Review

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Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture

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Abstract

The global expansion in aquaculture production implies an emerging need of suitable and sustainable protein sources. Currently, the fish feed industry is dependent on high-quality protein sources of marine and plant origin. Yeast derived from processing of low-value and non-food lignocellulosic biomass is a potential sustainable source of protein in fish diets. Following enzymatic hydrolysis, the hexose and pentose sugars of lignocellulosic substrates and supplementary nutrients can be converted into protein-rich yeast biomass by fermentation. Studies have shown that yeasts such as *Saccharomyces cerevisiae*, *Candida utilis* and *Kluyveromyces marxianus* have favourable amino acid composition and excellent properties as protein sources in diets for fish, including carnivorous species such as Atlantic salmon and rainbow trout. Suitable downstream processing of the biomass to disrupt cell walls is required to secure high nutrient digestibility. A number of studies have shown various immunological and health benefits from feeding fish low levels of yeast and yeast-derived cell wall fractions. This review summarises current literature on the potential of yeast from lignocellulosic biomass as an alternative protein source for the aquaculture industry. It is concluded that further research and development within yeast production can be important to secure the future sustainability and economic viability of intensive aquaculture.

Keywords: yeast; lignocellulosic biomass; wood; nutritional value; fish health; aquaculture

INTRODUCTON

Fish protein now contributes 17% of the global human population's intake of animal protein, and aquaculture accounts for a rapidly increasing share.[1] The increased feed demand of the rapidly expanding aquaculture sector has led to a concern that available feed resources will limit further growth. Fishmeal and fish oil were historically the major sources of protein and lipid in the intensive farming of carnivorous fish, and salmon farming has been criticised for reducing the amount of fish protein and lipids for human consumption.[2,3] Currently, there is insufficient fish oil and fishmeal on the world market to meet demand.

The recent development of salmon farming has shown reduced dependence on marine ingredients in the feed.[4,5] Following the rapid expansion in aquaculture and limited resources of fishmeal, plant ingredients have become the main protein sources in diets for most aquaculture species.[6,7] Thus, Norwegian salmon feed contained 66% plant ingredients in 2012.[5] Currently, the preferable plant protein sources in diets for carnivorous fish are refined and expensive products such as soybean protein concentrate. These plant ingredients are directly applicable for human consumption, and the use of human food as feed ingredients is doubtful, for ethical as well as economic reasons. Sustainability of food production should aim at maximised nutritional output for human consumption and minimised input of resources, with the lowest possible impact on the environment.[5] The change to plant ingredients implies that aquaculture uses large land areas and water resources. As arable land and clean water resources are already limited, this means that the sustainability of substituting marine feed ingredients with high quality plant products in fish feed is questionable.[5] Farmed fish species such as the Atlantic salmon (*Salmo salar*) are, however, more efficient than terrestrial farmed animals in retaining protein and energy.[4,5]

Microbial products, particularly yeast, are potential sustainable ingredients in aquafeeds due to the ability to convert low-value non-food biomass from forestry and agricultural industry into high-value feed with limited dependence on arable land, water and changing climatic conditions.[8] Under-utilised wood and co-products from agriculture and forestry can provide resources for production of feed ingredients from lignocellulosic biomass. Microbial conversion of the biomass to liquid biofuel has been an attractive research area in recent years.[9,10] Use of lignocellulosic biomass for production of high-value lipids and chemicals has also received considerable attention,[11−13] while there has been less interest in yeast biomass as an alternative protein source. Yeast cells are able to synthesise all precursors required for macromolecular constituents such as protein and nucleic acids from sugars and essential nutrients like nitrogen, inorganic phosphate and sulfate, and additional minerals and vitamins. The composition of yeast is dependent on strain, growth media and growing

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conditions; the content of crude protein varies between 40% and 55% of the dry matter including contents of nucleic acids.[14–16] The potential of different products from *Saccharomyces cerevisiae* for use in fish feed has been reviewed by Ferreira *et al.*[17]

The conversion of cellulose and hemicellulose to protein-rich biomass and ethanol by yeast fermentation is well established. Torula yeast, or *Candida utilis*, has been commercially available for more than 70 years as a nutritional supplement in animal feed.[18] During World War II, the Germans cultured Torula yeast as a protein source based on sulfite waste liquor from pulp and paper manufacture and wood sugar obtained by acid hydrolysis of wood.[19] Scientific progress and advances in technology have improved industrial yeast production from a wide range of lignocellulosic sources, including wastes or by-products from forestry and agriculture.[20,21] Recent studies with Atlantic salmon have documented excellent nutritional properties of *C. utilis* and *Kluyveromyces marxianus* yeasts grown on lignocellulosic biomass.[8] Both of these yeast species can metabolise a wide range of monosaccharides obtained from lignocellulosic biomass.[22–24] This is promising for future exploitation and economical industrial-scale production of yeast products based on lignocellulosic biomass as sustainable protein sources. Like *S. cerevisiae*, strains of *C. utilis* and *K. marxianus* have obtained the generally-regarded-as-safe (GRAS) status assigned by the US Food and Drug Administration (FDA) to substances not known to be hazardous to health and which are approved for use in foods.

Recent research on effects of yeast products in fish diets has focused mainly on the role as immunostimulants, gut health promotors and intestinal microbiota modulators at low inclusion rates. The literature pertaining to yeast products as a major nutrient source for different fish species is scarce, despite positive outlooks on future needs and prospects on new production technology development. The objective of this review is to summarise current knowledge on the potential of yeast products produced from lignocellulosic substrates as sustainable protein sources in aquaculture.

YEAST PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

In the late 20th century, the use of first-generation feedstock dominated the development of biofuel, but concerns exist about the impact this may have on biodiversity, water and land use, and competition with human food. So there is an increasing interest in second-generation lignocellulosic biomass such as by-products from the agricultural and forestry sectors, as this represents an abundant, natural, renewable and cheap resource for biorefinery. Lignocellulosic biomass is a heterogenous complex of the carbohydrate polymers cellulose and hemicellulose, and the aromatic lignin. Cellulose is generally the largest fraction in woody biomass, representing about 35–50% of the biomass weight, while hemicellulose represents about 20–30%.[25] Woody biomass has higher lignin content of about 20–30% and is structurally stronger and denser than agricultural biomass.[25] Woody biomass generally has a lower content of pentoses than agricultural biomass, but hard wood has a higher content of pentoses than soft wood species.[26]

Processing of lignocellulosic biomass for yeast production requires four major steps: (1) pre-treatment, (2) enzymatic hydrolysis, (3) fermentation and (4) downstream processing. Figure 1 shows the main steps in using woody biomass in the production of yeast. Pre-treatment of lignocellulosic biomass is essential to break the hemicellulose–lignin complex, separate out the lignin, and disrupt the crystalline structures of cellulose to make cellulose and hemicellulose more accessible before enzymatic hydrolysis to monosaccharides. The pre-treatment of the feedstock includes chemical, physical, thermal and biological methods.[10] Pre-treatment and saccharification of woody biomass, especially from softwood species, differ from non-woody materials because it is more resistant to microbial and enzymatic actions and requires more energy. Pre-treatment processing conditions must therefore be adapted to the properties of each different source of lignocellulosic biomass to get maximum yield of sugars for yeast production.[21,27]

Efficient conversion of crystalline polysaccharides to fermentable sugars is crucial for an economical and sustainable yeast production from lignocellulosic biomass. Maximum hydrolysis to monomeric sugars requires appropriate delignification and a cocktail of biomass-degrading enzymes.[10,28] Recent findings on enzymes that catalyse cleavage of glycosidic bonds in recalcitrant polysaccharides show the potential for increased efficiency of enzymatic conversion.[29,30] These enzymes increase the efficiency of hydrolytic enzymes by acting on the surface of the insoluble substrate.[30] Enzymatic hydrolysis may be carried out as separate hydrolysis and fermentation or simultaneous saccarification and fermentation.[13,28]

The sugars from lignocellulosic biomass can be converted into yeast biomass under aerobic conditions by fermentation, using suitable yeast strains and supplementary essential nutrients like nitrogen, inorganic phosphate and sulfate. *S. cerevisiae* strains are highly efficient in metabolising hexose sugars, and is predominant in ethanol production using cane and beet molasses as substrate. Wild-type strains of *S. cerevisiae* cannot ferment pentoses, but population genetics has been applied to develop non-recombinant *S. cerevisiae* strains that can grow on xylose.[31] Genetic engineering is also used to generate strains of *S. cerevisiae* that can grow on lignocellulosic feedstocks.[31–33] Fermenting mixed carbon components derived from lignocellulosic biomass is a challenge to cost-efficient production of yeast protein. However, recently, Wei *et al.*[34] were able to integrate the fermentation pathways of hexose and pentose sugars and an acetic acid reduction pathway into one *S. cerevisiae* strain using synthetic biology and metabolic engineering.

