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Use of yeasts in aquaculture nutrition and immunostimulation: A review

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ABSTRACT

With the technological advancement, application of yeasts in aquaculture becomes very popular, especially as an alternative source of proteins in addition to other proteins commonly used in the fish feed industry. Recently, yeast becomes a sustainable novel ingredient of aquafeed for its promising role in nutrition and immunostimulation of many fish species in aquaculture. Thus, yeast supplements and yeast-containing feed ingredients lead to the higher protection against diseases and to the better productivity of fishes resulting in the greater growth of the aquaculture industry. Moreover, rotifers, *Artemia*, and copepods can be produced well as live aquafeed by application of yeasts in aquaculture. Some yeasts used in probiotic products often improve immunity of fishes as well as attempt to enhance the water quality of aquaculture resulting in good production outcomes. Thus, yeast has been appeared as a novel and vital component of aquatic animal's feed in modern aquaculture. In this review, different aspects of usage of yeasts in aquaculture nutrition and immunostimulation have been discussed.

1. INTRODUCTION

Fish farming is currently the fastest food producing industry and now accounts for more than 50% of the world's production of seafood [1]. The world is moving toward the advancement of environment-friendly and cost-saving farming systems for sustainable fish productions, such as biofloc and aquamimicry, both of which rely on microorganism fermentation [2]. If the cultivated species have to confer on optimum growth, feed supplementation and disease tolerance, fish production industries would be beneficial and profitable [3]. The need of sharing precise knowledge on all relevant subjects is becoming a basic fundamental for aquaculture's responsible management [1]. During the past few years, traditional use of antibiotics in aquaculture has been criticized due to the potential development of antibiotic-resistant bacteria, the presence of antibiotic residues in fish flesh, and the destruction of microbial populations in the culture environment [4]. As an alternative to antibiotics, probiotics have attracted extensive scientific and commercial attention in the aquaculture industry [4]. Probiotics applications are playing role as an alternative approach to control microbiota in aquaculture farms, especially in fish hatcheries [4,5]. Due to the rapid expansion of aquaculture, there is a high demand of fishmeal, and their deficiency may impede the long-term development of the industry [6]. Aquaculture biotechnology has a great importance because of its potential role in discovery of new products as well as development of novel processes of economic importance. Hence, a lot of works has been done in aquaculture biotechnology to find out other forms of protein and oil, but suitable alternatives are still inadequate. Plant-based formulations are the least costly options, and many such formulations have specific protein suitable for fish growth and immunity [7]. Recently, yeasts have been considered as a suitable feed for both farmed and wild fishes because of its effective function in fish nutrition, immunity, and health which have been discussed in many literatures. Moreover, yeasts have been used either to feed *Artemia* and rotifers or to ferment feedstuff after natural or artificial colonization in gastrointestinal tracts of host [7]. Hence, the purpose of this review is to highlight the role of yeasts in aquaculture nutrition and immunostimulation in different types of culture technologies and aquatic species.

2. YEAST CELL FORMULATIONS AND NUTRITIONAL PROPERTIES

Yeast, also known as mold or fungus, is defined as a single-celled eukaryote, containing membrane-bound organelles such as the mitochondria, nucleus, and endomembrane system [8]. Different species of yeasts such as *Saccharomyces cerevisiae*, *Kluyveromyces*, *Torulaspora*, *Saccharomyce*, and *Torulopsis* are used as a source of protein in aquafeeds for culture of larvae of shrimps and marine fishes [9]. Yeast cell comprises diverse strain-specific structure as well as synthetic properties of the cell wall [10]. The chemical composition of whole yeast cell depends on strains of yeast, culture medium and

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growth conditions, and on subsequent post-fermentation processing and development of cellulosic biomass [11]. It has been reported that there is around 32–62% protein in yeast *S. cerevisiae* [12]. In addition, *Kluyveromyces fragilis* has been reported to account for around 50–55% of the protein content [12]. It is also reported that the 93–97% of dry yeast in average is dry matter which is composed of 40–60% crude nitrogen protein, 5–9% lipids, and 35–45% carbohydrates [12,13]. Many mineral components are available in yeast, such as sodium, copper, zinc, calcium, manganese, iron, and selenium [12]. In addition, yeast species such as *S. cerevisiae* have unsaturated fatty acids and linoleic and alpha-linolenic acids which can also be formed by fatty acid desaturase [12]. Furthermore, linoleic and alpha-linolenic acids have been reported to be produced by *Kluyveromyces lactis* yeast species. As shown in Figure 1, dry yeast products produced from the fermentation of low-value and non-food cellulosic materials are potential safe sources of protein in fish diets [11].

