0	6.5	7.5	6.5	7.1	3.2	7.3	7.1	8.0

Bold letters: essential amino acids. nd: not determined. Reported by Becker, 2007; Szabo et al., 2013; Kim et al., 2015; Tibbetts et al., 2015; Asiedu et al., 2018; Dineshbabu et al., 2019; de Morais et al., 2020; Niccolai et al., 2019; Torres-Tiji et al., 2020

Digestibility is one of the most important and determinative aspects when estimating protein availability in microalgae as aquafeed. The presence of a robust cell wall, which represents 10% of dry algal matter, is often a problem that hinders the action of digestive enzymes to access cell content, especially for carnivorous fish with a short digestion phase (e.g. salmonids). The analysis of different microalgae has revealed a range of digestibility values among genera, with higher values in cyanobacteria (*Arthrospira*, *Nostoc*) compared to chlorophycea (Table 8.4). This aspect seems to correlate with the chemical composition and structural organization of the cell wall (Niccolai et al., 2019). Something similar occurs in red microalgae, *Porphyridium* and *Rhodella*, which highlights their content in very digestible proteins given the presence of a polysaccharide mucilage instead of the classic cell wall (Gaignard et al., 2019).

Differences observed in the protein digestibility of microalgae become evident when running an analysis of nutritional indices associated with protein assimilation. Thus indices, like biological value (BV, nitrogen retained for growth or maintenance according to absorbed nitrogen), net protein utilization (NPU, nitrogen retained according to nitrogen intake), digestibility coefficient (DC, measuring both protein digestibility and the biological value of absorbed amino acids), and the protein efficiency ratio (PER, weight gain according to intake), confirm that some genera have suitable values, namely *Chlorella*, *Spirulina*, and *Scenedesmus* compared to, for example, egg proteins (Becker, 2007) (Table 8.4).

There is a variety of methods for cellular wall treatment to increase microalgae digestibility: enzymatic (cellulases), chemical (organic solvents or acids), and physical/mechanical (bead milling, high-pressure homogenization, or micro-fluidization) (Jones et al., 2020). In general terms, physical and mechanical methods are the most widely used because chemical and enzymatic methods can affect intracellular nutrients. Enzymatic treatment seems effective in *Chlorella* and *Nannochloropsis* by improving protein digestibility in fish species, like Atlantic salmon (*Salmo salar*) and juvenile Nile tilapia (*Oreochromis niloticus*), respectively (Tibbetts et al., 2017; Teuling et al., 2019), which may also result in additive or synergistic effects on nutrient utilization (Madeira et al., 2017). Technically speaking, the aquafeeds within the above compositional ranges can be formulated entirely from microalgae, but other factors to be considered, including feed attractiveness (e.g., smell, taste) and accessibility (e.g., cell/pellet size, buoyancy).

Different studies have reported employing microalgae as a protein source in feed for aquaculture (Macias-Sancho et al., 2014; Teuling et al., 2019; Ansari et al., 2020, Table 8.5). In any case, it is still difficult to reach 100% FM replacement or other protein sources of animal origin. Indeed, a recent comparative analysis performed in Nile tilapia (*O. niloticus*) revealed maximum FM substitution levels for *Nannochloropsis*, *Chlorella*, *Scenedesmus*, and *Spirulina* with 35%,

TABLE 8.4
Digestibility of Microalgae Biomass of Interest as a Food Source

Microalgae	Dry Matter Digestibility (%)	Crude Protein Digestibility (%)
Chlorella vulgaris	> 60	76
Tetraselmis sp.	> 60	62–70
Arthrospira sp.	78	81
Nostoc sphaeroides	> 60	82
Porphyridium purpureum	47	62–70
Nannochloropsis oceanica	> 60	50
Tisochrysis lutea	> 60	62–70

Adapted from data reported by Niccolai et al. (2019).