Natural yeast species that can co-ferment hexose and pentose sugars include *C. utilis* and *K. marxianus*.[22,35] Both *C. utilis* and *K. marxianus* can be grown on a broad substrate spectrum and are able to utilise pentoses such as xylose, the second most abundant carbohydrate in lignocellulosic biomass.[23,24] *C. utilis* is strictly aerobic and does not produce ethanol, whereas strains of *K. marxianus* can produce both ethanol and yeast biomass.[22,29] Yeast biomass may also be obtained as a by-product from production of biofuel or lipids.[10,13,36] After fermentation, the harvested yeast biomass is subjected to downstream processing like washing, cell disruption and drying.

CHEMICAL COMPOSITION OF YEAST

The chemical composition of whole cell yeast is dependent on strain, growth media and growing conditions, and downstream processing after fermentation.[14] Reported proximate composition of *S. cerevisiae, C. utilis* and *K. marxianus* yeasts is shown in Table 1. The average crude protein contents were similar for the three yeast species, but limited comparable data were found for *K. marxianus*. The reported contents of lipids and fibre in these yeast strains were generally low, but variable, possibly due to different analytical methods. Fatty acid composition may depend

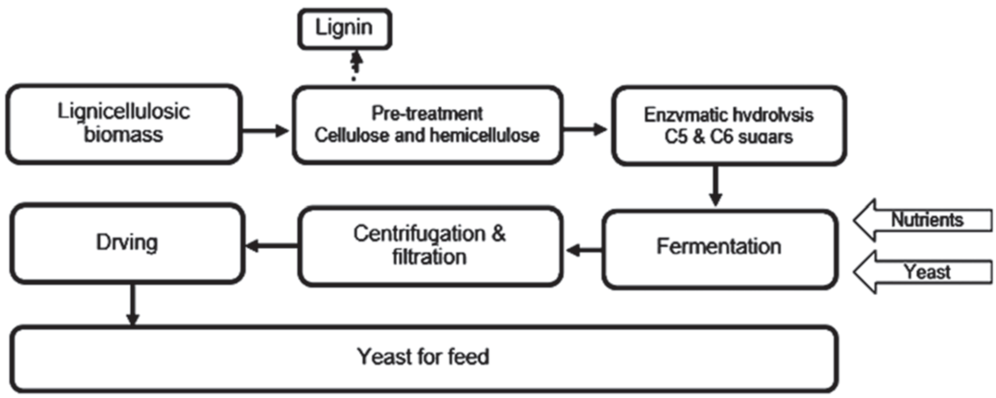

Figure 1. Flow chart of yeast production from lignocellulosic biomass involving four major steps: (1) pre-treatment of the biomass to remove lignin and to make cellulose and hemicellulose more accessible to hydrolysis; (2) enzymatic hydrolysis to convert cellulose and hemicellulose into C6 and C5 sugars; (3) fermentation of sugars, nitrogen, phosphate, and other nutrients; and (4) downstream processing into dry yeast product for use as a protein source in fish feed.

on growth stage and culture conditions,[14,37] but characteristically these yeast strains may contain mainly saturated fatty acids.[38] The nucleic acid contents in foods depend mainly on cell density,[39] and contents in yeasts are much higher than in plant and animal foods. The studied yeast species showed large variation in nucleic acid content among the different studies. This could be due to differences in growth rate, as rapidly dividing cells typically result in a higher level of nucleic acids than slower growth rates,[35,40] or different analytical methods.[41] Yeast is also a source of minerals such as phosphorus, calcium, sodium, zinc, iron, copper, manganese, and selenium.[42,43] Furthermore, yeasts such as *S. cerevisiae* and *C. utilis* are a moderately rich source of vitamins, predominantly B-vitamins, i.e. riboflavin, pyridoxine, niacin and pantothenic acid.[42,44]

The amino acid profile of yeasts compared with fish meal and soybean meal is shown in Table 2. The yeasts and fishmeal revealed similar contents of most indispensable amino acids on a crude protein basis, despite higher contents of non-protein nitrogen in the form of nucleic acids in yeast, but the content of methionine was lower in yeast than in fishmeal. Yeast protein is characteristically low in the sulfur-containing amino acids methionine and cysteine.[8,45] In general, the amino acid profile of the yeasts compiled in Table 2 agree well with reported ranges for yeast protein.[35] The data indicate that *S. cerevisiae* may have higher contents of methionine compared to the other yeast species, but slightly lower content of lysine. Except for the low methionine contents, yeast protein has a favourable amino acid composition compared with the fish requirements.[46] In agreement with previous studies,[38] the yeasts thus have potential as a protein source in a mixed diet for fish.

DIGESTIBILITY OF YEAST IN FISH

Digestibility is an important characteristic of yeast as an economically competitive protein source for aquaculture. Yeast products have a protein digestibility comparable to conventional protein sources in diets for different fish species. Studies with sea bass (*Dicentrarchus labrax*) showed that the apparent protein digestibility of *S. cerevisiae* was high (88.3%), but lower than that of high quality fishmeal (92.8%).[47] Increasing replacement of fishmeal with *S. cerevisiae* in the latter study also resulted in slightly decreasing apparent digestibility of dry matter and energy. Replacing fishmeal with up to 380 g kg^{-1} dried *S. cerevisiae* yeast in diets for pacu (*Piaractus mesopotamicus*, Holmberg 1887) had no significant effect on protein digestibility, whereas lipid digestibility was significantly increased.[48] In Gilthead sea bream (*Sparus aurata*), increased protein digestibility has been obtained when replacing fishmeal with 100 g kg^{-1} and 200 g kg^{-1} *S. cerevisiae* in the diets.[49] In the redclaw crayfish (*Cherax quadricarinatus*), the apparent protein digestibility of dried brewers yeast was of 92.6%, compared with 89.8% for fishmeal.[50] In contrast to the latter studies, rather poor protein and amino acid digestibility of intact and dried *S. cerevisiae* have been shown in salmonid fish species such as rainbow trout, *Oncorhynchus mykiss*,[43,51] Atlantic salmon,[8] and Arctic char (*Salvelinus alpinus*).[52] Lower energy and amino acid digestibility for intact than for extracted *S. cerevisiae* has been reported for Arctic char, while there was no significant difference in Eurasian perch (*Perca fluviatilis*).[52] This indicates differences in digestion efficiency among species, possibly due to different gastro-intestinal enzyme activity.[53]

High *in vitro* digestibility of *C. utilis* cells cultured in *Eucalyptus globolus* hydrolysates has been observed.[35] Results from studies with up to 347 g kg^{-1} *C. utilis* in diets for tilapia fry indicated that the apparent protein digestibility was similar to that of fishmeal.[54] Protein digestibility in rainbow trout was increased when freeze-dried *C. utilis* biomass was fed at levels up to 200 g kg^{-1}, corresponding to 35% of total dietary crude protein replacing fishmeal protein.[55] Very few studies have compared the digestibility of different yeasts in the same experiment. In studies with Atlantic salmon, replacement of 40% of low temperature (LT) fishmeal with *C. utilis* or *K. marxianus* yeast had no significant effect on digestibility of crude protein and energy, whereas substitution with *S. cerevisiae* significantly reduced protein and energy digestibility.[8] Digestibilities of individual amino acids were also unaffected by dietary inclusion of *C. utilis* and *K. marxianus*, except for a reduced digestibility of methionine and cysteine in the *K. marxianus* diet. Conversely, the diet containing *S. cerevisiae* revealed significantly lower amino acid digestibility than the fishmeal control except for glycine and cysteine.[8] The latter study[8] indicated considerable differences in digestibility among different whole cell yeast products.

