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Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production

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Abstract

Sustainability concerns associated with protein sources and currently used fishmeal and plant-based meal have necessitated the quests for novel sustainable ingredients for use in aquafeeds. Yeasts have been proposed as sustainable ingredients particularly because of their potential to valorise non-food lignocellulosic biomass into valuable protein resources. Prior to now, extensive studies exist on the role of yeast cell wall components in modulating health responses of fish. However, research on its use as a major protein source in fish diets is still in its infancy. The current review collates, synthesises and discusses the prospects of five major yeast species as future protein ingredients with respect to their nutritional adequacy in fish. Nutritional quality of *Saccharomyces cerevisiae*, *Cyberlindnera jadinii*, *Kluyveromyces marxianus*, *Blastobotrys adeninivorans* and *Wickerhamomyces anomalus* and their use as replacement for fishmeal and soy protein in the diets of Atlantic salmon and rainbow trout are discussed based on three protein quality indices: chemical score, essential amino acid index and ideal protein concept based on the first limiting amino acids, methionine. The crude protein contents of yeast (40–55%) are lower than that of fishmeal, but comparable with soya bean meal. Compared to fishmeal, the different yeast species have favourable amino acid profiles, except for methionine, lysine, arginine and phenylalanine which are the frequently limiting essential amino acids in Atlantic salmon and rainbow trout. This review also presents future area of research and emphasise the need for large-scale production of yeast at competitive price to constitute a feasible replacement for fishmeal and soy protein in aquaculture.

Key words: amino acids, aquafeeds, nutritional values, protein quality, protein-rich ingredients, yeast.

Introduction

Aquaculture is the fastest-growing food production sector in the world. With 5.8% annual growth rate since 2010, aquaculture continues to surpass other food production sectors (FAO 2018). Sustained growth of aquaculture is necessary to meet the future demand for animal protein as a result of continuous increase in human population. However, availability of resources for aquafeed production is a major constraint expected to exacerbate the rapidly expanding aquaculture sector. Traditionally, fishmeal and fish oil have been the major sources of protein and lipids for intensive farming of carnivorous fish species (Tacon & Metian 2008). The stagnation in the forage fish output implies that continuous high inclusion of fishmeal and fish oil in the diets is no longer sustainable (Tacon & Metian 2008). In recent time, salmon farming has shown reduced dependence on marine ingredients by replacement with plant ingredients, particularly soy protein concentrate (Ytrestøyl *et al.* 2015). This is evident in the reduction in fish-in:fish-out ratio (FIFO) over the years, from 2.57:1 in 2000 to about 0.82:1 at the end of 2015 (IFFO 2017). A major reason for using processed soy products such as, soy protein concentrate is that saponins and other anti-nutritional constituents in conventional soya bean meal can cause distal intestine enteritis and consequently regressed growth in Atlantic salmon and rainbow trout (Van den Ingh *et al.* 1991; Iwashita *et al.* 2009; Chikwati *et al.* 2012; Krogdahl *et al.* 2015). The transition to plant-based ingredients also raises serious ethical and sustainability concerns. The use of more plant-based ingredients in aquafeeds may contribute to intensified crop production, imposing

pressure on land and water use, energy, resource allocation and forest biodiversity (Pahlow *et al.* 2015; Fry *et al.* 2016). More importantly, the use of soy protein and other plant products in aquaculture reduces their availability for direct human consumption (Ytrestøyl *et al.* 2015). Thus, there is an emerging need for suitable and sustainable novel feed ingredients for aquaculture. More than ever, the quest for novel feed ingredients is gaining attention. At the forefront of this attention is microbial ingredients, particularly yeast, as potential feed ingredients.

One reason why yeasts are potential sustainable ingredients is their ability to convert low-value non-food biomass from forestry and agricultural industry into high-value feed with less dependence on arable land, water and changing climatic conditions (Anwar *et al.* 2014; Couture *et al.* 2019; Lapeña *et al.* 2020a; Lapeña *et al.* 2020b). Yeast cells contain appreciable crude protein (about 40–55%), and other bioactive components beneficiary to fish growth and development (Øverland *et al.* 2013; Hansen *et al.* 2019; Rawling *et al.* 2019; Vidakovic *et al.* 2020). Research on yeast products in fish diets have centred on their roles as nutritional supplements and functional supplements with beneficial effects on the immune responses and gut health in fish (Yilmaz *et al.* 2007; Torrecillas *et al.* 2012; Eryalçin *et al.* 2017). The cell walls represent 26–32% of the dry weight and contain mannan-oligosaccharides (MOS), β-glucan and chitin (Klis *et al.* 2002; Schiavone *et al.* 2014). Over the years, extensive scientific reviews have elucidated the health benefits of these cell wall components in various species, but little information exists on the role of yeast as macro-ingredient in fish feeds (Meena *et al.* 2013; Torrecillas *et al.* 2014). Therefore, this review aims at describing the potential of yeast as protein sources in fish feeds, particularly for Atlantic salmon and rainbow trout. Furthermore, this review focuses on *Saccharomyces cerevisiae* and four non-saccharomyces species that have been documented or are currently under investigation as aquafeed ingredients. The non-saccharomyces of interest are: *Cyberlindnera jadinii* (anamorph name *Candida utilis*), *Kluyveromyces marxianus*, *Blastobotrys adeninivorans* (synonym *Arxula adeninivorans*) and *Wickerhamomyces anomalus* (Øverland *et al.* 2013; Huyben *et al.* 2017; Hansen *et al.* 2019; Vidakovic *et al.* 2020; Lapeña *et al.* 2020a; Lapeña *et al.* 2020b).

Yeast as an efficient bio-converter of non-food biomass

Traditionally, molasses is used as principal raw material in the production of yeast. However, the surge in price and application of molasses in other industrial processes (CIBE 2017) has necessitated the needs for new substrate sources for yeast production. Because of serious environmental concerns such as biodiversity, water and land use, as well as, competition with human food, the first-generation feedstock (mainly food biomass) may be less desirable as substrates for yeast fermentation. Instead, second-generation feedstock, representing non-food biomass, is gaining increasing attention as carbon sources for yeast production. Second-generation feedstocks, such as lignocellulosic biomass, represent the most economical and renewable resources in the world for biofuel production (Anwar *et al.* 2014). Lignocellulosic biomass contains highly complex network of polysaccharides such as cellulose, hemicellulose and lignin, which are not easily hydrolysed by acid, alkaline or enzyme treatments. The main sources of lignocellulosic biomass are from the agricultural and forestry sectors. Yeast offers a great opportunity for conversion of highly non-hydrolysable lignocellulosic biomass into biofuel with tremendous industrial applications.

The presence of fermentable sugars as carbon sources is crucial for efficient yeast production. However, unlike molasses, lignocellulosic biomass first needs to be delignified and saccharified into fermentable sugars for yeast production. To obtain fermentable sugars for yeast fermentation, lignocellulosic biomass undergoes two major processing steps: pre-treatment and enzyme hydrolysis (Binder & Raines 2010; Anwar *et al.* 2014). Pre-treatment entails breaking down the highly complex polysaccharide structure of the lignocellulosic biomass, thereby disentangling them into lignin, cellulose and hemicellulose (Mosier *et al.* 2005; Binder & Raines 2010). In addition, pre-treatment also facilitates disruption of the crystalline structure of the cellulose and hemicellulose, making them more accessible before enzyme hydrolysis to monosaccharides. Methods commonly used for pre-treatment are physical, chemical or a combination of both methods (Mosier *et al.* 2005). Physical treatment uses mechanical milling, whereas chemical treatment mainly uses acid or alkaline treatment (Mosier *et al.* 2005). The choice of pre-treatment methods often depends on the nature and resistance of the biomass to enzymatic and microbial actions. Woody biomass requires more stringent pre-treatment conditions than non-woody biomass (Øverland & Skrede 2017). Enzyme hydrolysis occurs after pre-treatment to break down the biomass into fermentable sugars. It entails degrading the cellulose and hemicellulose into pentose and hexose sugars. The efficiency of enzymatic breakdown of cellulose is influenced by conditions such as temperature, time, pH, enzyme loading and substrate concentration (Horn *et al.* 2012). Figure 1 shows typical steps in production of yeast from molasses and lignocellulosic biomass.