TABLE 8.5
Studies Focused on Fishmeal Replacement for Algal Biomass in Aquaculture

Microalgae	Aquaculture Species	Challenge	Reference
Chlorella vulgaris	**Atlantic salmon** *(Salmo salar)*	Digestibility assays	Tibetts et al. (2017)
Nannochloropsis gaditana	**Nile tilapia** *(Oreochromis niloticus)*	Digestibility assays	Teuling et al. (2019)
Scenedesmus obliquus	**Nile tilapia** *(Oreochromis niloticus)*	Fishmeal replacement	Ansari et al. (2020)
Haematococcus pluvialis	**Yellow perch** *(Perca flavescens)* **Pacific white shrimp** (*Litopenaeus vannamei*)	Fishmeal replacement	Jiang et al. (2019, 2019) Ju et al. (2012)
Dunaliella tertiolecta	**Sea Urchin** *(Strongylocentrotus intermedius/ S. nudus)*	Dietary protein source	Qi et al. (2018)
Arthrospira (Spirulina platensis)	**Pacific white shrimp** *(Litopenaeus vannamei)*	Fishmeal substitution	Macias-Sancho et al. (2014)

50%, 50%, and 75%, respectively, which are similar to those observed for plants (Yarnold et al., 2019). Another study in the same species has shown that, although the maximum substitution values for *Scenedesmus obliquus* were around 7.5%, a significant improvement in growth and a higher PER were promoted. This study also revealed the profitability and sustainability of an integrated algae aquaculture system because the use of defatted algae after lipid extraction provided valuable source algae for FM replacement, while lipids were utilized for biodiesel synthesis purposes (Ansari et al., 2020).

The effect of partial FM protein replacement with defatted microalgae (*Haematococcus pluvialis*) meal has been evaluated as a protein ingredient in the diet of Pacific white shrimp (*Litopenaeus vannamei*), with a significantly higher growth rate and a lower feed conversion ratio at 12.5% replaced protein, which reveals a valuable alternative protein and pigmentation ingredient in shrimp feed (Ju et al., 2012).

At present, the challenge of using microalgae as a protein source in animal feed production is opening up a research area in the biotechnology field. However, it is true that today substitution levels are far from reaching 100% substitution because improvements in protein quality, suitable amino acid profile, digestibility indices, palatability, etc., are all still required.

A recent review by Hua et al. (2019), in which alternative protein sources to FM were evaluated according to the biological capacity of microalgae, reported positive research findings for its replacement efficacy in different aquaculture species, which suggests the high potential for employing microalgae as a protein source. However, this potential is affected by the technical, biological, and economic difficulties in the continuous production of high-quality microalgae biomass and in its downstream processing and subsequent scaling.

Major advances are required, including the establishment of extending algal collections for breeding purposes, genome sequencing, and optimal species identification (Yarnold et al., 2019). Indeed, the selection of breeds with a phenotype based on high protein content and an EAA profile is one of the proposals linked with improving their organoleptic qualities. Apart from all this, improving culture technology to reduce production costs and increase the use of microalgae remains a major challenge, for the near future, in the effort to support a sustainable 'circular' aquaculture industry (Torres-Tiji et al., 2020).

MACROALGAE

Marine algae, referred to by the generic term "seaweeds", and colloquially known as macroalgae, are one of the most prominent primary marine photosynthetic producers (Hong et al., 2014). They are divided into three major groups according to their photosynthetic pigments: Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). In recent years, they have been considered an alternative resource for sustainable biomass to produce biofuels, biochemicals, and food. Similar to microalgae, seaweeds are an outstanding source of proteins, polysaccharides, minerals, vitamins, and a series of biologically active substances. The culture and harvesting of algae are straightforward, and a significant amount of biomass can be obtained compared to microalgae (Kraan, 2013).

Within green algae, one of the most notable genera is *Ulva* spp., commonly known as sea lettuce. The brown algae group includes genera like *Ascophyllum nodosum*, *Laminaria ochroleuca* (kombu), *Undaria pinnatifida* (Japanese wakame), and *Himanthalia elongata*. Finally, red algae comprise genera such as *Eucheuma*, *Palmaria palmata*, *Gracilaria* spp., *Chondrus crispus*, *Porphyra* spp. (Japanese nori), and *Pyropia* spp., of which some are of interest for human consumption (Martínez-Hernández et al., 2018; Sudhakar et al., 2018; Hua et al., 2019; Yarnold et al., 2019).