Effects of processing of yeast on digestibility

The harvested yeast biomass should be subjected to cost-efficient downstream processing to preserve valuable nutrients and bioactive components, and to promote as high digestibility as

Table 1. Concentration (g kg^{-1} DM) of crude protein (N × 6.25), nucleic acids, lipids, fibre, carbohydrates, and ash of yeast

Organism	Crude protein	Nucleic acids	Lipids	Fibre	Carbohydrates	Ash	Reference
Saccharomyces cerevisiae	445	–	–	6.3¶	–	64	43
	485	75*	34§	122**	329¶¶	83	61
	539	–	06‡	23††	50***	68	116
	473	48*	–	–	77***	–	117
	475	60†	21‡	–	11.4***	66	8
	456	–	80§	–	232¶¶	103	42
	466	–	10§	–	–	63	64
	396	9.0*	5	314**	–	45	37
Candida utilis	501	–	–	–	–	–	55
	462	11.5†	24§	12¶	–	95	44
	482	–	16§	6.5¶	–	–	54
	598	99†	3.2‡	–	40***	58	8
Kluyveromyces marxianus	544	109†	9‡	–	9***	81	8

*RNA; †Total nucleic acids; ‡HCl-EE; §Ether extract (EE); ¶Crude fibre; **Total fibre; ††Acid detergent fibre (ADF); §§Neutral detergent fibre (NDF); ¶¶Total carbohydrates; ***Starch.

Table 2. Average amino acid composition (g 16 g N^{-1} ± standard deviation of non-hydrated amino acids) of yeast compared with fish meal and soybean meal

Amino acid	Fishmeal[46]	Soybean meal[46]	*Saccharomyces cereviciae*[8,37,43,61,64,116]	SD	*Candida utilis*[8,44,54,55,118]	SD	*Kluyveromyces marxianus*[8,119,120]	SD
Indispensable amino acids								
Arginine	5.74	7.38	4.68	0.60	5.20	0.71	4.34	0.20
Histidine	2.36	2.67	2.47	0.87	1.97	0.28	1.80	0.18
Isoleucine	4.53	4.94	4.43	0.76	4.29	0.34	4.20	0.14
Leucine	7.06	7.80	6.73	1.16	6.19	0.61	6.81	1.37
Lysine	8.18	5.53	6.95	0.70	7.71	1.16	7.39	0.65
Methionine	2.87	1.41	1.81	0.49	1.08	0.23	1.33	0.25
Phenylanine	3.84	5.26	4.18	0.72	3.64	0.51	3.96	0.25
Threonine	4.00	4.03	4.71	0.78	4.71	0.23	5.11	0.33
Tryptophan	1.05	1.41	1.08	0.15	1.17	0.12	0.98	
Valine	4.87	5.51	5.24	0.82	5.08	0.56	5.11	0.62
Dispensable amino acids								
Alanine	–	–	6.13	1.17	5.75	0.74	8.49	1.39
Aspartic acid	–	–	9.51	1.48	8.30	1.59	10.59	1.11
Cysteine	1.31	1.53	1.23	0.65	0.81	0.21	0.58	0.17
Glutamic acid	–	–	13.01	1.02	10.43	1.37	13.63	1.92
Glycine	–	–	4.32	0.77	4.15	0.27	4.47	0.45
Proline	–	–	3.69	0.71	3.85	0.83	3.99	0.66
Serine	–	–	4.48	0.94	4.07	0.74	5.34	0.34
Tyrosine	3.08	3.21	3.69	1.01	3.07	0.50	3.18	0.28

possible. There are few data on effects of processing on digestibility, and whether different yeasts require different processing methods. It has been suggested that the thick and rigid cell walls are a major problem inhibiting industrial production and utilisation of dietary yeast protein.[37,56–58] A poorly digestible cell wall may limit enzymatic access to cellular contents, depending on yeast cell characteristics as well as growth substrate and processing conditions. Rumsey *et al.*[51] showed that intact brewers yeast had lower protein and energy digestibility in rainbow trout than disrupted yeast cells, yeast extract and yeast protein isolate.

Cell walls represent 26–32% of the cell dry weight and contain varying proportions of mannan-oligosaccharides, β-glucan, chitin and nucleic acids depending on species and strain.[59,60] Various chemical, enzymatic, physical or mechanical methods can be used to rupture the yeast cell walls (reviewed by Nasseri *et al.*[23]). Digestibility of yeast nutrients can be increased by mechanical rupturing of cell walls[61] or enzymatic hydrolysis.[58] Enzymatic pre-treatment followed by high-pressure mechanical homogenisation have been shown to be efficient for *C. utilis*.[62] Yeast extract with higher protein content than in whole or hydrolysed yeast is obtained by removal of cell wall material; this represents the water-soluble cell contents. Increasing inclusion of yeast extract as a replacement for fishmeal in diets for shrimp (*Litopenaeus vannamei*) increased the apparent digestibility of protein,[63] most likely due to the combined effect of removal of cell walls and increased proportion of water-soluble low molecular weight proteins. In Arctic char, Langeland *et al.*[52] showed that the absence of intact cell walls had a positive effect on digestibility of *S. cerevisiae* protein. This confirms the finding that a phosphorylated *S. cerevisiae* protein concentrate, produced by rupturing of cell walls and centrifugation to remove debris, produced much higher protein digestibility in rats than whole yeast cells.[37] However, the cell wall fraction is rich in bioactive and immunostimulating compounds like β-glucan and mannan oligosaccharides. Hence, it seems likely that the whole yeast biomass, after rupturing of cell walls, may be the most attractive feed ingredient, by combining the properties as a source of nutrients and bioactive components. Extrusion of feed may cause partial disruption of yeast cell walls and potentially increase protein and amino acid digestibility.[64] Further research is warranted to determine optimal extrusion conditions for fish feed with contents of different yeast whole cell products.

EFFECT OF DIETARY YEAST ON GROWTH PERFORMANCE, NITROGEN UTILISATION AND CARCASS COMPOSITION

Growth responses and protein utilisation in fish fed yeasts may depend on a number factors, including yeast substrate, yeast strain, and post-fermention processing, as well as fish species and diet formulation. As an easily available natural industrial product, strains of *S. cerevisiae* have been used as a protein source in a number of studies with different fish species. Several growth performance studies have shown that intact *S. cerevisiae* can be used to partly replace fishmeal protein in diets for fish species such as sea bass;[47] the omnivore pacu, *Piaractus mesopotamicus*;[48] Nile tilapia, *Oreochromis niloticus*;[65] Atlantic salmon;[8] Thai Panga, *Pangasianodon hypophthalmus* × *Pangasius bocourti*;[66] and Arctic char.[64] A majority of these studies have been carried out with isonitrogenous and isoenergetic diets, with or without balancing amino acid composition with synthetic amino acids. Some studies have shown that partial substitution of fishmeal with *S. cerevisiae* increased growth rate and nitrogen retention.[47,48] Moderate levels of 250 g kg^{-1} brewers yeast (*S. cerevisiae*) in diets for rainbow trout, replacing casein, improved growth performance, whereas the very high levels of 500 g kg^{-1} and 750 g kg^{-1} reduced feed intake and growth.[67] Partially replacing fishmeal with 345 g kg^{-1} spray-dried and inactivated *S. cerevisiae* significantly reduced specific growth rate and feed conversion rate, but did not affect retention of digested N in Atlantic salmon.[8] Conversely, replacing fishmeal protein with 289 g kg^{-1} intact *S. cerevisiae* in extruded diets for Arctic char had no negative effects on growth performance.[64] The adverse effect of *S. cerevisiae* on growth and feed conversion ratio in the study by Øverland *et al.*[8] might be associated with poor digestibility of protein, amino acids and energy, possibly indicating an unfavourable yeast post-fermentation procedure.