Multi-functional values of yeast cell walls

The cell wall is an important component of the yeast cell architecture. It is vital for growth, shape, protection,

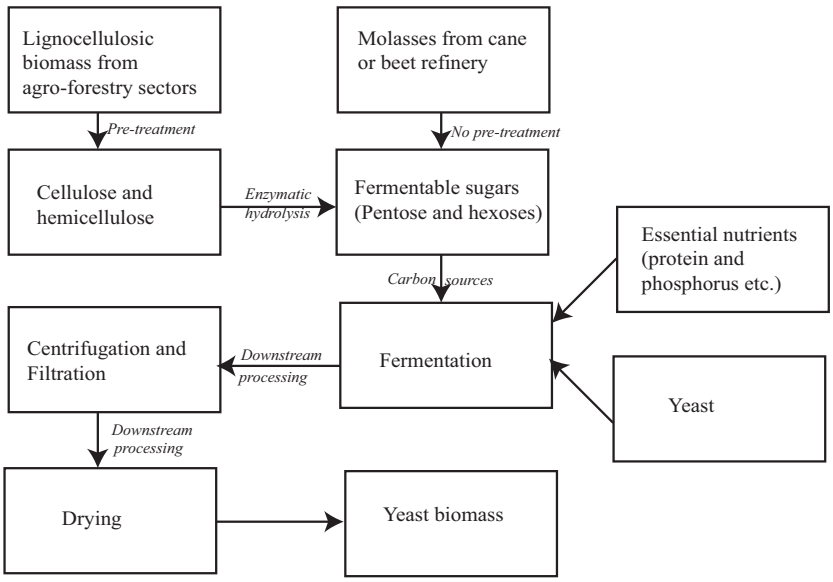

Figure 1 Fermentation process for converting low-value product into high-value yeast biomass (modified from Øverland and Skrede (2017)).

survival and morphogenesis of yeast. Generally, the cell wall represents 26–32% of the total dry weight of the cell (Fleet 1985; Nguyen *et al.* 1998; Klis *et al.* 2002). The cell wall principally contains about 85–90% polysaccharides and 10–15% protein (Nguyen *et al.* 1998; Schiavone *et al.* 2014). Glucan and mannan are the main polysaccharides, with small amounts of chitin. The cell wall structure of the extensively studied species *S. cerevisiae* typically contains 30–60% glucans, 25–50% of mannans and 5–10% of chitin (Fleet & Manners 1976; Fleet 1985; Schiavone *et al.* 2014). The mannan polysaccharides are in complex with the cell wall protein and are more correctly designated as mannoprotein. The chemical composition of the cell wall depends on the species and strains of yeast, fermentation substrates and the methods used for analysis (Papatryphon *et al.* 1999). The cell wall composition of yeast can be determined by chemical or enzymatic treatment or a combination of both methods, as previously highlighted by Magnelli *et al.* (2002) and Schiavone *et al.* (2014). These methods not only determine the content of total glucan, but also distinguish between the β-1,3 and β-1,6 glucan. Chemical analysis of yeast cell walls and separation into individual polysaccharide components continue to face further research aiming at producing well-refined, pure forms of these polysaccharides. Additionally, the current methods were developed for *S. cerevisiae* and there is possibility that further optimisation may be required for non-saccharomyces species.

In recent time, the use of derivatives from the yeast cell wall has become more prominent in the animal feed industry. This is in part due to governmental restrictions and elimination of prophylactic growth-promoting antibiotics in animal feeds within the European Union and United States. The ban of antibiotics in animal feeds consequently stimulated interest in using alternative products (including yeast derivatives) to support animal health and growth performance. There is evidence to show that dietary β-glucans enhance immune responses and survival of the host after a pathogen infection in fish, including Atlantic salmon (Robertsen *et al.* 1990; Bridle *et al.* 2005), rainbow trout (Siwicki *et al.* 2004; Guselle *et al.* 2007), European seabass (Bonaldo *et al.* 2007). Regardless of the health stimulating function performed by β-glucan, it seems to exert its mode of action in a dectin-1 dependent manner. Dectin-1 receptor is highly expressed on the surface of several immune cells such as dendritic cells, neutrophils, eosinophils, macrophages, monocytes and some T-cells (Volman *et al.* 2008). β-glucan binds to the dectin-1 receptor to activate NF-κB through intracellular signalling, which in turn leads to cytokine production, phagocytosis and respiratory burst (Volman *et al.* 2008). Yeast-derived β-glucans have also been used to adsorb or bind toxins, viruses and pathogenic bacteria (Volman *et al.* 2008).

Like β-glucan, MOS from yeast cell walls also exert beneficial and health stimulating effects in different animal species. Many reports have concluded that dietary inclusion of MOS can positively influence health and growth performance of fish, including Atlantic salmon (Refstie *et al.* 2010), rainbow trout (Staykov *et al.* 2007; Yilmaz *et al.* 2007), European sea bass (Torrecillas *et al.* 2011;

Torrecillas *et al.* 2012) and rohu (Andrews *et al.* 2009). Furthermore, dietary MOS can be used to modulate gut morphology (Eryalçin *et al.* 2017; Schmidt *et al.* 2017) and to enhance skin mucous barrier function in fish (Micallef *et al.* 2017). The most recognised mechanism of action associated with MOS is its ability to bind to enteropathogenic bacteria, preventing host colonisation (Torrecillas *et al.* 2014). This is carried out by binding to the mannose specific lectin-type receptor (Type 1 fimbriae) present on the surface of enteropathogenic bacteria through its branched α-mannosides, thereby preventing adhesion to the surface glycoproteins of intestinal villi (Firon *et al.* 1983; Torrecillas *et al.* 2014; Rawling *et al.* 2019). Several studies have documented the positive effects of both β-glucan and MOS in fish, while others have shown no effects on many of the parameters studied as shown in Table 1. The inconsistencies observed across different experiments may be due to the molecular structure of β-glucan or MOS used, dose and time of feeding, fish species used, stage of growth, culture conditions and health status of fish (Torrecillas *et al.* 2014). Shelby *et al.* (2009) and Lokesh *et al.* (2012) indicated that the effects of these oligosaccharides are more apparent in fish challenged with infection, suggesting their potency during clinical conditions. Detailed reviews on the role of yeast-derived β-glucan and MOS, and their mode of action in fish have been previously provided by Meena *et al.* (2013), Torrecillas *et al.* (2014) and Shurson (2018).

Table 1 Summary of growth and health beneficial effects of yeast-derived β-glucan and mannan-oligosaccharides in fish compared with control diets (without β-glucan or mannan-oligosaccharides inclusions)

Parameters	Positive effects	No effects	Responses considered as positive effects per category
β-glucans†			
Growth rate	1	7	Increased weight gain Reduced feed intake Increased specific growth rate
Feed: Gain	0	8	Reduced feed conversion ratio Increased feed efficiency
Immune response	15	3	Increased survival rate Protection against infection Upregulation of pro-inflammatory cytokines Downregulation of anti-inflammatory cytokines Improved serum biochemistry
Mannan-oligosaccharides‡			
Growth rate	12	19	Increased weight gain Reduced feed intake Increased specific growth rate Increased nutrient absorption
Feed: Gain	6	18	Reduced feed conversion ratio Increased feed efficiency
Immune response	15	5	Increased survival rate Protection against infection Upregulation of pro-inflammatory cytokines Downregulation of anti-inflammatory cytokines Improved serum biochemistry Improved gut barrier function

†Adapted from Meena *et al*. (2013).
‡Adapted from Torrecillas *et al*. (2014).

Nutritional composition of common yeast of interest for aquaculture

Saccharomyces cerevisiae has been the most commonly used yeast species in aquaculture, particularly for its health stimulating effects in various fish species. However, in recent time, there has been an increased focus on non-saccharomyces species with potential values in aquaculture. The utilisation of different substrates influences the chemical composition of different yeast species. For instance, yeast species such as *S. cerevisiae* are strictly efficient at metabolising hexose sugars, whereas others are efficient fermenters of pentose sugars. However, the strict preference for a specific type of sugar, can be resolved through genetic engineering (Wahlbom *et al.* 2003; Attfield & Bell 2006) or using yeast that can co-ferment both hexose and pentose sugars (e.g. *C. jadinii* and *K. marxianus*) (Parajó *et al.* 1995; Yanase *et al.* 2010) or through co-culture of two yeast strains (Azhar *et al.* 2017). Furthermore, environmental conditions such as temperature, oxygen and pH often influence the nutritional composition of whole yeast cells (Halasz & Lasztity 1991).

The nutritional compositions of *S. cerevisiae* and some non-saccharomyces species are presented in Table 2. It is noteworthy to mention that this study considers inactivated yeast or autolysed dry yeast, but not yeast extracts in the calculation of nutritional composition of yeast. The reported crude protein content ranges from 38 to 52% for the five yeast species, although limited data were found for *K. marxianus*, *B. adeninivorans* and *W. anomalus*. Yeast crude protein contains considerable amounts of non-protein nitrogen in the form of nucleic acids, about 10–25% of crude protein depending on yeast species, growth media, the growth rate and the methods used for analysis (Halasz & Lasztity 1991; Rumsey *et al.* 1991b; Øverland *et al.* 2013; Lapeña *et al.* 2020a). In most monogastric animals, elevated concentrations of plasma uric acid due to high dietary nucleic acids interfere with normal protein, fat, carbohydrate and uracil metabolism (Rumsey *et al.* 1992). However, this is not the case in some fish, as salmonids synthesise considerable level of urate oxidase, and are thereby able to metabolise relatively high levels of nucleic acids (Kinsella *et al.* 1985; Rumsey *et al.* 1991b;

Table 2 Nutritional composition (g/kg dry matter) of selected yeast species of commercial importance

	Saccharomyces cerevisiae†		*Cyberlindnera jadinii*‡		*Kluyveromyces marxianus*§		*Blastobotrys adeninivorans*¶		*Wickerhamomyces anomalus*¥	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry matter	939	27 (6)	943	29 (9)	943	5 (2)	948	5.6 (4)	943	7.5 (4)
Crude protein	501	102 (10)	463	66 (10)	531	28 (2)	382	8.4 (4)	528	1.2 (4)
Crude lipids	18	27 (8)	23	21 (10)	7	2 (2)	85	0.3 (4)	89	1.6 (4)
Ash	75	39 (9)	91	36 (10)	76	0 (2)	62	1.2 (4)	33	0.6 (4)
Gross energy	18	2 (6)	19	3 (5)	21	NA	22	0.2 (4)	23	0.1 (4)
Starch	46	33 (3)	37	0 (2)	8	NA	NA	NA	NA	NA
Nucleic acids	48	28 (4)	104	16 (2)	102	NA	NA	NA	NA	NA

Values in parenthesis are the number of studies used for calculating the mean and standard deviation for each yeast species.
Sources: †Chanda and Chakrabarti (1996), Pacheco *et al*. (1997), Cheng *et al*. (2004), Spark *et al*. (2005), Yamada and Sgarbieri (2005), Yalcin *et al*. (2011), Øverland *et al*. (2013), Kim *et al*. (2014), Vidakovic *et al*. (2016); ‡Valdivie *et al*. (1982), Martin *et al*. (1993), Chanda and Chakrabarti (1996), Olvera-Novoa *et al*. (2002), Rodríguez *et al*. (2011), Øverland *et al*. (2013), Sharma *et al*. (2018), Hansen *et al*. (2019), Sharma (thesis, unpublished); §Revillion *et al*. (2003), Øverland *et al*. (2013); ¶&¥Lapeña *et al*. (2020b), Lapeña *et al*. (2020a) and unpublished data from in-house trials.
NA, not available.