As shown in Figure 1, the pre-treatment of biomass includes flowing steps: Extraction of lignin, hydrolysis to make available cellulose and hemicellulose, enzymatic hydrolysis to turn hemicellulose and cellulose into C5 and C6 sugars, fermentation of sugars, ammonia, phosphorus and other nutrients, and downstream processing into dry yeast products for use in fish feed as a source of protein [11].

3. USE OF YEAST IN FISH FEEDING

The aquaculture industry requires 2–5 times much more fish meal to feed cultivated species than the farmed commodity provides [14]. Hence, scientists and fishermen are worried about the growing demand of fish feed resulted from comprehensive practices of aquaculture globally [15]. Using yeasts as a feed for fish farming are not a recent idea, as several studies have already been done on it, even before the early 1980s, though it has more importance on modern aquaculture biotechnology [9]. Because, using of yeasts as fish feed could be one of the more affordable and environmentally sustainable approach in aquaculture biotechnology resulting in reduction of fishmeal dependence [16]. Yeasts are an impressive protein-rich single-cell organism which have a low toxicity potential, can be grown on a wide variety of substrates, and are normally easy to cultivate [12]. In addition to vital amino acid such as lysine and amino acids containing sulfur, yeast is a provider of several important vitamins such as Vitamin B and folic acid [17]. A study on alternate sources of protein reveals that *S. cerevisiae* is an effective natural resource of protein when supplemented for fish meal in tilapia feed [18]. Similarly, another study shows that the 15% inclusion of yeast encourage the growth performance without reducing the quality of end-product [14]. Thus, some of the yeast species are used as supplementary feed in aquaculture which, in turn, improve the growth efficiency, resistance to fish disease, quality of water, and diversity of microbial communities, as shown in [Table 1].

The effects of partial and complete substitution of fishmeal with brewer's yeast on the growth, body composition, feed consumption, and digestibility of juvenile tilapia have been studied in several studies [7]. Some studies have reported that supplementation of 10% fishmeal with brewer's yeast, *S. cerevisiae*, has improved the nitrogen (N) gain and protein efficiency ratio in Nile tilapia (*Oreochromis niloticus*), but presented no substantial impact on growth efficiency [14]. Dietary dried yeast is evidently palatable to tilapia juveniles and also reported as ideal and successful diet for fostering their growth without impacting body composition [18]. Furthermore, fish feeding with various levels of dried yeast results in significant impact of body weight of fish as indicated by 20% greater growth by the dietary yeast inclusion in replacement of fish meal up to 40% [14]. Likewise, many yeast species have a high impact on growth performance, feed use, and the biochemical composition of the body of *O. niloticus* fingerlings. For example, the dietary supplementation of *S. cerevisiae* (1.0 g yeast kg^{-1} diet) has been reported to improve the growth performance of *O. niloticus* fingerlings and feed utilization [29]. Similarly, the effect of different levels of dietary supplementation of different yeast such as *S. cerevisiae*, *Wickerhamomyces anomalus*, *Cyberlindnera jadinii*, *Kluyveromyces marxianus*, and *Blastobotrys adeninivorans* has also been assessed in some studies [28].

4. YEASTS AS THE MAIN PROTEIN-RICH COMPONENT IN AQUAFEED

In the future, aquaculture will play a significant role in addressing the growing global food crisis [24]. However, fish feed manufacturers are using threatened fish stocks to supply feed in aquaculture industry for carnivorous species such as Atlantic salmon and rainbow trout [11,30]. The unavailability of aquafeed production resources could be a major constraint that is expected to exacerbate the rapidly expanding aquaculture sector [28]. Salmon farming has recently shown a decreased reliance on marine ingredients by replacing them with plant ingredients, specifically soy protein [31]. Ingredients from single-cell organisms are a comparatively large class of materials that in certain cases contain products extracted from bacteria, fungi (yeasts), microalgae, or combination of them [9]. Yeast cells contain substantial protein content (approximately 40–55%) and other bioactive compounds which are essential for fish development and growth performance [28,32]. It was reported that the