In general terms, and compared to terrestrial biomass, macroalgae stand out for their high carbohydrate content (25–60%), with protein values between 3–47% and low lipid contents (1–3%; dry weight) (Sudhakar et al., 2018). The wide range of protein levels depends on the seaweed type, with higher values reported for red seaweeds (10–47%), moderate ones for green algae (9–26 %) and the lowest values for brown seaweeds (3–19.5%), except *U. pinnatifida*. An analysis of the dried extract of different algae by a more exhaustive methodology (total amino acid residues, TAA) to minimize the overestimation associated with non-protein nitrogen, gave very high protein values in *U. pinnatifida* (51.6%) compared to *Ulva*, *Palmaria*, or *Chondrus* (18–37%), and even values of microalgae with a marked protein character, such as *Chlorella* or *Spirullina* (32–41%) (Martínez-Hernández et al., 2018).

Culture conditions are a key factor for determining protein levels. Under growth conditions, overestimations can arise in the assayed ranges because seaweeds have lower protein contents to ranges between 10–30% under non-limiting nutrient conditions (Vieira et al., 2018; Hua et al., 2019).

Regarding total amino acid content in seaweeds, generally the percentage is around 5.5%, which is lower than for other protein sources of plant origin, such as soya bean meal (22.3%), or animal sources, like fish (31.2%) (Angell et al., 2016). The analysis of the amino acid profile in some brown and red algae species generally revealed a higher EAA concentration than soya bean protein or even FM (Martínez-Hernández et al., 2018; Hua et al., 2019). Tryptophan, methionine, and leucine were the limiting EAAs in different seaweed species and, on the contrary, high lysine concentrations have been reported in red and green seaweeds. Accordingly, green and red algae seem to be interesting and suitable sources of protein for animal nutrition (Vieira et al., 2018).

The protein nutritional value is not only determined in terms of amino acid profiles, but also by protein digestibility. The digestibility of algae proteins often appears limited by present anti-nutritional compounds, such as polysaccharides or trypsin inhibitors, whose effect can be attenuated by a series of physicochemical enzymatic treatments (Fleurence et al., 2018).

Macroalgae and Aquaculture

Some aquaculture examples appear in which macroalgae have been traditionally used for feeding, and their use in mollusks such as abalone (*Haliotis* spp.) is noteworthy. With this species and a traditional diet of fresh algae, diets supplemented with macroalgae as a protein source have been assayed. A recent study on the greenlip abalone (*Haliotis laevigata*) has shown that replacing concentrates from green algae *Ulva* spp. in diet with 10% red algae *Gracilaria cliftonii* improves animal growth performance and survival (Duong et al., 2020).

Regarding fish feed, studies of the European sea bass (*Dicentrarchus labrax*) demonstrated that *Gracilaria* and *Ulva* can be incorporated into the diet as a protein source by up to 10% without affecting growth performance (Valente et al., 2006). In tilapia, reports reveal the positive effects of red algae *Gracilaria* on growth performance, provided the FM substitution level does not exceed 20% (Younis et al., 2018).

The profitability of using algae in aquaculture does not seem relevant because, despite their high protein content, it is not readily available, which entails raising costs to obtain necessary amounts. In general, given the marked presence of polysaccharides, the substitution percentage is limited to a 10% protein source in aquafeed formulations. Even so, their employment can be useful in herbivorous, or even omnivorous, species. Their use with carnivorous species as a functional additive is more highly recommended (Hua et al., 2019).

CONCLUSIONS

In summary, employing algae as an alternative protein source for aquafeed has revealed the more prominent role of microalgae compared to macroalgae because the former generally have a higher protein content and better tolerance to replacement percentages in diet, as reported for some species like tilapia (Yarnold et al., 2019). Microalgae can provide the most suitable bulk feed in fish diets, while macroalgae might be more suitable for terrestrial livestock and lower trophic-level aquaculture species (Shields and Lupatsch, 2012). Replacing FM with macroalgae in aquaculture feeding requires biomass processing to obtain more concentrated protein than can compete with other land and freshwater crops used as protein sources (Kim et al., 2013).