Andersen *et al*. 2006). Nucleic acids may have protein-sparing effects and enhance immune responses and growth of epithelial cells in several fish species including salmonids (Øverland & Skrede 2017). Despite higher contents of nucleic acid, yeasts show comparatively similar composition of amino acids with fishmeal and soy protein, except for sulphur-containing methionine and cysteine, which are characteristically low in yeast (Tables 3 and S1). The amino acid compositions, as shown in Table 3, vary among the different yeast species. The data indicate that *S. cerevisiae* have higher content of methionine and cysteine, but lower content of lysine than the other yeast species. Similarly, *B. adeninivorans* has lower content of arginine compared to other yeast species. Glutamic acid is consistently high in all the yeasts considered. The variation in amino acids profile of yeasts can be attributed to difference in species and strains, substrate media used, culturing conditions, downstream processing and analytical methods used during the production process (Øverland *et al*. 2013).

Yeasts have relatively low lipid content, high ash content and moderate levels of carbohydrates (Halasz & Lasztity 1991; Øverland *et al*. 2013). The fatty acid composition is characterised mainly by unsaturated fatty acids (Halasz & Lasztity 1991; Brown *et al*. 1996). The carbohydrates are predominately polysaccharides, with low amounts of mono- and oligosaccharides except trehalose (Halasz & Lasztity 1991). Aside from these macronutrients, yeasts are moderate sources of other valuable components such as vitamins (mostly B-group vitamins), minerals and enzymes (Lapeña *et al*. 2020a). Mineral contents vary between the different yeast species; and is greatly influenced by the amounts of corresponding minerals in the growth media. For instance, yeasts grown in media containing considerable amount of calcium (whey, calcium lignosulfonate, sulphite waste liquor) are known to be high in calcium content (Halasz & Lasztity 1991). This ability of yeast to efficiently incorporate minerals present in the culturing media, is the mechanism behind the production of selenium (Se) yeast. Selenium yeast is a type of specialty yeast produced commercially and marketed as a highly bioavailable form of Se (selenomethionine) and has a unique role of improving antioxidant status of animals (Schrauzer 2006; Han *et al*. 2017; Wang *et al*. 2018).

Nutritional adequacy of yeast as a sustainable protein ingredient for salmonids

Protein quality indices using the amino acid profile of yeasts, fishmeal, soya bean meal and their corresponding requirements in Atlantic salmon and rainbow trout, as shown in Table 3 (with Table S1), form the basis of this section. Comparatively, the total essential amino acid contents of yeasts in general meet the amino acids requirements of Atlantic salmon and rainbow trout (Fig. 2a,b). The protein quality of yeasts and the conventional fishmeal and soya bean meal throughout this calculations, are evaluated based on the estimated digestible amino acid contents. There is paucity of information on protein and amino acid digestibility of yeasts in literature. From the few available studies, protein digestibility values of yeasts in different fish species vary from 40 to 90% depending on species and strains of yeast, as well as the type of downstream processing used after fermentation (Rumsey *et al*. 1990; Barrows *et al*. 2011; Øverland *et al*. 2013; Sharma *et al*. 2018). These values are mainly reported for *S. cerevisiae* and *C. jadinii*; there are no data on protein digestibility coefficient of

Table 3 Average amino acid composition (g/16 g nitrogen) of selected yeast species of commercial importance

	Saccharomyces cerevisiae†	*Cyberlindnera jadinii*‡	*Kluyveromyces marxianus*§	*Blastobotrys adeninivorans*¶	*Wickerhamomyces anomalus*¥
Essential amino acids					
Arginine	4.3 (6)	5.1 (10)	4.1 (3)	2.3 (4)	4.7 (4)
Histidine	2.0 (6)	1.8 (10)	1.7 (3)	2.3 (4)	2.6 (4)
Isoleucine	4.3 (6)	4.1 (10)	4.0 (3)	4.3 (4)	5.0 (4)
Leucine	6.5 (6)	6.2 (10)	6.4 (3)	6.2 (4)	6.9 (4)
Lysine	6.4 (6)	6.9 (10)	6.8 (3)	6.7 (4)	6.9 (4)
Methionine	1.8 (6)	1.1 (10)	1.3 (3)	1.4 (4)	1.5 (4)
Phenylalanine	3.9 (6)	3.6 (10)	3.9 (3)	3.5 (4)	3.9 (4)
Threonine	4.4 (6)	4.6 (10)	5.0 (3)	3.7 (4)	4.6 (4)
Tryptophan	1.0 (6)	1.4 (6)	1.0 (1)	NA	NA
Valine	5.1 (6)	5.0 (10)	4.6 (3)	5.1 (4)	4.5 (4)
Non-essential amino acids					
Alanine	5.9 (6)	5.4 (7)	7.9 (3)	5.0 (4)	5.0 (4)
Aspartic acid	9.1 (6)	8.6 (6)	10.1 (3)	7.1 (4)	8.0 (4)
Glycine	4.2 (6)	3.8 (7)	4.1 (3)	3.9 (4)	4.2 (4)
Glutamic acid	12.5 (6)	12.1 (7)	13.3 (3)	10.8 (4)	11.0 (4)
Cysteine	1.3 (6)	0.8 (8)	0.6 (3)	0.6 (4)	0.7 (4)
Tyrosine	3.5 (6)	2.9 (7)	3.0 (3)	4.0 (4)	2.7 (4)
Proline	3.8 (6)	3.4 (6)	3.6 (3)	4.4 (4)	3.7 (4)
Serine	4.2 (6)	4.3 (7)	5.3 (3)	3.0 (4)	3.8 (4)

Values in parenthesis are the number of studies used for calculating the average for each yeast species.
Sources: †Pacheco *et al*. (1997), Cheng *et al*. (2004), Øverland *et al*. (2013), Kim *et al*. (2014), Vidakovic *et al*. (2016); ‡Prior *et al*. (1981), Valdivie *et al*. (1982), Martin *et al*. (1993), (Nigam 1998), Olvera-Novoa *et al*. (2002), Øverland *et al*. (2013), Sharma *et al*. (2018), Hansen *et al*. (2019), Sharma (thesis, unpublished); §Anderson *et al*. (1988), Øverland *et al*. (2013); ¶&¥Lapeña *et al*. (2020b), Lapeña *et al*. (2020a) and unpublished data from in-house trials.
NA, not available.

K. marxianus, *B. adeninivorans* and *W. anomalus*. For this reason, it becomes apparently impossible to compare the nutritional values of individual yeasts based on their specific protein and amino acid digestibility. Therefore, to bypass this limitation, the digestible amino acid contents of yeasts (i.e. *S. cerevisiae*, *C. jadinii*, *K. marxianus*, *B. adeninivorans and W. anomalus*) (presented in Table S2) used for all the necessary calculations were based on amino acid digestibility coefficient of 80% – the expected target digestibility coefficient for yeast to be able to nutritionally compete with the conventional ingredients. Furthermore, amino acid digestibility coefficients of 90% and 85% were used for fishmeal and soya bean meal, respectively, throughout this article (presented in Table S2) (Glencross *et al.* 2004; Barrows *et al*. 2011). Radar charts of digestible amino acids indicate that the contents of some amino acids in yeasts are below the requirements of Atlantic salmon and rainbow trout (Fig. 2c,d); these amino acids below the requirements of fish are otherwise referred to as limiting amino acids. To gain further insights into the limiting amino acids in the different yeast species, protein quality indices such as, chemical score, essential amino acids index (EAAI) and ideal protein concept are employed in this article.