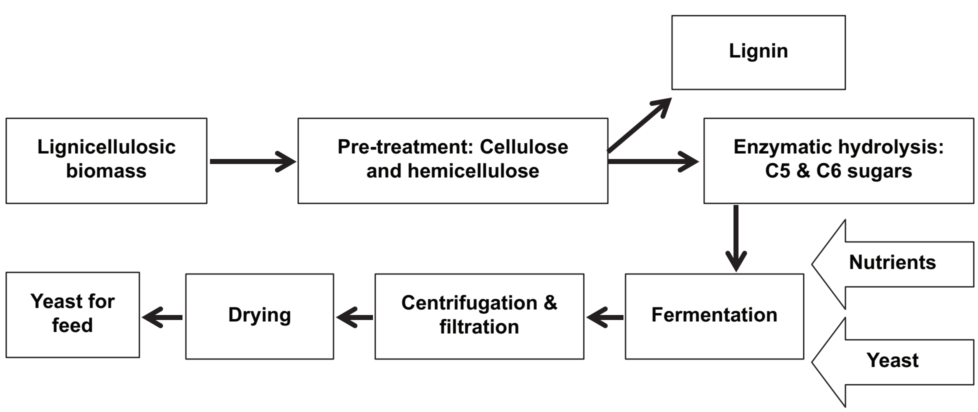

Figure 1: Pre-treatment steps for low-value and non-food cellulosic materials to improve their value and downstream processing into dry yeast products for use as a source of protein in fish feed [11].

Table 1: The yeast species commonly used in aquaculture nutrition for higher growth and immunity of aquatic animal as well as for improvement of water quality.

Yeast species	Aquaculture species	Effects	Method used	References
Saccharomyces cerevisiae	Freshwater catfish gulsa tengra, *Mystus cavasius* and Nile tilapia (*Oreochromis niloticus*)	Antioxidants and immune system enhancement, resistance to *Aspergillus flavus* infection, and growth efficiency enhancement	Feed additive or supplementary feed	[16,18]
Candida utilis	Atlantic salmon, rainbow trout, and whiteleg shrimp.	Source of protein for fish feed ingredients	Feed ingredients	[19]
Aspergillus awamori	Turbot fish *Scophthalmus maximus* L.	Enhancing the nutritional value and health status with soybean meal for fish feed	Fermentation with soybean meal	[20]
Pichia fermentans and *Meyerozyma caribbica*	Java barb *Barbonymus gonionotus*	Enhance the growth performance	Used as diets	[21]
Candida tropicalis	Multiple aquaculture species	Water nitrogen removal, biofloc formation and enhance the microbial communities	Water additives	[22]
Debaryomyces hansenii	Multiple aquaculture species	An immunostimulant with antioxidant properties and positive effects on the health of the fish intestines.	Feed additive	[23]
Rhodotorula mucilaginosa and *Bacillus licheniformis*	*Litopenaeus vannamei*	Enhanced bowel wellbeing, immune systems and tolerance to ammonia	Feed ingredient	[24]
Kluyveromyces lactis	Atlantic salmon (*Salmo salar*) and gilthead seabream (*Sparus aurata*)	Increase the growth efficiency and optimization the immune activities	As protein sources in diets	[25]
Metschnikowia sp. in combination with *Rhodotorula sp.*	Juvenile of sea cucumber (*Apostichopus japonicus*)	For enhance the growth, improve digestive enzyme production, add nutritional content, and promote the immune system.	Feed additive	[26]
Hanseniaspora opuntiae	Juvenile of sea cucumber (*Apostichopus japonicus*)	Improving immunity and disease resistance to infection with *Vibrio splendidus*	Feed additive	[27]
Cyberlindnera jadinii, Kluyveromyces marxianus, Blastobotrys adeninivorans and Wickerhamomyces anomalus	Atlantic salmon and rainbow trout	Increase the growth performance and replacement for fishmeal and soy protein in fish feed	Protein ingredients	[28]

yeasts used in rainbow trout diets are *Candida* species and almost 40% of fishmeal is effectively substituted without any reduction in production or efficiency [9]. However, researchers have shown that feeding yeast to supply 100% of the protein in the rainbow trout diet leads to dangerous amounts of kidney uric acid and blood anemia [17]. Hence, as promising replacements for fish meal in fish diets, single-cell proteins, including yeast and bacteria, can be used, and low-value refined yeast and non-food lignocellulosic biomass in aquaculture diets can be a healthy source of protein, but this replacement must be partial or balanced for ensuring health safety of consumers [30]. However, yeasts are capable of converting low-value non-food biomass from agricultural industries into high-value feeds that are less dependent on arable land, water, and changing climate conditions [33-35]. A monosaccharide with five or six carbon atoms of lignocellulosic substrates and complementary nutrients can be transformed by fermentation into protein-rich yeast biomass after enzymatic hydrolysis [11]. Yeast, *S. cerevisiae*, contains various immunostimulating compounds indicating the potentiality of use of whole yeast as natural immunostimulants in common fish diets such as seabream *Sparus aurata* [36]. In addition, the yeast can be used in culture of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) [37]. Some studies have been done on feeding yeast protein on the growth and feed utilization parameters of *Clarias gariepinus*, *O. niloticus,* Atlantic salmon, rainbow trout, and whiteleg shrimp which are cultured separately [16,18,19]. The results have shown 30% and 50% increase of growth of *C. gariepinus* and *O. niloticus* fingerlings, respectively. It can, therefore, be inferred that fishmeal should then be substituted at these levels with single-cell yeast protein to reduce the expense of aquafeed for the sustainable production of aquaculture for these species [38].