Unlike *S. cerevisiae*, there are few studies with *C. utilis* and *K. marxianus* in diets for farmed fish. *C. utilis* is recognised for rapid growth to high cell density based on numerous inexpensive substrates including lignocellulosic biomass and wastes.[18] It is commonly used as a food additive, partly due to umami flavour, high palatability, and documented safety. In studies with rainbow trout, *C. utilis* biomass grown on peat could partly replace fishmeal protein without significant reduction of growth performance.[55] Studies with Atlantic salmon showed that *C. utilis* grown on a lignocellulosic sugar substrate could be fed at 283 g kg^{-1} of the diet, replacing 40% of the fishmeal protein in isonitrogenous diets, without adverse effects on growth, feed intake or feed conversion ratio.[8] Nitrogen retention was significantly enhanced in salmon fed *C. utilis* as compared with the fishmeal control diet, despite lowered level of methionine (the likely limiting essential amino acid for protein synthesis), higher level of non-protein nitrogen, and unaffected digestibility of nitrogen and amino acids. In previous studies with tilapia fry, up to 350 g kg^{-1} *C. utilis* was fed without adverse effects on growth and feed conversion ratio.[54] Recent studies with shrimp (*Litopenaeus vannamei*) showed that growth and survival rate were similar when feeding diets containing varying proportions of *C. utilis* and fishmeal.[68] They showed that by applying a nitrogen stable isotope technique (^{15}N), the relative body growth incorporation of dietary nitrogen from *C. utilis* consistently increased with increasing proportion in the diets. Previous studies have indicated that *K. marxianus* yeast is a promising protein source for aquaculture.[57] The results obtained by Øverland *et al.*[8] support this by documenting that a diet containing 302 g kg^{-1} *K. marxianus*, replacing 40% of the crude protein from fishmeal, promoted similar growth, feed conversion ratio, and nitrogen retention in Atlantic salmon as the fishmeal-based control diet. No other study apparently has examined the suitability of inactive *K. marxianus* as a major protein source in aquaculture.

Recovery of microbial biomass as a by-product from fuel ethanol biorefineries is a new concept to produce yeast protein sources. In studies with carp (*Cyprinus carpio*), significantly improved growth and feed conversion ratio was obtained when dried yeast from distillers grains and solubles, containing 340 g kg^{-1} protein on a DM basis, replaced 15% or 20% of a high quality fishmeal.[36] A higher level of this yeast (460 g kg^{-1}, replacing 50% of fishmeal protein) resulted in similar growth and feed conversion as the fishmeal control diet. Grain distillers dried yeast containing 520 g kg^{-1} crude protein in the dry matter has been evaluated as a protein source for rainbow trout,[69] and the results showed no significant differences in growth performance when 25% and 37.5% of the fishmeal were replaced with this yeast product on a digestible protein basis. Further fishmeal replacement resulted in linear reductions in growth and poorer feed conversion ratio, although the diets were balanced for digestible lysine, methionine and threonine. The reduction in growth performance at high inclusion levels was not associated with reduced feed intake.[69]

In addition to amino acids and protein, yeast cells synthesise nucleic acids as substantial N-containing components. In fast-growing yeast cells, about 10–15% of the total nitrogen is in the form of nucleic acids.[40] In contrast to indispensable amino acids, nucleotides are endogenously synthesised and not considered essential nutrients, but dietary nucleic acids may be partially salvaged and used by the animals, thus influencing growth performance and nitrogen balance.[70] Rumsey *et al.*[71] showed that feeding rainbow trout high levels of nucleic acids, corresponding to up to 500 g kg^{-1} dietary *S. cerevisiae*, had no effect on feed intake, but increased growth and nitrogen retention. This may be consistent with the results obtained by Øverland *et al.*,[8] where nitrogen retention was increased by feeding *C. utilis* containing 93 g kg^{-1}

nucleic acids as a substitute for fishmeal for Atlantic salmon, thus reducing the proportion of amino acid nitrogen in total dietary nitrogen. Similarly, replacing fishmeal with *K. marxianus*, containing 102 g kg^{-1} nucleic acids, in salmon diets had no negative effect on nitrogen retention.[8] This indicates that the nucleic acids in yeast may be directly incorporated in the body or spare non-essential amino acid nitrogen through endogenous utilisation. In contrast, no clear nitrogen sparing effect of yeast or dietary nucleic acids have been found in turbot (*Psetta maxima*) or rainbow trout.[72] The digestibility of nucleic acid nitrogen seems to be high in many species,[41] and a variable proportion is excreted in the urine.[73] The urolytic pathway in Atlantic salmon seems to be able to control high dietary levels of nucleic acids.[74] This may partially explain the positive effect of dietary inactive yeast on growth and nitrogen utilisation in studies with different fish species.

Carcass composition

Fillet characteristics of fish fed yeast products may depend on fish growth and body condition.[54,75] However, studies with different fish species have shown no or only minor effects of replacing fishmeal by different yeast products on whole body or muscle proximate composition.[36,65,66,69] Carcass composition was not significantly affected by protein source when *C. utilis* replaced fishmeal in diets for rainbow trout,[55] or fishmeal and blood meal in diets for fingerling grey mullet (*Mugil cephalus*).[76] Nor were there any clear effects on carcass composition when using high levels of *C. utilis* in diets for tilapia (*Oreochromis mossambicus* Peters).[54]

The fatty acid composition of fish muscle reflects the dietary fatty acid profile. The fatty acid profile of fish muscle may thus be slightly modified by using yeast as a lipid source. However, use of diets with a low proportion of dietary lipids from yeast products may have no or minor effects on fillet fatty acid profile. This has been clearly shown in studies with live *K. marxianus* cells as a substitute for fishmeal in diets for tilapia (*Oreochromis niloticus* Linnaeus).[77] Likewise, fillet colour measurements in Thai Panga have revealed no effects of dietary substitution of fishmeal with *S. cerevisiae*.[66] In general, the knowledge from using yeast biomass in nutritionally balanced fish diets indicates no major effects on characteristics of fish as human food. Assuming use of GRAS yeast strains, the evaluation as fish feed ingredients should be mainly based on effects on fish growth performance and health.

EFFECT OF DIETARY YEAST AND YEAST PRODUCTS ON FISH HEALTH

Yeast is commonly used in aquaculture as a growth promoter and immunostimulant in functional feeds. Yeasts contain various bioactive components such as β-glucans, mannose polymers covalently linked to proteins (manno proteins), minor amounts of chitin, and nucleic acids. Positive health effects have been documented when adding yeast to fish diets at low levels (10–40 g kg^{-1}), especially with whole *S. cerevisiae* yeast or yeast products, but few studies evaluate the health beneficial effects of yeast when used as a protein source. Low levels of *S. cerevisiae* yeast have shown to enhance growth performance, immune responses, and/or protection against bacterial infection, and to increase disease resistance in several fish species, such as salmonids,[58,78] hybrid striped bass,[79,80] gilthead seabream,[81] seabream,[82] hybrid tilapia (*Oreochromis niloticus*),[83] common carp,[60] Indian common carp (*Labeo rohita*),[84] channel catfish (*Ictalurus punctatus*),[85] Japanese seabass (*Lateolabrax japonicas*),[86] and Nile tilapia.[87] Yeasts such as *C. utilis* have also been shown to modulate immune responses in rainbow trout,[88,89] and *K. marxianus* has shown probiotic properties in an *in vitro* colonic model system.[90] Feeding live bakers yeast (10 g kg^{-1}) improved growth performance and feed utilisation, and resistance to *Aeromonas hydrophila* in Nile tilapia[91] and enhanced intestinal colonisation or accelerated the maturation of the digestive system of early feeding fry.[92] When used in moderate levels of 200 g kg^{-1} in diets for Atlantic salmon, *C. utilis* and *K. marxianus* affected the bacterial composition in the distal intestine, particularly by reducing the relative amount of *Firmicutes* bacteria.[93] Inclusion of 200 g kg^{-1} inactive *C. utilis* whole yeast cells, and also *K. marxianus*, counter-acted soybean meal induced enteritis (SBMIE) in the distal intestine of Atlantic salmon,[93] while *S. cerevisiae* did not protect against SBMIE. Adding 10–20 g kg^{-1} dietary yeast cell wall extracted from *S. cerevisiae* improved growth performance and intestinal mucus development of Japanese seabass fed a diet with 210 g kg^{-1} SBM.[86] Similarly, adding 2 g kg^{-1} bakers yeast cell wall fraction rich in MOS to a diet with moderate (140 g kg^{-1}) inclusion of SBM eliminated SBMIE in the distal intestine of Atlantic salmon.[78] The protective mechanisms of yeast against SBMIE could include a beneficial effect on gastrointestinal morphology, reduced inflammatory responses, and stimulating wound healing processes and modulation of gastrointestinal microbiota.