Chemical score and EAAI

The protein value of ingredients can in principle be evaluated based on chemical scoring system proposed by Mitchell and Block (1946) and recently modified by Veldkamp and Bosch (2015) to quantify protein quality of novel feed ingredients. This method is used to determine the single essential amino acid in maximum deficit compared to a reference protein. Nine essential amino acids (excluding tryptophan), were used in calculating the chemical score and EAAI to test the concept of ideal protein based on the amino acid requirements of juvenile Atlantic salmon and rainbow trout (Table S1). Tryptophan was exempted because contents in most yeasts are scarcely reported in literature. As shown in Figure 2, digestible amino acids values are closer to Atlantic salmon requirements, compared to total amino acids values. Therefore, for each ingredient, chemical score was calculated from the ratio of each digestible amino acid and the corresponding requirements in Atlantic salmon and rainbow trout. The resultant ratios were then compared with fishmeal as the reference protein source. The chemical score for *S. cerevisiae*, *C. jardinii*, *K. marxianus*, *B. adeninivorans*, *W. anomalus*, soya bean meal and

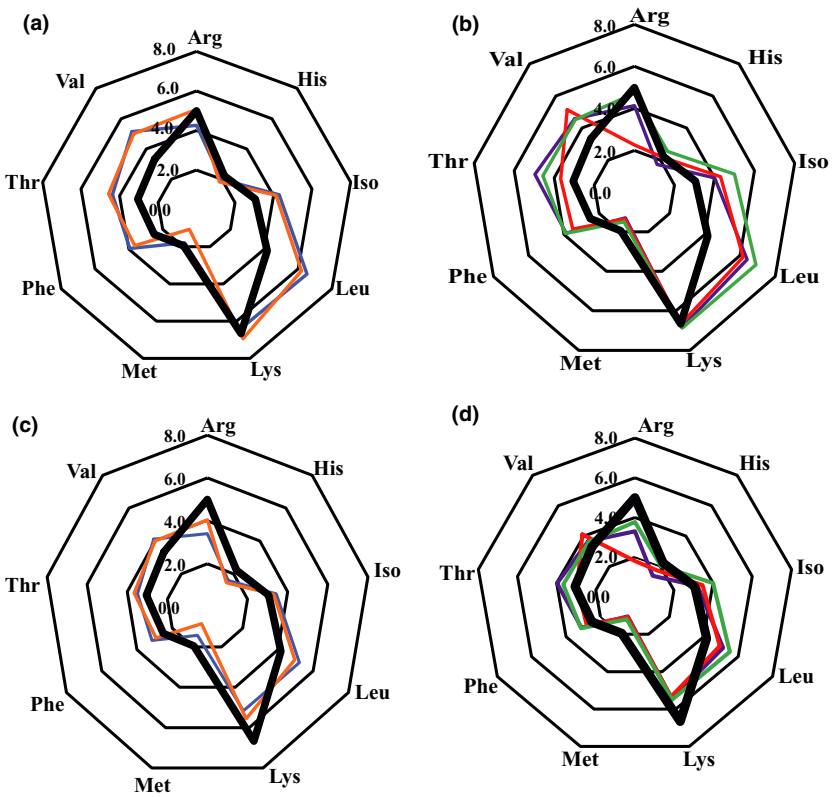

Figure 2 Radar plots (in g/16 g nitrogen) showing the comparison of total (a, b) and digestible amino acids (c, d) in the selected yeast species with the corresponding requirements in Atlantic salmon (similar trends were observed for rainbow trout, not presented to avoid repetition)[†,‡,§]. [†]The digestible amino acids content was calculated from the total amino acids and protein digestibility coefficient of 80% for all the yeast species in both fish species. [‡]SC, *Saccharomyces cerevisiae*; CJ, *Cyberlindnera jadinii*; KM, *Kluyveromyces marxianus*; BA, *Blastobotrys adeninivorans*; WA, *Wickerhamomyces anomalus*; AS, Atlantic salmon. [§]Arg, Arginine; His, Histidine; Iso, Isoleucine; Leu, Leucine; Lys, Lysine; Met, Methionine; Phe, Phenylalanine; Thr, Threonine and Val, Valine. All essential amino acids except tryptophan which values are missing for some yeast ingredients. (—) SC; (—) CJ; (—) KM; (—) BA; (—) WA; (—) AS.

fishmeal are shown in Table 4. The results indicated that *S. cerevisiae*, *C. jadinii*, *K. marxianus*, *B. adeninivorans and W. anomalus* had comparable chemical score with soya bean meal, but lower than fishmeal for both Atlantic salmon and rainbow trout. Veldkamp and Bosch (2015) considered chemical score as the measure of limiting amino acids. Methionine was the first limiting amino acid in most yeast species, except *B. adeninivorans* where arginine was the most limiting (Table 4).

A major limitation of chemical score is that it considers each amino acid as an individual entity, whereas all amino acids work in synchrony during protein synthesis. To sidestep this limitation, a model integrating all the nine essential amino acids (same as in chemical score) was used in estimating the EAAI. The EAAI was calculated according to the method proposed by Oser (1951) and recently used by Smith (2017) and Veldkamp and Bosch (2015), and presented in Equation (1). The EAAI method integrates all the essential amino acids into the nutritional evaluation of protein. The EAAI was defined by Veldkamp and Bosch (2015), as the adequacy between the concentration of all the essential amino acids in the dietary protein and the requirement of the target animal. A protein source completely matching the requirement of a target animal has an EAAI equals to 100, whereas those which amino acids profiles fall below the target animal requirement has EAAI less than 100. In this paper, the EAAI of *S. cerevisiae*, *C. jadinii*, *K. marxianus*, *B. adeninivorans*, *W. anomalus* and soya bean meal were reported relative to fishmeal as the reference protein source, as shown in Figure 3. Consistent with chemical score, the EAAI of *S. cerevisiae*, *C. jadinii*, *K. marxianus*, *B. adeninivorans*, *W. anomalus* and soya bean meal were lower than for fishmeal. Furthermore, *W. anomalus* showed the highest EAAI among the yeast candidates, whereas *B. adeninivorans* had the lowest value. Oser (1951) previously asserted that protein quality rating of an ingredient should be based on the contribution a protein makes in respect to all the essential amino acids rather than simply the first limiting amino acid, because each amino acid has its own specific peculiarity and are all

Table 4 Chemical score of selected yeast species and reference protein ingredients for Atlantic salmon and rainbow trout†

	SC	CJ	KM	BA	WA	SBM	FM
Arginine	67.3	80.1	65.3	35.7	74.5	123.2	100.0
Histidine	74.2	68.0	64.8	81.3	95.0	105.3	100.0
Isoleucine	81.6	78.5	76.1	81.8	94.9	91.3	100.0
Leucine	75.8	72.0	74.4	71.7	80.2	96.0	100.0
Lysine	73.4	79.0	77.8	75.9	78.2	77.7	100.0
Methionine	53.2	31.6	38.5	40.3	44.3	43.9	100.0
Phenylalanine	86.1	79.7	84.7	76.4	85.7	115.0	100.0
Threonine	90.1	94.3	102.4	75.8	94.3	86.1	100.0
Valine	85.2	82.8	75.7	85.1	74.9	96.0	100.0

BA, *Blastobotrys adeninivorans*; CJ, *Cyberlindnera jadinii*; FM, Fishmeal; KM, *Kluyveromyces marxianus*; SBM, soya bean meal; SC, *Saccharomyces cerevisiae*; WA, *Wickerhamomyces anomalus*.

†First, the digestible content of each amino acids was calculated from the total amino acids and protein digestibility coefficients of 80%, 85% and 90% for yeast species, soya bean meal and fishmeal, respectively. The chemical score was then calculated as the ratio of these digestible amino acids and the corresponding requirements in Atlantic salmon and rainbow trout. The values presented are expressed relative to chemical score of fishmeal as the reference protein which is 100 and assumed to be ideal for Atlantic salmon and rainbow trout.

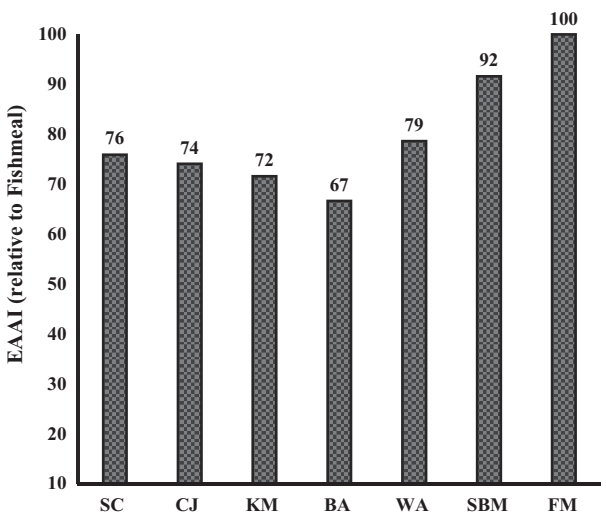

Figure 3 Essential amino acid index (EAAI) of selected yeast species and reference protein ingredients in both Atlantic salmon and rainbow trout†,‡. †The EAAI were calculated based on Equation (1) from the digestible amino acids content of each ingredient and their corresponding requirements in both target fish species. †The EAAI presented are expressed relative to fishmeal as the reference protein which is 100 and assumed to be ideal for Atlantic salmon and rainbow trout. ‡SC, *Saccharomyces cerevisiae*; CJ, *Cyberlindnera jadinii*; KM, *Kluyveromyces marxianus*; BA, *Blastobotrys adeninivorans*; WA, *Wickerhamomyces anomalus*; SBM, soya bean meal and FM, Fishmeal.

equally essential. Thus, EAAI give a true representation of nutritive value of an ingredient, compared to chemical score.

$$\mathrm{EAAI} = \sqrt[n]{\frac{\mathrm{aa1}}{\mathrm{AA1}} \times \frac{\mathrm{aa2}}{\mathrm{AA2}} \times \frac{\mathrm{aa3}}{\mathrm{AA3}} \ldots\ldots\ldots \frac{\mathrm{aa}n}{\mathrm{AA}n}} \quad (1)$$

Sources: Oser (1951), Veldkamp and Bosch (2015) and Smith (2017).

Where, aa is the percentage of each of the essential amino acids in observed protein source.

AA is the requirement of each of the essential amino acids in the target animals.

n is the total number of amino acids used in the calculation.