5. YEAST AS PROBIOTICS

There are different limitations in industrial fish farming, such as burdens, deterioration of water quality, and malnutrition resulting in compromised immune system. Diseases have been found to spread quickly and usually have threatened the intensive systems of fish farming, and causing the major economic losses [3]. The use of antibiotics is a common strategy for controlling the fish diseases which often lead to the emergence of antibiotic resistant pathogens and their dissemination to the human body [39-42]. In addition, the presence of leftover medicinal properties in the body and ecosystem of fish often raises the risk to human health [26]. Another concern posed by the widespread use of antibiotics in the management of aquaculture diseases has been the proliferation of populations of multidrug and/or extremely drug resistance bacteria that cause many incurable infectious diseases [43]. Hence, instead of using antibiotics, novel techniques should be developed to control the pathogenic microorganisms in aquaculture [3]. Probiotics are usually regarded safe for consumption, although in rare situations, they can induce pathogenic interactions and unintended health risks [44]. The concern of probiotics as an environmentally sustainable solution for enhancing the quality of water for better production of fishes is growing day by day [4,25,45,46]. Furthermore, research into whiteleg shrimp *Penaeus vannamei* has demonstrated that the use of mixed probiotic cultures improves viability, feed conversion, and the final yield of farmed shrimp [47]. The most widely used probiotics in aquaculture alongside yeasts are bacteria of the genus *Bacillus* [48]. Studies have shown that polysaccharides of fungi are prebiotics component that is generally recognized as a nutritional ingredient for the control of health and growth conditions in aquaculture activity [3]. Another study has reported that Indian shrimp *Fenneropenaeus indicus* shows substantial improvement in immune response and growth by feed on beta-glucans of marine yeast [49]. It has been reported that a mixture of yeast supplementation can promote growth, improve the function of the digestive enzyme, add nutritional contents, and induce the intrinsic immune responses of Apostichopus japonicus juveniles [26]. Similarly, the yeast supplementation causes promising positive development as well as the inhibition of diseases in freshwater catfish *Mystus cavasius* [18]. Moreover, commercial probiotic products are currently being prepared from different species of yeast such as *S. cerevisiae,* as shown in Table 2, and their use is controlled by regulation and careful recommendations for better management of aquaculture system [50].

However, probiotics are typically used to control specific pathogens through competitive exclusion or improvement of fish immunity systems [4]. However, some yeast species such as *Debaryomyces hansenii*, *Rhodotorula sp*., *Metschnikowia zobelii,* and *Trichosporon cutaneum* which are commonly found in the intestines of fish are known to accelerate the development of the digestive system in fish [53]. Likewise, some yeast species present in the gastrointestinal tract of healthy fish are reported as an essential part of the fish gut's microbiota [53]. Moreover, bioactive substances with potential application in mariculture could be produced by the marine yeasts mixed with dietary supplements as probiotics. Therefore, in aquaculture, the yeast has been deemed an exceptional probiotic nominee [26]. The evidences of using yeasts as probiotics are growing which, in turn, help us to consider these yeasts as a potential candidate for boosting growth, survival, and intestinal maturation, and also to improve the immune systems of larvae and juveniles of fish [7].