Enhanced growth performance and reduced mortality after pathogen challenge and increased disease resistance are attributed to several immunostimulatory properties of yeast, including stimulation of both humoral and cellular immune responses. These responses include blood neutrophil oxidative radical and extracellular superoxide anion production of head kidney macrophages,[79] enhanced serum leucocytes and lysozyme activities and complement activity,[58,83] enhanced cellular immune responses of the innate immune system as measured by phagocytic index, respiratory burst activity,[83,84] number of erythrocytes and lymphocytes,[84] and increased antioxidant status by increasing catalase, superoxide dismutase, and total antioxidant activity in serum.[94] Enhanced growth performance and health in fish fed yeast might also be attributed to improved digestive enzyme activity,[95] and by supplying digestive enzymes that aid in the digestion of complex carbohydrates,[84] or by enhancing digestive capacity by improving gastrointestinal morphology and thereby increasing the absorptive surface.[8,58,95,96] Yeast also provides dietary nucleotides that enhance immune responses in several fish species, including Atlantic salmon,[97] hybrid striped bass,[80] and grouper (*Epinephelus malabaricus*),[98] enhances intestinal epithelial cells in Atlantic salmon,[97] and increases weight of the gastrointestinal tract in Atlantic salmon.[8] Modulation of intestinal microbiota may also be a contributing factor as reported for several fish species, including salmonids,[58] common carp,[60] hybrid striped bass,[80,99] hybrid tilapia,[83] juvenile beluga sturgeon (*Huso huso*),[100] and Nile tilapia.[87] Modulation of intestinal microbiota and protection against bacterial infection might be associated with the mannan oligosaccharides in the yeast cell walls, which prevent colonisation of pathogens and thereby eliminating them from the intestinal tract, or by increasing the gastrointestinal tract mucus secretion and by that improving the gastrointestinal barrier function and protection.[101]

The effect of yeast on immunity and survival rate of fish is inconsistent, however. This could be attributed to factors such as type of yeast products,[93] concentration,[83,86] feeding duration of the yeast products,[85] and fish age, size and species. The fermentation conditions or downstream processing conditions during manufacture may also affect the nucleic acid levels[71] and the cell wall properties of the yeast and thus the health promoting effects. Fish fed diets supplemented with nucleotides have shown enhanced resistance to viral, bacterial and parasitic infection, thus indicating that nucleotides are conditionally or semi-essential nutrients for fish.[99] Grinding yeast cells or extracting β-(1,3)-glucan from yeast also have implications for the health promoting properties.[60,102]

CONCLUSIONS AND FUTURE PERSPECTIVES

The human world population is estimated to increase from 6.9 billion in 2010 to 8.2 billion by 2030, and probably to more than 9 billion by 2050.[1] The food supply will have to increase and become more efficient to grow given the availability of natural resources and existing technology.[103] The total supply of seafood has been growing more rapidly than the global population.[1] Almost 90% of the capture fisheries are now fully exploited or over-exploited. Aquaculture has grown to almost half of total fish supply, and is expected to continue to grow at a rapid rate in the coming decades. The rapid growth of aquaculture production has been driven by productivity growth, technological progress, globalising trade, and favourable economics of large-scale intensive farming.[104]

Increasing production of carnivorous species like salmonids has greatly enhanced the demand of commercial feed, and further expansion will depend on a sufficient supply of high-quality sustainable feed resources. The increased production has been made possible through diet modifications, mainly replacing fishmeal and oil with plant ingredients derived from agricultural commodity crops like soybean and rapeseed. Conceivably, future salmon farming may be a net producer of fish protein and oil.[5,105–107] Challenges associated with a further increase in plant protein sources include contents of anti-nutrients,[108–110] potential contents of mycotoxins,[111] and insecticides.[112] The increasing use of genetically modified plants[113] is subject to regulatory constraints and may limit acceptability in some markets and among groups of consumers. The sustainability of substituting marine feed ingredients with plant products in fish feed is questionable, considering the need of water and arable land, use of scarce global phosphorus fertilisers, use of pesticides, and environmental human health implications.[114] Although these concerns are not specific to plant products used in fish feed, it is urgent to identify non-conventional sources of protein that can be converted into high-quality feed ingredients for aquaculture. Especially the further expansion in the production of carnivorous species like salmon may require economically competitive and sustainable feed ingredients produced from novel non-food resources.

Efforts towards sustainability in the aquaculture industry may benefit from combining the approaches of different research disciplines. Fast-growing yeasts, produced from non-edible feedstocks and categorised as GRAS microorganisms, may be attractive candidates for industrial upscaling to important feed resources. A number of studies with different yeast products and fish species have shown that yeast products produced from lignocellulosic substrates can be used as attractive protein sources with the additional benefit of improving immune function and intestinal health of the fish. Yeast production from lignocellulosic biomass offers the advantage of making food production less dependent on arable land and climate, and relieving pressure on resources for direct human food production. No single cost-efficient technology may meet all the challenges of large-scale industrialisation,[20,115] and yeast production from lignocellulosic biomass is less efficient compared to feedstocks such as sugar or starch. Currently, the process cost of yeast protein production from lignocellulosic biomass may be too high and there is a need to develop efficient processes for economic utilisation. Production of multiple co-products such as lignin, biochemicals and biofuel in biorefineries can contribute to reduce the cost as the technology matures. In the future, this may open diverse options that reach beyond current use of land areas for production of food and feed. Continued research and development in yeast production from lignocellulosic substrates can be an important contribution to securing the sustainability and economic viability of future aquaculture.

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REFERENCES

1 United Nations Food and Agriculture Organization (FAO), *Economic Analysis of Supply and Demand for Food up to 2030 – Special Focus on Fish and Fishery Products*, FAO Fisheries and Aquaculture circular no. 1089. FAO, Rome (2014).
2 Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J *et al.*, Effect of aquaculture on world fish supplies. *Nature* **405**:1017–1024 (2000).
3 Naylor RL, Hardy RW, Bureau D, Chiu A, Elliot M, Farrell AP *et al.*, Feeding aquaculture in an era of finite resources. *PNAS* **106**: 15103–15110 (2009).
4 Torrissen O, Olsen RE, Toresen R, Hemre GI, Tacon AJ, Hardy RW *et al.*, Atlantic salmon (*Salmo salar*): The "super-chicken" of the sea? *Rev Fish Sci* **19**:257–278 (2011).
5 Ytrestøyl T, Aas TS and Åsgård T, Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. *Aquaculture* **448**:365–374 (2015).
6 Hardy RW, Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. *Aquacult Res* **41**:770–776 (2010).
7 Shepherd CJ and Jackson AJ, Global fishmeal and fish-oil supply: inputs, outputs and markets. *J Fish Biol* **83**:1046–1066 (2013).
8 Øverland M, Karlsson A, Mydland LT, Romarheim OH and Skrede A, Evaluation of *Candida utilis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* **402–403**:1–7 (2013).
9 Somerville C, Youngs H, Taylor C, Davis SC and Long SP, Feedstocks for lignocellulosic biofuels. *Science* **329**:790–792 (2010).
10 Balan V, Current challenges in commercially producing biofuels from lignocellulosic biomass. *ISRN Biotechnology* Article ID 463074 (2014).
11 Huang C, Chen X, Xiong L, Chen X, Ma L and Chen Y, Single cell oil production from low-cost substrates: The possibility and potential of its industrialization. *Biotechnol Adv* **31**:129–139 (2015).
12 Enshaeieh M, Abdoli A, Madani M and Bayat M, Recycling of lignocellulosic waste materials to produce high-value products: single cell oil and xylitol. *Int J Environ Sci Technol* **12**:837–846 (2015).
13 Jin M, Slininger PJ, Dien BS, Waghmode S, Moser BR, Orjuela A *et al.*, Microbial lipid-based lignocellulosic biorefinery: feasibility and challenges. *Trends Biotechnol* **33**:43–54 (2015).
14 Halasz A and Lasztity R, *Use of Biomass in Food Production*. CRC Press, Boca Raton (1991).
15 Reed G and Nagodawithana TW, *Yeast Technology*. Van Nostrand Reinhold, New York (1991).
16 Harrison JS, Food and Fodder Yeasts, in *The Yeasts*, ed. by Rose AH and Harrison JS. Academic Press, London, pp. 399–433 (1993).
17 Ferreira IMPLVO, Pinho O, Vieira E and Tavarela JG, Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. *Trends Food Sci Technol* **21**:77–84 (2010).
18 Bekatorou A, Psarianos C and Koutinas AA, Production of food grade yeasts. *Food Technol Biotechnol* **44**:407–415 (2006).