Ideal protein concept based on limiting methionine

In this paper, we have established through chemical score that methionine is the first limiting amino acid in the selected yeast species. However, from Table 4, it was evident that aside from methionine, there are other essential amino acids responsible for lower values of EAAI recorded for the selected yeasts compared to fishmeal. To deepen our knowledge further on these other amino acids, a multivariate statistical analysis was conducted on the levels of digestible amino acids in the selected yeast, soya bean meal and fishmeal and their corresponding requirements in both Atlantic salmon and rainbow trout. The levels of digestible arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine of the ingredients were expressed as percentage of digestible methionine (Table S3), according to Faria-Filho *et al*. (2005). Likewise, the corresponding requirements of these amino acids in Atlantic salmon and rainbow trout were also expressed as percentage of methionine (Table S3). Linear discriminate function analysis (DFA) (Seron *et al.* 1998) was performed on these data to identify the amino acids (other than methionine) that better contribute to the differentiation of these ingredients from the amino acid requirements of Atlantic salmon and rainbow trout. Methionine (100%) was excluded because it was the basis for standardising the data and because our aim was to identify other amino acids responsible for the discrimination. The eight remaining amino acids were used as the predictor variables and were linearly combined to obtain three discriminant functions. The first two functions (function 1 = 43.4% and function 2 = 37.8%) explained 81.2% of the variation associated with the multivariate structure on the discriminant analysis function plot (Fig. 4). The discriminant power of the model was significant ($P < 0.001$) based on Wilk's Lambda test of significance. As expected, the scattered distribution on the DFA plot showed that amino acids from fishmeal was not clearly differentiated from the amino acid requirement of Atlantic salmon and rainbow trout (located on the left side of the quadrant), but was discriminated by function 1 from *S. cerevisiae*, *C. jadinii*, *K. marxianus*,

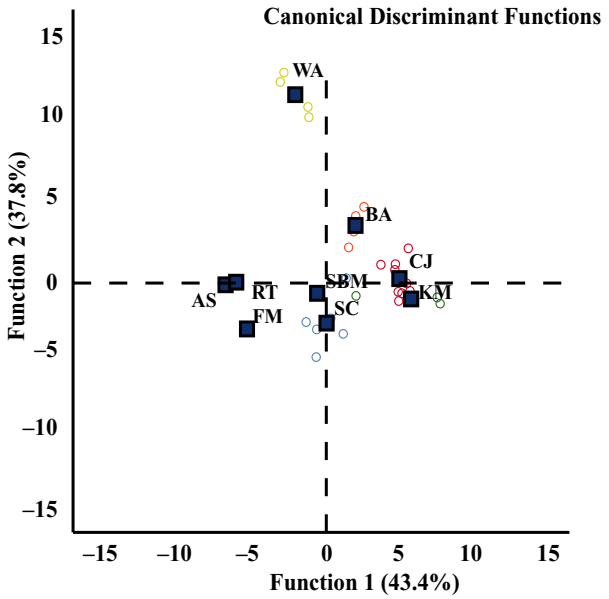

Figure 4 Plot showing the discrimination of the selected protein sources following discriminant function analysis (DFA) of their digestible essential amino acid profile from the corresponding amino acid requirements of Atlantic salmon and rainbow trout. SC, *Saccharomyces cerevisiae*; CJ, *Cyberlindnera jadinii*; KM, *Kluyveromyces marxianus*; BA, *Blastobotrys adeninivorans*; WA, *Wickerhamomyces anomalus*; FM, Fishmeal; SBM, soya bean meal; AS, Atlantic salmon and RT, rainbow trout. (◯) SC; (◯) CJ; (◯) KM; (◯) BA; (◯) WA; (◯) FM; (◯) SBM; (◯) AS; (◯) RT; (■) Group centroid.

B. adeninivorans and soya bean meal (located on the right side of the quadrant). The discriminant power of function 1 was highly influenced by lysine and phenylalanine as indicated by higher positive values of standardised coefficient of variables (Table S4). Function 2, on the other hand, discriminated the amino acid profiles of *W. anomalus* from fishmeal, Atlantic salmon and rainbow trout. Histidine, leucine and isoleucine were the amino acids responsible for the discrimination along function 2 (Table S4). Consistent with the results obtained with chemical score and EAAI, there was no clear discrimination between amino acid profiles of *S. cerevisiae*, *C. jadinii*, *K. marxianus*, *B. adeninivorans* and soya bean meal. Cross-validation of the discriminant model revealed that, among the yeasts, all data points were correctly assigned for *S. cerevisiae*, *B. adeninivorans and W. anomalus*. However, the model inaccuracy revealed that two data points for *C. jadinii* were wrongly classified for *K. marxianus*. The data suggest that apart from methionine, lysine and phenylalanine are also responsible for the variation between the amino acid profiles of selected protein sources (i.e. *S. cerevisiae*, *C. jadinii*, *K. marxianus*, *B. adeninivorans* and soya bean meal) and fishmeal, and their ability to match the amino acids requirements of Atlantic salmon and rainbow trout. On the contrary, histidine, leucine and isoleucine accounted for the discrimination observed with *W. anomalus*.

From this section, there are indications based on EAAI that *W. anomalus* has the best suited amino acids for Atlantic salmon and rainbow trout among all the yeast considered; whereas *B. adeninivorans* has the least suited amino acid profile. The yeasts *S. cerevisiae*, *K. marxianus* and *C. jadinii* are in-between. Furthermore, the chemical score, EAAI and ideal protein concept based on limiting amino acid used in this article are quick and inexpensive methods to support important conclusions on nutritional value of yeasts, especially on their amino acid (im)balance with respect to the requirements in target fish species. As such, with the emergence of different novel ingredients, these methods could be of valuable assistance in the feed industry for pre-screening of ingredients before delving into the actual fish trials. Despite the benefits accrued with these methods, they are confronted with certain limitations, which are briefly highlighted below.

Methodological constraints

Assumption of a single amino acid digestibility value for all yeasts adopted in this paper may lead to underestimation or overestimation of protein value. Similarly, the digestibility of individual amino acids present in yeasts could have provided the best estimate to predict their nutritional values. These two limitations were not catered for because of the paucity of information on protein and individual amino acid digestibility of the five considered yeasts, implying the need for future research. Taken into consideration the digestibility of protein and individual amino acids, therefore, becomes imperative when predicting the nutritional values of yeasts. Additionally, the chemical score and EAAI models endeavour to take into consideration all essential amino acids present in an ingredient. These methods, however, fail to consider practical scenarios when these yeasts are used in combination with non-target ingredients in typical compound feeds for fish. It is left to be seen whether ingredient–ingredient interaction between these yeasts and non-target ingredients will dampen and/or improve the nutritional quality of yeast covered in this review. Furthermore, the protein quality indices used in this report failed to take into consideration animal related factors, such as feed intake, passage rate, retention time, endogenous losses and rearing conditions which may have significant bearing on how different nutrients are utilised and metabolised by the different fish species. Moreover, other macronutrients (aside protein), micronutrients, anti-nutritional factors and feed processing conditions, which may positively or negatively impact the nutritional values of an ingredient are also not covered by these models.

Nutritional values for different fish species

Despite the numerous studies available on the functional benefits of yeast cell wall derivatives in fish (Meena *et al.* 2013; Torrecillas *et al.* 2014), only few studies have considered yeast as a macro protein ingredient in fish feeds. Of the limited available studies, *S. cerevisiae* is the most widely studied as shown in Table 5, and this may be connected with its ubiquitous availability as by-products generated from many industrial processes, including beer, alcohol and bio-ethanol production. In fact, *S. cerevisiae* is regarded as the second most valuable by-product from brewing industry (Ferreira *et al.* 2010) and has potential as valuable raw material for different industrial applications, including feed for different fish species. A majority of studies in aquaculture have shown that *S. cerevisiae* (Table 5) could be used to partly replace fishmeal or soy protein without adverse effect on growth performance of aquatic species, such as Atlantic salmon (Øverland *et al.* 2013), rainbow trout (Huyben *et al.* 2017; Vidakovic *et al.* 2020), Artic charr (Vidakovic *et al.* 2016), catfish (Essa *et al.* 2011; Peterson *et al.* 2012), goldfish (Gumus *et al.* 2016), lake trout (Rumsey *et al.* 1990), Nile tilapia (Abass *et al.* 2018), sea bass (Oliva-Teles & Gonçalves 2001), shrimp (Guo *et al.* 2019) and sea bream (Fronte *et al.* 2019). In general, these studies showed positive responses even at high replacement level of fishmeal protein, except few where high inclusion of *S. cerevisiae* linearly depressed growth and nutrient utilisation in fish. Examples of these are in rainbow trout (Hauptman *et al.* 2014), Atlantic salmon (Øverland *et al.* 2013), Nile tilapia (Ozório *et al.* 2012), Southern African dusky kob (Madibana & Mlambo 2019) and Mirror carp (Omar *et al.* 2012). Fermentation media, yeast strain and post-fermentation processing, as well as fish species and diet formulation are factors that may be responsible for the decreased growth and nutrient utilisation with increasing levels of *S. cerevisiae* in some fish species (Øverland & Skrede 2017). Dietary supplementation of intact *S. cerevisiae* may also be used to modulate intestinal microbiota in fish, such as rainbow trout (Huyben *et al.* 2017) and Beluga sturgeon (Hoseinifar *et al.* 2011).