VEGETABLE PROTEIN SOURCES

Introduction

Fish oil and FM have traditionally been considered the most important standard dietary protein and lipid sources for many fish species owing to their high protein content, balanced EAA profile, and considerable quantities of lipid and minerals. Moreover, they offer excellent nutrient digestibility and are low in anti-nutritional factors (ANFs) (Daniel, 2018). These characteristics explain the predominance of FM over other raw materials in aquaculture feed. The production of both FM and fish oil is based on marine fish waste that does not reach commercial sizes or has no market value (Naylor et al., 2009). However, these products are available only in limited quantities; they are usually expensive, and are considered unsustainable from the environmental and ecological points of view. For these reasons, societal and economic pressures are placed on aquaculture production to search for alternative protein sources to FM (Figure 8.4). In omnivorous fish species, the elimination of FM from the diet has been more easily achieved than in carnivorous fish and crustacea, where it is more difficult to implement (Turchini et al., 2019). In general, the main and more widely studied alternative to FM in aquafeed is vegetable protein sources. It has been shown that up to 50% FM protein can be replaced with vegetable proteins in carnivorous fish diets without negatively affecting fish growth or welfare (Hardy, 2010).

Raw materials of vegetable origin abound. The use of these vegetable meals offers a series of advantages and disadvantages. They are less expensive than those of animal origin and, therefore, feed manufacturers substitute them because they are cost-effective. They are normally endowed with a certain binding power, associated with the presence of digestible substances. They are also a source of group B vitamins. However, the growth performance obtained with vegetable meal is inferior to that of the fish fed FM-based diets as they do not meet fish nutritional requirements (Gajardo et al., 2017). Their n-3 HUFA content is zero, and they are less palatable and poorly digestible because they contain significant amounts of ANFs, such as phytate, saponins, lectins, pectins,

FIGURE 8.4 Examples of vegetables used as a protein source in aquafeed: potatoes, coffee, soybean, palm kernel, canola, green pea, lupin, Lemna, corn, faba bean, and peanut (Courtesy of E. Rufino, University of Granada, Spain).

hemicellulose, pentosans, cellulose, lignin, among others (Lall and Anderson, 2005). Furthermore, starch is the main energy source in vegetable meal, which is not always well tolerated by fish.

Many studies have explored the possibility of vegetable proteins being used to replace FM (Caruso, 2015). Each vegetable protein source has its own characteristics and FM substitution efficiency depends on each species' nutritional needs. When choosing one or another, it is necessary to bear in mind that food must be available all year round at a low cost for growers; handling, transport, and processing requirements prior to feeding must be minimal; nutritional value has to be high in protein and carbohydrates and low in fiber; and they must be well accepted by the species that they intend to feed (Kaushik et al., 1995).

The present work reviews information about the innovative vegetable protein sources that allow FM to be replaced without affecting farmed fish growth and quality.

LEGUMES

The most frequently used vegetable protein source to replace FM is legumes: soya bean, bean, green peas, and lupins (Pereira and Oliva-Teles, 2002; Thiessen et al., 2003). Legumes have low fat content and are an excellent source of protein, dietary fiber, and a variety of micronutrients, as well as other compounds like phytochemicals (Messina, 1999).

Soya Bean

Among legumes, the importance of soya bean meal (SBM) is worth highlighting, which is the most important protein source as feed for fish feeding and as partial or entire FM replacement. SBM is the by-product left after removing oil from soya beans (glycine max) (Storebakken et al., 2000). It provides a high protein content, a good amino acid profile, and good digestibility. All this, together with adequately processing its seeds to eliminate ANFs, guarantees a sufficient availability of amino acids to achieve acceptable development (Oliveira et al., 1999). Moreover, the cost of SBM is lower compared to FM. Hence many studies have reported that partially substituting FM for SBM affects the performance of many fish species, such as trout (*Oncorhynchus mykiss*) (Harlioğlu and Yilmaz, 2011), common carp (*Cyprinus carpio*) (Uran et al., 2008), Japanese sea bass *Lateolabrax japonicus* (Zhang et al., 2014), juvenile tench *Tinca tinca L.* (García et al., 2015), gilthead sea bream *Sparus aurata L.* (Kokou et al., 2012), Nile tilapia *Oreochromis* *niloticus* (Abdel-Warith et al., 2013), Atlantic salmon (*Salmo salar*) (Furuya et al., 2004), shrimp (Molina-Poveda et al., 2013). All this reveals that adequate SBM inclusion levels in diet differs depending on the studied species. It is known that SBM inclusion over 50% decreases fish growth. Therefore, acceptable substitutions lie between 40% and 50%, depending on the species. The use of SBM involves several limitations due to ANFs and low methionine levels, which could lead to intestinal problems in some carnivorous fish (Gatlin et al., 2007). Furthermore, SBM can have an environmental impact, including deforestation, water pollution, and pesticide use, among others (Sánchez-Muros et al., 2014).