19 Litchfield JH, Single-cell protein, in *A Revolution in Biotechnology*, ed. by Marx JL. Cambridge University Press, New York, pp. 71–81 (1989).
20 Mussatto SI, Dragone G, Guimarães PMR, Silva JPA, Carneiro LM, Roberto IC *et al.*, Technological trends, global market, and challenges of bio-ethanol production. *Biotechnol Adv* **28**:817–830 (2010).
21 Pereira FB, Romaní A, Ruiz HA, Teixeira JA and Domingues L, Industrial robust yeast isolates with great potential for fermentation of lignocellulosic biomass. *Bioresour Technol* **161**:192–199 (2014).
22 Fonseca GG, Heinzle E, Wittmann C and Gombert AK, The yeast *Kluyveromyces marxianus* and its biotechnological potential. *Appl Microbiol Biotechnol* **79**:339–354 (2008).
23 Nasseri AT, Rasoul-Amini S, Morowvat MH and Ghasemi Y, Single cell protein: Production and process. *Am J Food Technol* **6**:103–116 (2011).
24 Rodrussamee N, Lertwattanasakul N, Hirata K, Suprayogi, Limtong S, Kosaka T *et al.*, Growth and ethanol fermentation ability on hexose and pentose sugars effect under various conditions in thermotolerant yeast *Kluyveromyces marxianus*. *Bioenergy Biofuels* **90**:1573–1586 (2011).
25 McKendry P, Energy production from biomass (part 1): overview of biomass. *Bioresour Technol* **83**:37–46 (2002).
26 Zhu JY and Pan XJ, Woody biomass pre-treatment for cellulosic ethanol production: Technology and energy consumption evaluation. *Bioresour Technol* **101**:4992–5002 (2010).
27 Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holzapple M *et al.* Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour Technol* **96**:673–686 (2005).
28 Narra M, James JP and Balasubramanian V, Simultaneous saccharification and fermentation of delignified lignocellulosic biomass at high solid loadings by a newly isolated thermotolerant *Kluyveromyces* sp. for ethanol production. *Bioresour Technol* **179**:331–338 (2015).
29 Lane MM and Morrissey JP, *Kluyveromyces marxianus*: A yeast emerging from its sister's shadow. *Fungal Biol Rev* **24**:17–26 (2010).
30 Horn SJ, Vaaje-Kolstad G, Westereng B and Eijsink VGH, Novel enzymes for the degradation of cellulose. *Biotechnol Biofuel* **5**:45 (2012).
31 Attfield PV and Bell PJL, Use of population genetics to derive nonrecombinant *Saccharomyces cerevisiae* strains that grow using xylose as a sole carbon source. *FEMS Yeast Res* **6**:862–868 (2006).
32 Jeffries TW and Jin Y-S, Metabolic engineering for improved fermentation of pentoses by yeasts. *Appl Microbiol Biotechnol* **63**:495–509 (2004).
33 Nielsen J, Larsson C, van Maris A and Pronk J, Metabolic engineering of yeast for production of fuels and chemicals. *Curr Opin Biotechnol* **24**:398–404 (2013).
34 Wei N, Oh EJ, Million G, Cate JHD and Jin Y-S, Simultaneous utilization of cellobiose, xylose, and acetic acid from lignocellulosic biomass for biofuel production by an engineered yeast platform. *ACS Synth Biol* **4**:707–713 (2015).
35 Parajó JC, Santos V, Domínguez H, Vázquez M and Alvarez C, Protein concentrates from yeast cultured in wood hydrolysates. *Food Chem* **53**:157–163 (1995).
36 Omar SS, Merrifield DL, Kühlwein H, Williams PEV and Davies SJ, Biofuel derived yeast protein concentrate (YPC) as a novel feed ingredient in carp diets. *Aquaculture* **330–333**:54–62 (2012).
37 Yamada EA and Sgarbieri VC, Yeast (*Saccharomyces cerevisiae*) protein concentrate: preparation, chemical composition, and nutritional and functional properties. *J Agric Food Chem* **53**:3931–3936 (2005).
38 Brown MR, Barrett SM, Volkman JK, Nearhos SP, Nell JA and Allan GL, Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture. *Aquaculture* **143**:341–360 (1996).
39 Gil A, Modulation of the immune response mediated by dietary nucleotides. *Eur J Clin Nutr* **56**:S1–S4 (2002).
40 Ugalde U and Castrillo JI, Single cell proteins from fungi and yeasts. *Appl Mycol Biotechnol* **2**:123–149 (2002).
41 Mydland LT, Frøyland JRK and Skrede A, Composition of individual nucleobases in diets containing different products from bacterial biomass grown on natural gas, and digestibility in mink (*Mustela vison*). *J Anim Phys Anim Nutr* **92**:1–8 (2008).
42 Chanda S and Chakrabarti S, Plant origin liquid waste: A resource for single-cell protein production by yeast. *Bioresour Technol* **57**:51–54 (1996).
43 Cheng ZJ, Hardy RW and Huige NJ, Apparent digestibility coefficients of nutrients in brewer's and rendered animal by-products for rainbow trout (*Oncorhynchus mykiss* (Walbaum)). *Aquacult Res* **35**:1–9 (2004).
44 Valdivie M, Compte X and Fundora O, The utilization of Torula yeast in diets for White Leghorn birds during growth and laying period. *Anim Feed Sci Technol* **7**:185–190 (1982).
45 Kuhad RC, Singh A, Tripathi KK, Saxena RK and Eriksson K-EL, Microorganisms as an alternative source of protein. *Nutr Rev* **55**:65–75 (1997).
46 National Research Council, *Nutrient Requirement of Fish and Shrimp*. The National Academy Press, Washington DC (2011).
47 Oliva-Teles A and Gonçalves P, Partial replacement of fishmeal by brewers yeast (*Saccharomyces cerevisiae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture* **202**:269–278 (2001).
48 Ozório ROA, Turini BGS, Môro GV, Oliveira LST and Portz L, Growth, nitrogen gain and indispensable amino acid retention of pacu (*Piaractus mesopotamicus*, Holmberg 1887) fed different brewers yeast (*Saccharomyces cerevisiae*) levels. *Aquacult Nutr* **16**:276–283 (2010).
49 Salnur S, Gultepe N and Hossu B, Replacement of fish meal by yeast (*Saccharomyces cerevisiae*): Effects on digestibility and blood parameters for gilthead sea bream (*Sparus aurata*). *J Anim Vet Adv* **8**:2557–2561 (2009).
50 Pavasovic A, Anderson AJ, Mather PB and Richardson NA, Effect of a variety of animal, plant and single cell-based feed ingredients on diet digestibility and digestive enzyme activity in redclaw crayfish, *Cherax quadricarinatus* (Von Martens 1868). *Aquaculture* **272**:564–572 (2007).
51 Rumsey GL, Hughes SG, Smith RR, Kinsella JE and Shetty KJ, Digestibility and energy values of intact, disrupted and extracts from brewer's dried yeast fed to rainbow trout (*Oncorhynchus mykiss*). *Anim Feed Sci Technol* **33**:185–193 (1991).
52 Langeland M, Vidakovic A, Vielma J, Lindberg JE and Kiessling A, Digestibility of microbial and mussel meal for Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*). *Aquacult Nutr* **22**:485–495 (2016).
53 Langeland M, Lindberg JE and Lundh T, Digestive enzyme activity in Eurasian perch (*Perca fluviatilis*) and Arctic charr (*Salvelinus alpinus*). *J Aquacult Res Dev* **5**:208 (2013).
54 Olvera-Novoa MA, Martìnez-Palacios CA and Olvera-Castillo L, Utilization of torula yeast (*Candida utilis*) as a protein source in diets for tilapia (*Oreochromis mossambicus* Peters) fry. *Aquacult Nutr* **8**:257–264 (2002).
55 Martin AM, Goddard S and Bemister P, Production of *Candida utilis* biomass as aquaculture feed. *J Sci Food Agric* **61**:363–370 (1993).
56 Murray AP and Marchant R, Nitrogen utilization in rainbow trout fingerlings (*Salmo Gairdneri* Richardson) fed mixed microbial mass. *Aquaculture* **54**:263–275 (1986).
57 Kim JK, Tak K-T and Moon J-H, A continuous fermentation of *Kluyveromyces fragilis* for the production of a highly nutritious protein diet. *Aquacult Nutr* **18**:41–49 (1998).
58 Tukmechi A and Bandboni M, Effects of *Saccharomyces cerevisiae* supplementation on immune response, haematological parameters, body composition and disease resistance in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792). *J Appl Ichthyol* **30**:55–61 (2014).
59 Nguyen TH, Fleet GH and Rogers PL, Composition of the cell walls of several yeast species. *Appl Microbiol Biotechnol* **50**:206–212 (1998).
60 Gopalakannan A and Arul V, Enhancement of the innate immune system and disease-resistant activity in *Cyprinus carpio* by oral administration of β-glucan and whole cell yeast. *Aquacult Res* **41**: 884–892 (2010).
61 Pacheco MTB, Caballero-Còrdoba GM and Sgarbieri VC, Composition and nutritive value of yeast biomass and yeast protein concentrates. *J Nutr Sci Vitaminol* **43**:601–612 (1997).
62 Baldwin, CV and Robinson CV, Enhanced disruption of *Candida utilis* using enzymatic pretreatment and high-pressure homogenization. *Biotechnol Bioeng* **43**:46–56 (1994).
63 Zhao L, Wang W, Huang X, Guo T, Wen W, Feng L *et al.*, *The effect of replacement of fish meal by yeast extract on the digestibility, growth and muscle composition of the shrimp* Litapenaeus vannamei. *Aquacult Res* doi: 10.1111/are.12883 (2015).
64 Vidakovic A, Langeland M, Sundh H, Sundell K, Olstorpe M, Vielma J *et al.*, Evaluation of growth performance and intestinal barrier function in Arctic Charr (*Salvelinus alpinus*) fed yeast (*Saccharomyces*