Limited studies have documented the use of non-saccharomyces yeasts as major protein ingredients in farmed fish (Table 6). *Candida* yeast, especially *C. jadinii*, has been used at different dietary inclusion levels in several species, including Atlantic salmon (Øverland *et al.* 2013; Hansen *et al.* 2019; Sahlmann *et al*. 2019), rainbow trout (Mahnken *et al.* 1980), Coho salmon (Mahnken *et al.* 1980) and shrimp (Babu *et al.* 2013). Similarly, studies have reported possible replacement of fishmeal protein with *K. marxianus* (Øverland *et al.* 2013), *Yarrowia lipolytica* (Hatlen *et al.* 2012), *Rhodotorula mucilaginosa* (Chen *et al.* 2019) and *W. anomalus* (Huyben *et al.* 2017; Vidakovic *et al.* 2020) in various fish species. In general, these studies have shown positive results on performance and overall health status of fish. Furthermore, yeast has been used as an abatement strategy to counteract distal intestine inflammation in Atlantic salmon (Grammes *et al.* 2013; Hansen *et al.* 2019). However, inconsistent responses have been observed on the ability of yeast to alleviate intestinal inflammation in Atlantic salmon. According to Grammes *et al*. (2013), *C. jadinii* supplemented at 20% dietary inclusion level counteracts soya bean meal induced enteritis in Atlantic salmon fed 20% soya bean meal-based diets during the seawater phase. On the contrary, in a recently published article *C. jadinii* addition did not counteract mild intestinal inflammation changes observed in Atlantic salmon reared in freshwater (Hansen *et al.* 2019). In a work by Grammes *et al*. (2013), *K. marxianus* and *S. cerevisiae* had little or no counteracting effect on intestinal inflammation in Atlantic salmon. Thus, the disparity in these results may be due to a number of factors, including yeast species and strain, fermentation media, yeast inclusion levels and rearing phase and age of fish. From the available studies, it is evident that different yeast species can be used as major protein ingredients in fish feeds. However, the optimal inclusion levels of many of these yeasts remain largely undetermined. Therefore, future research is warranted to unravel the optimal inclusion levels of yeasts for different aquaculture species.

Strategies to increase the utilisation of yeast in fish feeds

In spite of the documented nutritive values of yeasts in various fish species (Tables 5 and 6), the incorporation of yeast into commercial aquafeeds is currently constrained by a number of factors. These constraints and possible solutions to overcome them are discussed in the following part of this review.

Nutrient optimisation of yeast through diet formulation

Dietary crystalline amino acids supplementation could be a strategy to augment the imbalance of amino acids present in yeasts. However, post-prandial availability differs between these two classes of amino acids (i.e. the intrinsic amino acids in yeasts and crystalline amino acids); crystalline amino acids tend to be more readily available than intrinsic ones within the intestinal lumen (Berge *et al.* 1994; Yamamoto *et al.* 1998; Larsen *et al.* 2012). Therefore, through diet optimisation, an effective synchronisation strategy between the intrinsic and the crystalline amino acids is warranted in the future to improve dietary utilisation of yeasts as a major protein ingredients in fish feeds. The effects of feeding frequency on amino acid synchronisation and consequently on protein utilisation, are well-documented in fish, such as

Table 5 Bibliographic review of research with *Saccharomyces cerevisiae* as macro-ingredient in aquaculture feeds

Fish	Duration	Experiment	Results	Reference
African catfish (*Clarias gariepinus*)	186 days	*S. cerevisiae* supplemented at 0–2% dietary inclusion levels	*S. cerevisiae* could be used to improve performance and profitability of African catfish	Essa *et al*. (2011)
Artic charr (*Salvelinus alpinus*)	99 days	Intact and extracted *S. cerevisiae* replacing 40% fishmeal protein	Intact and extracted *S. cerevisiae* could replace 40% fishmeal protein without compromising feed conversion ratio (FCR) in Artic charr	Vidakovic *et al*. (2016)
Goldfish (*Carassius auratus*)	84 days	Replacement of 0–45% dietary fishmeal protein with *S. cerevisiae*	Up to 45% replacement of fishmeal with *S. cerevisiae* improved performance of goldfish	Gumus *et al*. (2016)
Lake trout (*Salvelinus namaycush*)	84 days	six different preparations of *S. cerevisiae* supplementing 50% crude protein in the diets	*S. cerevisiae* could replace up to 50% crude protein in the diet without deleterious effect on growth performance and feed efficiency, optimal result was observed with disrupted yeast cell.	Rumsey *et al*. (1990)
Nile tilapia (*Oreochromis niloticus*)	51 days	*S. cerevisiae* supplemented at 0–40% inclusion level of the experimental diets	Above 15% inclusion level of *S. cerevisiae* linearly decreased growth performance and nutrient utilisation of Nile tilapia	Ozório *et al*. (2012)
Pacu (*Piaractus mesopotamicus*)	54 days	*S. cerevisiae* replacing 0–100% dietary fishmeal protein	50% replacement of dietary fishmeal in the diets of Pacu optimally improved feed efficiency and growth performance.	Ozório *et al*. (2010)
Sea bass (*Dicentrarchus labrax*)	84 days	Partial replacement of fishmeal protein with 0–50% *S. cerevisiae*	*S. cerevisiae* could partially replace up to 50% fishmeal protein in Sea bass, without adverse effect on performance and nutrient retention.	Oliva-Teles and Gonçalves (2001)
Thai Panga (*Pangasianondon hypophthalmus* × *Pangasius bocourt*i)	252 days	*S. cerevisiae* substituting 0–75% dietary fishmeal protein	*S. cerevisiae* reduced fish performance, as reflected in significant lower weight gain and FCR compared to fishmeal control. Meat quality was, however, not affected by *S. cerevisiae* supplementation.	Pongpet *et al*. (2016)
Giant freshwater prawn (*Macrobrachium rosenbergii*)	90 days	*S. cerevisiae* replacing 0–60% fishmeal protein in diets of giant freshwater prawn reared in either a recirculating aquaculture system (RAS) or a biofloc system	It was possible to substitute 60% fishmeal protein with *S. cerevisiae* in giant freshwater prawn diets, especially for prawn reared in biofloc system	Nguyen *et al*. (2019)
Gilthead sea bream (*Sparus aurata*)	92 days	*S. cerevisiae* replacing 20% fishmeal protein (4.6% dietary inclusion level)	*S. cerevisiae* could partially replace 20% fishmeal protein without adverse effect on growth performance and gut morphology	Fronte *et al*. (2019)
Hybrid striped bass (*Morone chrysops* × *M. saxatilis*)	Trial 1 - 42 days; Trial 2 - 56 days	In both trials, yeast biomass represented 0–4% dietary inclusion levels	*S. cerevisiae* could be used to enhance growth, feed efficiency and disease resistance of hybrid striped bass	Li and Gatlin (2003)
Nile tilapia (*Oreochromis niloticus*)	84 days	*S. cerevisiae* replacing 0–100% fishmeal protein in diets of Nile tilapia reared in either a recirculating aquaculture system (RAS) or a biofloc system	*S. cerevisiae* could completely replace fishmeal protein in diets of Nile tilapia. Better results were observed in Nile tilapia reared in biofloc environment than in RAS system.	Nhi *et al*. (2018)
Pacific white shrimp (*Litopenaeus vannamei*)	42 days	*S. cerevisiae* replacing 0–24% fishmeal or soya bean meal protein	*S. cerevisiae* could be used as partial replacement for FM or SBM in shrimp diets, without deleterious effect on growth performance, protein retention efficiency and survival	Guo *et al*. (2019)
Pacific white shrimp (*Litopenaeus vannamei*)	56 days	Diets supplemented with 1% yeast hydrolysate or yeast biomass	1% inclusion of yeast hydrolysate or yeast biomass could improve growth performance, enhance innate immunity and strengthen resistance to ammonia nitrogen stress in shrimp.	Jin *et al*. (2018)
South African dusky kob (*Argyrosomus japonicus*)	42 days	Diets supplemented with 0–30% inactivated *S. cerevisiae*	At 5% inclusion level, *S. cerevisiae* that does not compromise growth and health of dusky kob. Growth depressed at dietary supplementation above 5%.	Madibana and Mlambo (2019)

Table 5 (continued)

Fish	Duration	Experiment	Results	Reference
Beluga sturgeon (*Huso huso*)	42 days	*S. cerevisiae* supplemented at 0–2% dietary inclusion levels	*S. cerevisiae* could be used to improve growth performance and modulates intestinal microbiota, without detrimentally affecting haematological parameters of beluga sturgeon.	Hoseinifar *et al*. (2011)
Rainbow trout (*Oncorhynchus mykiss*)	70 days	Fishmeal protein was replaced with 0–60% *S. cerevisiae* or a mixture (70:30 biomass mix) of W. *anomalus* and *S. cerevisiae*	40% replacement of fishmeal protein with yeast caused no adverse effect on growth performance, nutrient digestibility or intestinal health of rainbow trout	Vidakovic *et al*. (2020)
Rainbow trout (*Oncorhynchus mykiss*)	70 days	Fishmeal protein was replaced with 0–60% *S. cerevisiae* or a mixture (70:30 biomass mix) of W. *anomalus* and *S. cerevisiae*	40% and 60% replacement of fishmeal protein with a mixture of W. anomalus and *S. cerevisiae* modulated the gut microbiota, while 20% replacement and diets with only *s. cerevisiae* had little or no effects in rainbow trout.	Huyben *et al*. (2017)
Nile tilapia (*Oreochromis niloticus*)	84 days	*S. cerevisiae* supplemented with 0–7% in diets.	*S. cerevisiae* enhanced fish tolerance to acute heat and hypoxia condition. It was concluded that *S. cerevisiae* could enhance the growth performance, stress resistance and disease resistance of Nile tilapia.	Abass *et al*. (2018)
Rainbow trout (Oncorhynchus mykiss)	63 days	Grain Distiller Dried Yeast (GDDY) replacing 0–100% fishmeal protein	Further replacement of fishmeal protein beyond 35% GDDY generally decreased fish performance.	Hauptman *et al*. (2014)
Mirror carp (*Cyprinus carpio*)	56 days	Yeast Protein Concentrate (YPC) replacing 0–50% fishmeal protein	YPC could replace half of fishmeal protein in mirror carp without depressing growth performance and health status of the fish. Optimal performance was observed with 15% and 20% replacement of fishmeal protein with YPC	Omar *et al*. (2012)
Channel catfish (*Ictalurus punctatus*)	62 days	NuPro® meal replacing 0–125% fishmeal	NuPro® could replace up to 100% fishmeal without adverse effect on performance of Channel catfish	Peterson *et al*. (2012)
Atlantic salmon (*Salmo salar*)	89 days	*S. cerevisiae* substituted 40% fishmeal protein	*S. cerevisiae* depressed growth performance and nutrient utilisation	Øverland *et al*. (2013)
Atlantic salmon (*Salmo salar*)	28 days	20% each yeast was used in combination with 20% SBM to investigate yeast potential in counteracting SBMIE. FM and SBM were, respectively, used as negative and positive controls	Histopathological examination of the distal intestine showed that *S. cerevisiae* could not be used to counteract SBMIE in Atlantic salmon	Grammes *et al*. (2013)