One source that derives from SBM is soya protein concentrate (SPC). It is produced by SBM fractionation, which is a highly refined ingredient as most of the ANFs present in SBM are removed during processing (González-Rodríguez et al., 2015). Mambrini et al. (1999) reported that the 50% supplementation of dietary protein using SPC as an FM replacement showed good amino acid digestibility in rainbow trout. According to Paripatananont et al. (2001), 8% SPC inclusion could replace 22% FM in *Penaeus monodon* diet. In carp, SPC can be incorporated into diet up to 40% without affecting survival and growth (Escaffre et al., 1997). According to Swick et al. (1995) 40–50% FM can be replaced with 25–30% SBM in shrimp without compromising animal growth. Furthermore, González-Rodríguez et al. (2015) studied the effects of substituting FM for SPC in tench juveniles. The fish fed between 0% and 45% replacement diets obtained significantly lower feed conversion ratios and higher protein productive values than those fed diets with higher replacement levels.

Lupin

Other legumes represent alternative protein sources to be used for aquaculture feed. Lupin (*Lupinus albus*) is a legume that belongs to the same family of plants as peas and soya beans. Lupin seeds have been described for aquaculture diet for their high protein content (30–40 g/100 g), availability, and low cost. Lupin seeds have a CP content between 31% and 42%, which is higher than the content of most other grain legumes (Rajeev and Bavitha, 2015). In all aquaculture species for which a nutritional assessment has been made on the value of lupins, they are an acceptable and nutritionally useful ingredient. Lupin seed meal could be a good alternative vegetable protein of high nutritive quality when used up to 30% or 40% in rainbow trout diet (Glencross et al., 2004). The nutritive quality of trout diets, including lupin seed meal, at 10%, 20%, 30%, and 40% dietary protein content has been studied by de la Higuera et al. (1988). These authors concluded that 30% raw lupin seed meal could be included in trout diet. The studies by Molina-Poveda et al. (2013) found that the body weight gain in shrimp gradually decreased with increasing lupin meal concentrations in diet, and displayed excellent growth at the 50% FM replacement level with defatted and dehulled lupin meal (Glencross et al., 2001). The results reported by Anwar et al. (2020) showed that SPC can be replaced with up to 25% white lupin meal in the carp diet with no reduction in growth performance, feed utilization, body composition, gut integrity, or health status.

Green Pea

Green pea (GP) (*Pisum sativum* L.) is another legume that has been used in livestock feeds for a long time as a source of energy and protein but has only been recently evaluated in feed for aquatic species (Sonesson et al., 2005). Early studies by Kaushik et al. (1995) demonstrated that dehulling, extrusion, and milling improved GP digestibility. GP has been studied as an alternative protein source for FM and impacts growth performance, feed utilization, and phosphorus excretion for Asian sea bass, *Lates calcarifer.* The obtained results revealed that GP can replace FM at the 10% level in diets for sea bass without adverse effects on growth, feed utilization or body composition, which may also contribute to environmental protection and to lower feed costs to sustain aquaculture (Ganzon-Naret, 2013). Likewise, Borlongan et al. (2003) confirmed that GP meal could be used as a dietary feed ingredient and can replace up to 20% total dietary protein in the milkfish (*Chanos chanos*) diet.