cerevisiae), fungi (*Rhizopus oryzae*) and blue mussel (*Mytilus edulis*). *Aquacult Nutr* doi: 10.1111/anu.12344 (2015).
65 Ozório ROA, Portz L, Borghesi R and Cyrino JEP, Effects of dietary yeast (*Saccharomyces cerevisiae*) supplementation in practical diets of tilapia (*Oreochromis niloticus*). *Animals* **2**:16–24 (2012).
66 Pongpet J, Ponchunchoovong S and Payooha K, Partial replacement of fishmeal by brewer's yeast (*Saccaromyces cerevisiae*) in the diets of Thai Panga (*Pangasianodon hypopthhalamus* x *Pangasius bocourti*). *Aquacult Nutr* doi: 10.1111/anu.12280 (2015).
67 Rumsey GL, Kinsella JE, Shetty KJ and Hughes SG, Effect of high dietary concentration of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). *Anim Feed Sci Technol* **33**:177–183 (1991).
68 Gamboa-Delgado J, Fernández-Díaz B, Nieto-López M and Cruz-Suárez LE, Nutritional contribution of torula yeast and fish meal to the growth of shrimp *Litopenaeus vannamei* as indicated by natural nitrogen stable isotopes. *Aquaculture* **453**:116–121 (2016).
69 Hauptman BS, Barrows FT, Block SS, Gaylord TG, Paterson JA, Rawles SD *et al.*, Evaluation of grain distillers dried yeast as a fish meal substitute in practical-type diets of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **432**:7–14 (2014).
70 D'Mello JPF, Utilization of dietary purines and pyrimidines by non-ruminant animals. *Proc Nutr Soc* **41**:301–308 (1982).
71 Rumsey GL, Winfree RA and Hughes SG, Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **108**:97–110 (1992).
72 Fournier V, Gouillou-Coustans MF, Métailler R, Vachot C, Moriceau J, Delliou HL *et al.*, Nitrogen utililisation and ureogenesis as affected by dietary nucleic acid in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). *Fish Phys Biochem* **26**:177–188 (2002).
73 Roth FX and Kirchgessner M, Alimentär zugeführte Nukleinsäuren im N-Stoffwechsel von Monogastriden. *Arch Tierernährung* **30**:77–88 (1980).
74 Andersen Ø, Aas TS, Skugor S, Takle H, van Nes S, Grisdale-Helland B *et al.*, Purine-induced expression of urate oxidase and enzyme activity in Atlantic salmon (*Salmo salar*). Cloning of urate oxidase liver cDNA from three teleost species and the African lungfish *Protopterus annectens*. *FEBS J* **273**:2839–2850 (2006).
75 Peterson BC, Booth NJ and Manning BB, Replacement of fish meal in juvenile channel catfish, *Ictalurus punctatus*, diets using a yeast-derived protein source: the effects on weight gain, food conversion ratio, body composition and survival of catfish challenged with *Edwardsiella ictaluri*. *Aquacult Nutr* **18**:132–137 (2012).
76 Luzzana U, Valfrè F, Mangiarotti M, Domenehini C, Radaelli G, Moretti VM *et al.*, Evaluation of different protein sources in fingerling *Mugil cephalus* practical diets. *Aquacult Int* **13**:291–303 (2005).
77 Ribeiro CS, Moreira RG, Cantelmo OA and Esposito E, The use of *Kluyveromyces marxianus* in the diet of red-stirling tilapia (*Oreochromis niloticus*, Linnaeus) exposed to natural climatic variation: effects on growth performance, fatty acids, and protein deposition. *Aquacult Res* **45**:812–827 (2014).
78 Refstie S, Baeverfjord G, Seim RR and Elvebø O, Effects of dietary yeast cell wall β-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. *Aquaculture* **305**:109–116 (2010).
79 Li P and Gatlin III DM, Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture* **219**:681–692 (2003).
80 Li P and Gatlin III DM, Dietary brewers yeast and the prebiotic Grobiotic™ AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. *Aquaculture* **231**:445–456 (2004).
81 Ortuño J, Cuesta A, Rodríguez A, Esteban MA and Meseguer J, Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L.). *Vet Immunol Immunopathol* **85**:41–50 (2002).
82 Rodríguez A, Cuesta A, Ortuño J, Esteban MA and Meseguer J, Immunostimulant properties of a cell wall-modified whole *Saccharomyces cerevisiae* strain administered by diet to seabream (*Sparus aurata* L.). *Vet Immunol Immunopathol* **96**:183–192 (2003).
83 He S, Zhou Z, Meng K, Zhao H, Yao B, Ringø E *et al.*, Effects of dietary antibiotic growth promoter and *Saccharomyces cerevisiae* fermentation product on production, intestinal bacterial community, and nonspecific immunity of hybrid tilapia (*Oreochromis niloticus* female × *Oreochromis aureus* male). *J Anim Sci* **89**:84–92 (2011).
84 Tewary A and Patra BC, Oral administration of baker's yeast (*Saccharomyces cerevisiae*) acts as a growth promoter and immunomodulator in *Labeo rohita* (Ham.). *J Aquac Res Dev* **2**:109 (2011).
85 Welker TL, Lim C, Yildirim-Aksoy M and Klesius PH, Effect of short-term feeding duration of diets containing commercial whole-cell yeast or yeast subcomponents on immune function and disease resistance in channel catfish, *Ictalurus punctatus*. *J Anim Physiol Anim Nutr* **96**:159–171 (2012).
86 Yu HH, Han F, Xue M, Wang J, Tacon P, Zheng YH *et al.*, Efficacy and tolerance of yeast cell wall as an immunostimulant in the diet of Japanese seabass (*Lateolabrax japonicus*). *Aquaculture* **432**: 217–224 (2014).
87 El-Boshy ME, El-Ashram AM, AbdelHamid FM and Gadalla HA, Immunomodulatory effect of dietary *Saccharomyces cerevisiae*, β-glucan and laminaran in mercuric chloride treated Nile tilapia (*Oreochromis niloticus*) and experimentally infected with *Aeromonas hydrophila*. *Fish Shellfish Immunol* **28**:802–808 (2010).
88 Siwicki AK, Anderson DP and Rumsey GL. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Vet Immunol Immunopathol* **41**:125–139 (1994).
89 Sheikhzadeh N, Heidarieh M, Pashaki AT, Nofouzi K, Farshbafi A and Akbari M, Hilyses® fermented *Saccharomyces cerevisiae* enhances the growth performance and skin non-specific immune parameters in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol* **32**:1083–1087 (2012).
90 Maccaferri S, Klinder A, Brigidi P, Cavina P and Costabile A, Potential probiotic *Kluyveromyces marxianus* B0399 modulates the immune response in Caco-2 cells and peripheral blood mononuclear cells and impacts the human gut microbiota in an *in vitro* colonic model system. *Appl Environ Microbiol* **78**:956–964 (2012).
91 Abdel-Tawwab M, Abdel-Rahman AM and Ismael NEM, Evaluation of commercial live baker's yeast, *Saccharomyces cerevisiae*, as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged *in situ* with *Aeromonas hydrophila*. *Aquaculture* **280**:185–189 (2008).
92 Gatesoupe FJ, Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture* **267**:20–30 (2007).
93 Grammes F, Reveco FE, Romarheim OH, Landsverk T, Mydland LT and Øverland M, *Candida utilis* and *Chlorella vulgaris* counteract intestinal inflammation in Atlantic salmon (*Salmo salar* L.). *PLoS ONE* **8**:e83213 (2013).
94 Wang J, Zhang, D, Sun Y, Wang S, Li P, Gatlin III DM *et al.*, Effect of a dairy-yeast prebiotic (GroBiotic®-A) on growth performance, body composition, antioxidant capacity and immune functions of juvenile starry flounder (*Platichthys stellatus*). *Aquacult Res* **47**:398–408 (2016).
95 Heidarieh M, Mirvaghefi AR, Akbari M, Sheikhzadeh N, Kamyabi-Moghaddam Z, Askari H *et al.*, Evaluations of Hilyses™, fermented *Saccharomyces cerevisiae*, on rainbow trout (*Oncorhynchus mykiss*) growth performance, enzymatic activities and gastrointestinal structure. *Aquacult Nutr* **19**:343–348 (2013).
96 Barnes ME, Durben DJ, Reeves SG and Sanders R, Dietary yeast culture supplementation improves initial rearing of McConaughty strain rainbow trout. *Aquacult Nutr* **12**:388–394 (2006).
97 Burrels C, Williams PD, Southgate PJ and Wadsworth SL, Dietary nucleotides: a novel supplement in fish feeds. 2. Effects on vaccination, salt water transfer, growth rates and physiology of Atlantic salmon (*Salmo salar* L.). *Aquaculture* **199**:171–184 (2001).
98 Lin Y-H, Wang H and Shiau SY, Dietary nucleotide supplementation enhances growth and immune responses of grouper, *Epinephelus malabaricus*. *Aquacult Nutr* **15**:117–122 (2009).
99 Li P and Gatlin III DM, Nucleotide nutrition in fish: Current knowledge and future applications. *Aquaculture* **251**:141–152 (2006).
100 Hoseinifar SH, Mirvaghefi A and Merrifield DL, The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). *Aquaculture* **318**:90–94 (2011).
101 Sweetman JW, Torrecillas S, Dimitroglou A, Rider S, Davies SJ and Izquierdo MS, Enhancing the natural defences and barrier protection of aquaculture species. *Aquacult Res* **41**:345–355 (2010).
102 Li XH, Chen YP, Cheng YF, Yang WL, Wen C and Zhou YM, Effect of yeast cell wall powder with different particle sizes on the growth