FM, fishmeal; SBM, soya bean meal; SBMIE, soya bean meal induced enteritis.

common carp (Nwanna *et al.* 2012), rainbow trout (Peragón *et al.* 1992; Barroso *et al.* 1999), channel catfish (Zarate *et al.* 1999) and Nile tilapia (Lanna *et al.* 2016). Therefore, the use of different feeding frequency in yeast diets supplemented with crystalline amino acids could be an interesting area of research in the future.

Hitherto, dietary enzyme supplementations have been used to improve nutritional values of feedstuff in fish (Castillo & Gatlin III 2015; Adeoye *et al.* 2016; Maas *et al.* 2018). This approach could also be used to increase nutrient digestibility and utilisation of yeast in fish. The yeast cell walls contain a complex network of polysaccharides that are unsusceptible to endogenous enzymes produced by aquaculture species. However, this challenge could be ameliorated by dietary supplementation with exogenous enzymes capable of degrading the yeast cell wall and enhance the utilisation of nutrients. Currently, there is a paucity of literature specifically on the role of exogenous enzymes to enhance nutritional value of yeast in various fish species. However, enzymes specific for yeast cell wall components such as mannanase, glucanase, chitinase and glucosidase are commercially available in the market. Therefore, the technical feasibility of unlocking the nutritional potential of various yeast species with these commercially available enzymes, either singly or as cocktail of enzymes could be an interesting area of research in the future.

Promoting increased nutrient digestibility through cost-effective downstream processing

Øverland and Skrede (2017) suggested that downstream processing of yeast after harvesting is imperative to preserve

Table 6 Bibliographic review of research with non-saccharomyces as macro-ingredients in aquaculture feeds

Fish	Yeast species & duration	Experiment	Results	Reference
Black tiger shrimp (*Panaeus monodon*)	CA & 30 days	Diet contained 10% inclusion level of CA. Diets were given to shrimp at different frequencies (daily, once in three days, once in seven days and once in five days), followed by white spot syndrome virus (WSSV) challenge	CA administered once every 7 days could enhance protective ability of P. monodon against WSSV	Babu *et al*. (2013)
Atlantic salmon (*Salmo salar*)	CU & 56 days split into two periods: 0–28 days freshwater and 28–56 days in salt-water	CU supplemented at 25% dietary inclusion level. The diet was used in a crossover design between the freshwater and salt-water phases of the fish	Feeding yeast containing diets throughout the experiment improved fish performance compared to those receiving control diet. In addition, yeast significantly downregulated the secretion of IFNγ, TNFα, IL-1β, IL-8 and modulated the expression of aquaporin 8 (aqp8ab) superoxide dismutase (sod1) and major histocompatibility complex 1 (mhc1).	Sahlmann *et al*. (2019)
Atlantic salmon (*Salmo salar*)	CU & 48 days	CU supplemented at 30% dietary inclusion level	CU could be included in the diet of Atlantic salmon without negatively affecting weight gain and overall fish health status	Sharma *et al*. (2018)
Atlantic salmon (*Salmo salar*)	CU & 28 days	Graded levels of CU were used in combination with 40% soya bean meal to investigate the potential of CU to counteract SBMIE. FM and SBM based diets were used as the negative and positive controls, respectively.	CU supplementation supports fish performance but was unable to counteract the mild histology changes observed in the distal intestine of SBM fed fish.	Hansen *et al*. (2019)
Shrimp (*Litopenaeus vannamei*)	CU & 29 days	CU replacing 0–100% fishmeal on protein basis	CU could be used to replace up to 60% fishmeal protein without deleterious effect on shrimp performance	Gamboa-Delgado *et al*. (2016)
Atlantic salmon (*Salmo salar*)	CU, KM & 89 days	Each yeast substituted 40% fishmeal protein	CU and KM could replace 40% fishmeal protein without adverse effects on growth performance, nutrient digestibility and retention.	Øverland *et al*. (2013)
Atlantic salmon (*Salmo salar*)	CU, KM & 28 days	20% each yeast was used in combination with 20% SBM to investigate yeast potential in counteracting SBMIE. FM and SBM were, respectively, used as negative and positive controls	Histopathological examination of the distal intestine showed that CU could be used to counteract SBMIE in Atlantic salmon, whereas KM could not.	Grammes *et al*. (2013)
Rainbow trout (*Oncorhynchus mykiss*)	CU	CU replacing 0–35% fishmeal protein	CU could be used in rainbow trout's diet without dietary imbalance or significance loss of growth performance	Martin *et al*. (1993)
Coho salmon (*Oncorhynchus kisutch*)	C & 196 days	Candida yeast replacing 0–100% fishmeal protein	More than 25% replacement of fishmeal protein with Candida yeast depressed growth of Coho salmon. Methionine supplementation could be used to enhance performance at higher level of yeast inclusion.	Mahnken *et al*. (1980)

Table 6 (continued)

Fish	Yeast species & duration	Experiment	Results	Reference
Rainbow trout (*Salmo gairdneri*)	C & 162 days	Candida yeast replacing 0–40% fishmeal protein	Candida yeast could replace up to 40% fishmeal protein without compromising performance and health status of rainbow trout	Mahnken *et al*. (1980)
Atlantic salmon (*Salmo salar*)	YL & 95 days	YL supplemented 0–30% dietary inclusion levels	Up to 20% dietary inclusion of YL did not compromise fish performance, but apparent digestibility of nutrients linearly declined with increased inclusion of yeast biomass. Yeast supplementation, however, increased the ratio of omega 3 (n-3) fatty acids in the fillet.	Hatlen *et al*. (2012)
Nile tilapia (*Oreochromis niloticus*)	RM & 56 days	RM supplemented at 0–1% dietary inclusion level	Dietary supplementation of RM could be used to enhance growth performance, nutrient composition, immune response and antioxidant capacity of Nile tilapia	Chen *et al*. (2019)
Rainbow trout (*Oncorhynchus mykiss*)	WA + SC & 70 days	Fishmeal protein was replaced with 0–60% mixture (70:30 biomass mix) of WA + SC	40% replacement of fishmeal protein with yeast caused no adverse effect on growth performance, nutrient digestibility or intestinal health of rainbow trout	Vidakovic *et al*. (2020)
Rainbow trout (*Oncorhynchus mykiss*)	WA + SC & 70 days	Fishmeal protein was replaced with 0–60% mixture (70:30 biomass mix) of WA + SC	40% and 60% replacement of fishmeal protein with a mixture of WA + SC modulated the gut microbiota, while 20% replacement and diets with only *s. cerevisiae* had little or no effects in rainbow trout.	Huyben *et al.* (2017)

C, *Candida* sp.; CA, *Candida aquaetxtris*; CU, *Candida utilis*; FM, fishmeal; KM, *Kluyveromyces marxianus*; RM, *Rhodotorula mucilaginosa*; SBM, soya bean meal; SBMIE, soya bean meal induced enteritis; WA + SC, *Wickerhamomyces anomalus* mixed with *Saccharomyces cerevisiae* in a 70:30, respectively biomass mix; YL, *Yarrowia lipolytica*.

valuable nutrients and bioactive components and to improve nutrient digestibility. The rigid cell walls of yeast limits accessibility of digestive enzymes to the intracellular contents and consequently affects utilisation of dietary yeast protein (Murray & Marchant 1986; Rumsey *et al.* 1990; Yamada & Sgarbieri 2005). To the authors' knowledge, this was first investigated by Rumsey *et al.* (1991a) and showed that cell wall disruption improved protein and energy digestibility of brewers' yeast cells, yeast extract and yeast protein isolate compared to intact cells. Other authors have shown that partial or complete disruption of yeast cell walls enhance nutrient digestibility and overall utilisation in Atlantic salmon (Hansen *et al.* 2021), shrimp (Zhao *et al.* 2017) and Arctic charr (Langeland *et al.* 2016). The treatments for rupturing the yeast cell walls range from chemical, enzymatic, physical, to mechanical methods (Nasseri *et al.* 2011; Lapeña *et al.* 2020b). Chemical rupturing can be done by exposing the cell walls to acid or alkaline treatments or a combination of both methods (Schiavone *et al.* 2014). Enzymatic hydrolysis can be performed by autolysis, with the aid of endogenous enzymes encapsulated by the yeast cell walls, or by exogenous enzymes targeting the specific layer of the cell walls (Schiavone *et al.* 2014; Hansen *et al.* 2021). Mechanical disintegration of the cell wall can be done either by crushing, crumbling, grinding, pressure homogenisation or ultrasonification (Nasseri *et al.* 2011; Hansen *et al.* 2021). Cost-effectiveness and intended use of the final yeast products should be of paramount concern while making decisions on the choice of downstream processing to be used. Some downstream methods may be excessively harsh to preserve the bioactive components prevalent on the surface of the cell walls. Therefore, a well-structured balance should be maintained when producing yeast products with nutritional and health beneficial values.