Faba Bean

Faba bean (FB) (*Vicia faba*) is a widespread and relatively unexploited legume crop in Europe with potentially favorable characteristics, including low ANF levels – with the highest concentration in the seed coat, which can be removed during the dehulling process (El-Shemy et al., 2000). FB can be successfully used as an FM replacement because of its relatively high protein content (20–40%) and good amino acid profile (El-Shemy et al., 2000). A study by Ouraji et al. (2013) demonstrated that rainbow trout fingerlings could tolerate up to 30% FB inclusion in diet and that 15% inclusion levels were beneficial for growth performance. Similar studies have been performed in *Atlantic salmon*, in which the inclusion rates of 35% FB protein concentrate were well accepted and had no negative impact on either growth or immune capacity (De Santis et al., 2015).

LEMNA AND PEANUT

Lemna sp. are widely used as a model plant to treat wastewater (Nasar et al., 2014) and to partially replace FM, with good Indian major carp growth performance (Kaur et al., 2012). Peters et al. (2009) evaluated the nutritional quality of duckweed meal (*Lemna obscura*) as an ingredient for preparing food for red tilapia. They concluded that up to 25% can be included in the diet of fingerlings without affecting growth, as long as it is combined with other high protein ingredients. Solid residue from peanut, obtained after oil extraction, is known as peanut meal. It contains 48% protein, but it is deficient in amino acids, like methionine and lysine. Liu et al. (2011) suggested that 50% FM replacement with peanut meal (diet with 14% peanut meal) could be included in practical *L. vannamei* diets. The potential of peanut leaves has also been studied, as has their utilization by fish for their high protein content (22.3%), which was comparable to that of FM, and can replace the latter by up to 20% without negative effects on growth performance (Yue et al., 2012).

CORN GLUTEN MEAL

Corn gluten meal (CGM) is another important alternative protein source in aquafeed production. CGM is considered the major protein portion obtained from the wet milling process to separate starch, germ, protein, and fiber components from corn (Anderson and Lamsal, 2011). Compared to other vegetable protein sources, CGM is a cost-effective alternative protein source for aquafeed, given its high available protein content (60–70% of DM), low fiber and ANF content, competitive price, and steady supply (Glencross, 2016). CGM has been shown to successfully replace more than half the FM protein used in the diets of juvenile cobia, Japanese seabass, and sea bream and has no negative effect on growth performance (Luo et al., 2012; Pereira et al., 2003). However, in turbot, CGM negatively affects gut health by inducing enteritis and by decreasing intestinal immunity and antioxidant capacity (Bai et al., 2019).

CANOLA

The tested seed meal of Canola (*Brassica napus*) has experimentally achieved success. Canola is a vegetable oil deriving from rapeseed and is rich in the marine fatty acid DHA. It has high protein content and is also used as a feed ingredient in animal nutrition (Chakraborty et al., 2019). Canola meal has been used in the diet of several species, such as salmon, trout, carp, catfish, sea bass, tilapia, perch, sea bream, and shrimp, with similar results to those found in SBM (Enami, 2011). The first studies by Hardy and Sullivan (2011) suggested 20% canola meal inclusion in the rainbow trout (*Salmo gairdneri*) diet had no effect on growth. Similar results with canola have been observed for rainbow trout (Shafaeipour et al., 2008). According to Webster et al. (1997), incorporating canola meal into channel catfish (*Ictalurus punctatus*) diet is cost-effective compared to other vegetable protein ingredients used in commercial catfish feed. Buchanan et al. (1997) observed how enzyme-treated canola meal in the diets of juvenile *Penaeus monodon* led to better fish conversion ratios and greater weight gain compared to others. Furthermore, Soares et al. (2001) suggested 35–40% canola meal inclusion to replace 48.17% of soya bean meal in Nile tilapia diet.