performance, serum metabolites, immunity and oxidative status of broilers. *Anim Feed Sci Technol* **212**:81–89 (2016).
103 Reynolds LP, Wulster-Radcliffe MC, Aaron DK and Davis TA, Importance of animals in agricultural sustainability and food security. *Science* **145**:1377–1379 (2015).
104 Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen, K *et al.*, Aquaculture; global status and trends. *Philos Trans R Soc London B* **365**:2897–2912 (2010).
105 Torstensen BE, Espe M, Sanden M, Stubhaug I, Waagbø R, Hemre G-I *et al.*, Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. *Aquaculture* **285**:193–200 (2008).
106 Crampton VO, Nanton DA, Ruohonen K, Skjervold P-O and El-Mowafi A, Demonstration of salmon farming as a net producer of fish protein and oil. *Aquacult Nutr* **16**:437–446 (2010).
107 Bendiksen EÅ, Johnsen CA, Olsen HJ and Jobling M, Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture* **314**:132–139 (2011).
108 Francis G, Makkar HPS and Becker K, Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* **199**:197–227 (2001).
109 Gatlin III DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW *et al.*, Expanding the utilisation of sustainable plant products in aquafeeds: a review. *Aquacult Res* **38**:551–579 (2007).
110 Krogdahl Å, Penn M, Thorsen J and Bakke AM, Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquacult Res* **41**:333–344 (2010).
111 Anater A, Manyes L, Meca G, Ferrer E, Luciano FB, Pimpão CT *et al.*, Mycotoxins and their consequences in aquaculture: A review. *Aquaculture* **451**:1–10 (2016).
112 Schlechtriem C, Pucher J and Michalski B, Dietary burden calculations relating to fish metabolism studies. *J Sci Food Agric* **96**:1415–1419 (2016).
113 Flachowsky G, Chesson A and Aulrich K, Animal nutrition with feeds from genetically modified plants. *Arch Anim Nutr* **59**:1–40 (2005).
114 Fry PF, Love DC, MacDonald GK, West PC, Engstom PM, Nachman KE *et al.*, Environmental health impacts of feeding crops to farmed fish. *Environment Int* **91**:201–214 (2016).
115 Kumar R, Singh S and Singh OV, Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. *J Ind Microbiol Biotechnol* **35**:377–391 (2008).
116 Kim BG, Liu Y and Stein HH, Energy concentration and phosphorus digestibility in yeast products produced from the ethanol industry, and in brewer's yeast, fish meal, and soybean meal fed to growing pigs. *J Anim Sci* **92**:5476–5484 (2014).
117 Spark M, Paschertz H and Kamphues J, Yeast (different sources and levels) as protein source in diets of reared piglets: effects on protein digestibility and N-metabolism. *J Anim Physiol Anim Nutr* **89**:184–188 (2005).
118 Nigam JN, Single cell protein from pineapple cannery effluent. *World J Microbiol Biotechnol* **14**:693–696 (1998).
119 Singh K and Neelakantan S, Amino acid composition of yeast single cell grown on paneer whey. *J Dairy Res* **56**:813–815 (1989).
120 Anderson PJ, McNeil KE and Watson K, Thermotolerant single cell protein production by *Kluyvermyces marxianus* var. *marxianus*. *J Ind Microbiol* **3**:9–14 (1988).