Manipulating the protein quality of yeast through genetic engineering

Research has shown that efforts to increase protein content of yeasts through manipulation of the fermentation media seems to produce minimal improvement, as observed by

Lapeña *et al.* (2020b) and Lapeña *et al.* (2020a) when yeast quality were optimised by using different fermentation media and growing conditions. Therefore, it becomes imperative to devise other means for increasing protein content and improving the protein quality of yeast. Genetic engineering has potential as a tool for production of high-protein novel yeast strains. Traditionally, novel production strains have been developed by mutagenesis (Guthrie & Fink 2002), breeding (Walker 1998) and evolutionary engineering (Francis & Hansche 1972). More recently, there are different attempts to manipulate the metabolic pathways in order to favour the protein secretion process in yeasts (Tang *et al.* 2015; Bao *et al.* 2017). According to Chiang (2004), metabolic engineering has the potential to develop novel biosynthesis pathways to produce new molecules or existing products that are traditionally made by expensive and complex chemical synthesis routes. Understanding the underlying mechanism behind the protein secretory pathway and its interaction with other cellular processes is key to stimulating protein secretion, and concomitantly protein production in yeast (Huang *et al.* 2017; Wang *et al.* 2019). Improved fermentation capacity and balancing of amino acids in *S. cerevisiae* yeast were achieved by tuning many other cellular processes, particularly energy metabolism (Huang *et al.* 2017). Wang *et al.* (2019) identified nine different genes with functions in cellular metabolism, protein modification and degradation, as well as cell cycle, which upon silencing improved protein production in engineered *S. cerevisiae* cells. Although the two previously cited reports focused on the use of yeast as cell factories to enhance production of specific protein (α-amylase in this case), we suggest that such methodology may be replicated to improve overall protein production of yeast. As such, research into genetic engineering using Crispr technology, gene editing, gene insertion and other forms of advanced techniques should be given utmost attention going forward, in order to create high-quality genetically modified yeast strains that can compete nutritionally with the conventional protein sources in fish feeds.

Increase investment portfolio for yeast production

An additional important constraint hindering the use of yeast as a major protein ingredient in fish feed is limited market availability in terms of quantity needed for commercial aquafeeds. To be considered as viable replacement for conventional fishmeal and soy protein, an alternative protein source must, apart from being nutritionally adequate, be commercially available with consistent supply to the end users. To our knowledge, yeasts are currently not economical as major protein ingredients in aquafeed. However, due to the potential sustainability of such ingredients, large corporate players in the yeast industry, such as Lallemand® (https://www.lallemand.com/), Phileo-Lesaffre® (https://phileo-lesaffre.com/en/) and emerging start-ups like Arbiom® (https://arbiom.com/), as well as Research Centres like Foods of Norway (https://www.foodsofnorway.net/) and others are investing in upscaling and optimising the production process for many yeast species. It is therefore, expected that constraints associated with availability and price will be resolved in the near future.

Impacts on environmental sustainability

Responsible sourcing is crucial to the contribution of feed ingredients to the overall sustainability index of most fish feed industries, and concomitantly fish farms. In this regard, the competitiveness of yeast as a major protein ingredient in fish feeds compared to conventional protein sources depends on its overall environmental contributions to the feed industry. Therefore, for better understanding of environmental impacts attributable to yeast as fish protein ingredients, there is need for holistic life cycle assessment of the process involved during production. Life cycle assessment is an analytical technique used to measure the overall environment impacts within all stages of a product lifecycle. This methodology is not alien to the currently used feed ingredients by the aquaculture industry (Pelletier *et al.* 2009; Henriksson *et al.* 2013; Henriksson *et al.* 2017; Smárason *et al.* 2017; Silva *et al.* 2018; Couture *et al.* 2019). Indeed, several of the formerly mentioned studies have documented the environmental footprint of various ingredients constituting the compound feeds, however, the environmental costs of yeast as potential major fish feed ingredient is conspicuously missing in literature. One major sustainability benefit of microbial products is that they are produced in a closed/controlled environment (fermenters) with strict biosecurity as opposed to GMO crops in open field. To our knowledge, only one study has conducted a direct comparison between the environmental impacts of yeast and that of conventional ingredients in fish feeds (Couture *et al.* 2019). In this study, attributional life cycle assessment (ALCA) was used to document the environmental benefits of replacing soy products with yeast in the diets of Atlantic salmon based on seven resource use and emission indicators: climate change impacts, acidification, freshwater eutrophication, marine eutrophication, land occupation, water consumption and primary production requirement. The authors first compared the environmental impacts of soy protein concentrate and yeast protein concentrate at the level of meal, and subsequently extended the model to measure the impacts when these ingredients are incorporated into two different complete feeds (with other non-target ingredients) of Atlantic salmon (Couture *et al.* 2019). At the level of meal, yeast protein concentrate exhibited drastically lower impacts in all

categories compared to soy protein concentrate. The author, however, further observed that the environmental benefits accrued with the yeast are dampened by high impacts from the non-target ingredients used in the complete feeds (Couture *et al.* 2019). This implies that a proper combination of ingredients with less environmental footprint is needed to achieve more sustainable aquafeeds, indicating that diversifying alternative protein sources in modern fish diets is likely to be the way forward. Although the results of this assessment showed a potential of yeast to provide better environmental performance than conventional feed resources, more study is needed in the future to substantiate this claim.

Regulation/legislation for use of yeast in animal feeds

The European Commission (EC) Regulation No 68/2013 on the catalogue of feed materials, classified yeast under products obtained by fermentation using micro-organisms, but in which the micro-organisms have been inactivated before use as animal feed (Commission Regulation (EC) 2013). Commission Regulation (EC) 1829/2003 guides the authorisation of genetically modified feed and food materials (Commission Regulation (EC) 2003). This regulation aimed to ensure high protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed (Commission Regulation (EC) 2003). Currently under these guidelines, only inactivated *S. cerevisiae* and *C. jadinii* among the yeast reviewed are allowed for use as macro-ingredients in feed within the EU. Similarly, these same yeasts are listed as GRAS (Generally Recognised as Safe Substances) under the Food and Drug Administration (FDA) Code of Federal Regulations (21 CFR), indicative of their authorisation as macro-ingredients in the feeds. In contrast, *K. marxianus, B. adeninivorans* and *W. anomalus* are currently unauthorised for use as major feed sources in both the EU and the US. However, it is important to state that *K. marxianus* and *W. anomalus* are listed under qualified presumption of safety biological agents catalogue of European Food Safety Association and listing them in the catalogue of feed material should not be an issue (https://zenodo.org/record/3828466#.Xu2_gGgzbLY). Research in the area of nutritional values, toxicology, safety (to both recipient animals and man), as well as environmental impacts of these three aforementioned yeasts are currently ongoing in different parts of the world. Therefore, dossier application seeking for their authorisation as novel feed ingredients is warranted in the future. It is of note to mention that Commission Regulation No 258/97 (Commission Regulation (EC) 1997) detailed the established procedures for submitting the dossier application for novel food and food ingredients in the EU.

Concluding remarks and future research consideration

With respect to the opinions expressed in this review article, the use of yeast as a sustainable protein ingredient in fish feed appear as technically feasible. Yeast is efficient in converting non-food lignocellulosic biomass to valuable products. Yeasts contain lower crude protein and lipids compared to conventional fishmeal. The amino acid compositions of five yeasts under study are comparable with the fishmeal meal and soy protein currently used in aquafeeds, except for methionine, arginine, lysine and phenylalanine, which are the most frequently limiting essential amino acids for juvenile Atlantic salmon and rainbow trout. Genetic modification or improved nutrient digestibility through exogenous enzymes supplementation and the use of cost-effective downstream processing could be a feasible approach to improve the overall protein quality in yeast. For yeast to become competitive with fishmeal and soy protein in aquafeeds, there is a need for additional investment in large-scale production and at affordable costs for feed manufacturers and fish farmers. Finally, of the five yeast species considered in this article, only *S. cerevisiae* and *C. jadinii* are currently allowed for use in animal feeds under the existing EU and US legislations. In the future, more concerted efforts should be dedicated at reviewing the existing legislations to accommodate more yeasts that are found to be safe for fish, environment and for human consumption of the final products.

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Supporting Information

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

Table S1. Amino acid (g/16g nitrogen) compositions of fishmeal and soybean meal, and their corresponding requirements in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*).

Table S2. Digestible amino acid contents in g/16g nitrogen (mean values) of selected yeasts, fishmeal, and soybean meal.

Table S3. Ideal amino acid profiles (mean values) of selected yeast species, fishmeal and soybean meal relative to digestible methionine and their corresponding requirements in Atlantic salmon and rainbow trout.

Table S4. Summary of standardized coefficient of variables and variance structure described by the discriminant function analysis (DFA).