6. MECHANISM OF YEASTS TOWARD IMMUNOSTIMULATION OF FISHES

Yeasts are a good source of β-glucan which is well-known for its role in immunostimulation in fishes and other vertebrates [54]. Hence, yeasts are commercially applied in aquaculture as a probiotic to induce immune responses in host fishes by the binding of pathogen-associated molecular pattern (PAMP) to pattern recognition receptor (PRR). Such PAMP-PRR interaction and the downstream signaling are responsible for enhancement of the activities of the immune system in fishes [54]. In this immunostimulation mechanism, β-glucan and β-glucan-derived active metabolites such as short-chain fatty acids (SCFAs) act as PAMP after entrance of yeasts into the fish body. Different receptors such as Dectin-1, Toll-like receptors (TLRs), C-type lectin receptor, and complement receptor Type 3 (CR3; CD11b/CD18) have been known to bind to β-glucans as PRRs [54,55]. TLRs, generally TLR-2 and TLR-4, together with lactosylceramide and scavenger receptor can bind yeast ligands, and can be characterized as β-glucan receptor for inducing signaling through the MyD88 pathway which, in turn, causes subsequent release of cytokines for providing anti-fungal immunity [54,55]. However, information on PRR for SCFAs is still inadequate. The binding of β-glucans and/or SCFAs to known or unknown PRRs has been reported to stimulate the immune system of fishes for enhancement of phagocytosis, production of reactive oxygen species, leukotriene, interleukin and cytokines, and other immune responses associated with Th1 immunity [Figure 2] [54-57]. In this mechanism, professional antigen presenting cells such as macrophages are activated by β-glucan to engulf pathogens and to process and present antigens more efficiently by phagocytosis and autophagy which, in turn, activate other immune cells [54-56]. Leukotriene released by activated macrophages is known to generate anti-infection immunity. Similarly, interleukin and cytokines produced by activated macrophages have been reported to activate T cells, B cells, NK cells, and other macrophages for better defense against pathogens. Thus, all these immune responses are vital for the protection against many pathogens. In such defensive mechanisms, Dectin-1 has been reported as the main PRR that can regulate their own signaling and

Table 2: Some of the yeast species used in aquaculture as probiotics for better growth performance, higher resistance to disease, and improved immune system of aquatic animals.

Yeast species	Aquaculture species	Effects	References
Kluyveromyces lactis M3	Gilthead seabream (*Sparus aurata*)	Immunostimulant activitvation for finfish	[25]
Rhodotorula sp. H26 and *Metschnikowia sp.* C14	Sea cucumber, Apostichopus japonicus	Improve the innate immune response	[26]
Saccharomyces cerevisiae	Convict cichlid (*Amatitlania nigrofasciata*) and *Oreochromis* niloticus	Improve feed utilization and increase survival rate and immunostimulants	[51]
Debaryomyces hansenii BUU01 and Rhodotorula sp. BUU02	Whiteleg shrimp (*Litopenaeus vannamei*)	Enhanced survival and growth and decreased *Vibrio* levels, along with a rise in the number of beneficial probiotics and a reduction in the amount of likely digestive tract pathogenic bacteria (*Vibrio parahaemolyticus* and *Vibrio cholerae*).	[52]
Metschnikowia zobelii in combination with *Rhodotorula sp.*	Sea cucumber (*Apostichopus japonicus*)	Improving digestive enzyme development, adding nutritional content, and stimulating the immune system to enhance growth.	[26]
Hanseniaspora opuntiae	Sea cucumber (*Apostichopus japonicus*)	Improving immunity and disease resistant to *Vibrio splendidus* infection	[27]
Rhodotorula benthica D30	Sea cucumber, (*Apostichopus japonicus*)	Improving immunity system and disease resistant	[47]

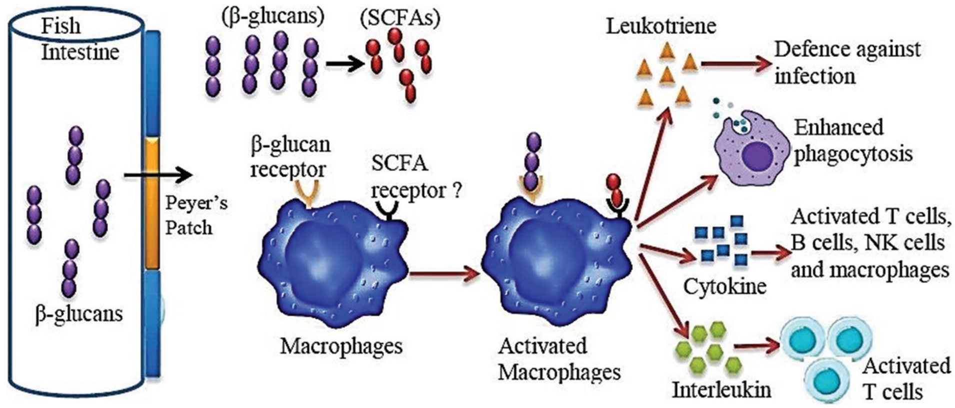

Figure 2: Mechanism of yeast-derived β-glucans toward immunostimulation of fishes.

can be activated by β-glucans to initiate immune response. However, Dectin-1 can synergize with other receptors to start specific immune responses to β-glucans [54-57].