POTATO PROTEIN CONCENTRATE

Potato protein concentrate (PPC) isolate, obtained by a thermal coagulation process from processing waste potato juice (a by-product of the potato starch manufacturing industry), is a promising FM substitute candidate for its high crude protein content (more than 750–800 g/kg per weight) and nutritionally balanced EAA (Refstie and Storebakken, 2001). Early studies into aquaculture with PPC diets gave a slightly negative result for use and showed that solanine and chaconine, anti-nutritional compounds, influenced diet quality (Tacon and Jackson, 1985; Xie and Jokumsen, 1998). Similarly, the inclusion of up to 26% in rainbow trout feed implies no adverse effect on growth (Tusche et al., 2012). The utility of PPC has also been detailed in Atlantic salmon (*Salmo salar*), and there are reports that formulating feed to contain 21% PPC has no negative effect on growth (Refstie and Storebakken, 2001). Xie et al. (2001) showed that protein digestibility reached 93% in Gibel carp (*Carassius gibelio*) that were fed a diet comprising 32% PPC, which is the equivalent to that of FM. Experiments with *Tilapia zilli* revealed that up to 15% sweet potato leaf meal can be included in diets without compromising either growth or feed efficiency (Adewolu et al., 2008). Other results obtained by Takakuwa et al. (2020) showed that PPC can replace up to 20% FM, which is originally contained at 60% in the diet of greater amberjack (*Seriola dumerili*), and with no effect on growth or feed efficiency. However, the protein digestibility of PPC is lower, and the viscera of the fish fed PPC tend to be small.

PALM KERNEL MEAL

Palm kernel meal (PKM) is another alternative in aqua feed ingredients. It is cultivated mainly for its high oil content. PKM is the by-product that forms as a result of the palm kernel oil extraction process. Its incorporation as fish feed is restricted for its lower protein content (< 20%) (Chakraborty et al., 2019). Ng and Cheng (2002) reported that 20% PKM dietary inclusion in tilapia led to better growth performance. The Nile tilapia fingerlings fed 60% PKM displayed similar growth performance to that of the fish fed with an FM-based diet (Omoregie et al., 1993). Studies by Ng et al. (2002) revealed that commercial feed enzyme-treated PKM incorporated into the diet of red tilapia resulted in better growth performance than the fish fed raw PKM. The results obtained in that study indicated that the inclusion of enzyme-treated PKM up to 30% did not involve any significant decrease in fish feed utilization and growth. Souza et al. (1997) reported how pacu fish (*Piaractus mesopotamicus*) fed diets containing 70% PKM showed less digestibility (54%) compared to copra meal (a coproduct of coconut oil production). Studies performed in hybrid catfish have demonstrated that 20% PKM could be incorporated and have no negative impacts on growth performance (Ng et al., 2002).

OTHER VEGETABLE PROTEIN SOURCES

Other vegetable protein sources have been investigated. Castillo et al. (2002) studied employing coffee pulp to feed tilapia fingerlings, and concluded that this input can be included in diet up to 25% without affecting production indices (Bayne et al., 1976). It also highlights that those diets, in which coffee pulp has been used, are cheaper than conventional diets. Likewise, Delgado et al. (2006) evaluated diets for tilapia containing four roatan banana flour levels (10%, 20%, 30%, 40%). These authors concluded that diet with 10% banana flour presented the best results for weight gain, SGR, and feed conversion index. Diets prepared by combining 30% fish offal meal, 5% FM, and 24% mulberry leaf meal for herbivorous *H. fossilis* fingerlings achieved 75% FM substitution (Mondal et al., 2011).

CONCLUSIONS

In conclusion, the results obtained by the current studies performed with vegetable products are promising and encourage further experimentation to meet seafood production and environmental sustainability. Currently, good results are being obtained when replacing FM and combining several plant sources and when vegetable protein sources are supplemented with amino acids.

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ABBREVIATIONS

ALA –: linolenic acid
ARA –: arachidonic acid
CP –: crude protein
DHA –: docosahexaenoic acid
DW –: dry weight
EPA –: eicosapentaenoic acid
LA –: linoleic acid
BFT –: Biofloc technology
CW –: clear-water system
EAA –: essential amino acids
FA –: fatty acids
FBW –: final body weight
FCR –: feed conversion ratio
FM –: fishmeal
HI –: *Hermetia illucens*
HUFA –: highly unsaturated fatty acids
IBW –: initial body weight,
MUFA –: monounsaturated
PER –: protein efficiency ratio
PKM –: palm kernel meal
PPC –: potato protein concentrate
PUFA –: polyunsaturated fatty acids
SGR –: specific growth rate
SFA –: satutared fatty acids
TM –: *Tenebrio molitor*