7. USE OF YEAST IN LIVE AQUAFEED CULTURE TECHNIQUE

With the impressive advances in larval rearing technology, worldwide hatchery expansion requires the effective development of rotifers, copepods, and *Artemia* culture technique to ensure their adequate supply as an important larval food. Due to the size, nutritional value, and behavior of rotifers, they are the most vital live food species used for the rearing of fish larvae [58]. Moreover, *Artemia nauplii* has become one of the most reliable live foods for fish and is commonly used to feed early stages of cultivated aquatic species in aquaculture [59]. Rotifers (*Brachionus* sp.) are spread as a living capsule, supplying the necessary nutrients needed for the proper growth and production of cultivated fish larvae [60]. Notably, algae and baker's yeast are forms of sources of food that have been used to culture these live aquafeeds such as rotifers and *Artemia* [61]. However, single-cell algae are small in size and contain high amount protein, but their cultivation is comparatively difficult and costly than yeast [62]. Because of these significant constraints of microalgae production, yeasts are considered a potential replacement of microalgae. An important impact on the production of the gnotobiotic *Artemia* has been reported where *S. cerevisiae* strain wild-type bacteria-free baker's yeast has been provided to *Artemia* as a main food under a regulated gnotobiotic culture system in the laboratory [63]. In addition, a medium containing a mixture of yeast, starch, and albumen has been shown to be suitable for the growth of rotifers. However, due to lack of knowledge and data about the feeding rate, feeding frequency, and range of fed compositions, the amount of yeast use differs tremendously in rotifer culture [64]. In a study, the optimum feeding rate and feeding frequency of *Brachionus plicatilis* are then determined using a moderately diet process dependent on various yeast quantities [65]. The findings reveal that the feeding rate of the rotifers is adequate to an average of 0.3 g of barker yeast to a million rotifers and that, with rising feeding frequency, the population growth rate and egg-bearing ratio can be increased from twice to 3 times [65]. In addition, marine yeast and baker's yeast have been used in combination to feed rotifer *B. plicatilis* in other studies which have contributed to the birth rate and total productivity of rotifers [66]. Hence, yeast is preferred as a supplement or protein substitute in the culture of live food. Moreover, studies have shown that yeast raises the density of *Tisbe furcata* copepods in culture and may be a potential candidate in the future for feed supplements for other copepod organisms [67]. Other experiments have also shown that the use of commercial *S. cerevisiae* baker's yeast with a soybean component to feed copepods has attained the highest relative population density compared to marine microalgae [68].

8. CONCLUSIONS AND FUTURE RESEARCH ASPECTS

As discussed in this review article, the yeast applications in aquaculture have become very important and feasible which is entered into the fish feed industry as an alternative source of protein. Furthermore, it gives positive impact in aquaculture by increasing weight of many fish species such as tilapia and catfish. Moreover, yeast can be used as a promising feed for live aquafeed aquaculture. Yeast has also been used as probiotics to provide better immunity against pathogens as well as to improve water quality of aquaculture resulting in better production of fishes. In addition, yeast supplements and yeast-containing feed ingredients are used as a sustainable feed resource in aquaculture. However, complete replacement of other types of protein with yeast-protein in aquafeed has been discouraged because of some negative impacts on kidneys of some fishes which, in turn, may impose health risk to the consumers. Even though, use of yeasts appears as a potential protein ingredient in aquafeed, data are lacking on the safe and effective ratio of replacement of other protein with yeast-protein in aquafeed for most fish species. Hence, future researches on this field should be focused on the exploration of effective as well as the safest amount of yeast to be used in aquafeed for specific fish species in a particular culture technique. Therefore, more scientific researches on yeast are the demand of time for the development of novel and promising approaches to use yeast in aquaculture industry in near future which will be safe for fish, environment, and human.

9. AUTHORS' CONTRIBUTIONS

Mohammed A. Mahdy, Mamdoh T. Jamal and Md Fazlul Haque wrote the first draft of the manuscript. Mamdouh Al-Harbi and Bandar A. Al-Mur managed the literature search. Md Fazlul Haque edited and finalized the manuscript.

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11. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

12. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

13. DATA AVAILABILITY

The data used to support the findings of this study are included within the article.

14. PUBLISHER'S NOTE

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