



# Use of active yeasts in shrimp nutrition

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## ABSTRACT

Shrimp aquaculture is an industry that uses fishmeal in diets, which is an ingredient difficult to obtain at low cost and increasingly scarce. It is important to find strategies that help shrimp nutrition by using alternative ingredients that replace or complement fishmeal and help develop a more environmentally friendly industry. Yeasts can be added to diets for aquatic organisms and are microbial products generated by biotechnological systems or a by-product from agri-food industries. This study determined the potential use of active yeasts (*Candida insectorum*, *C. parapsilosis*, *C. sake*, *C. utilis*, *Debaryomyces hansenii*, *Rhodospiridium paludigenum*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Yarrowia lipolytica*) in juvenile and postlarvae shrimp of *Litopenaeus schmitti*, *Fenneropenaeus indicus*, and *L. vannamei*. The results show that active yeasts can be used at different doses, as partial substitute for a fishmeal-soybean meal and incorporated into diets for juvenile shrimp or used directly in diets for postlarvae. However, it is necessary to carry out studies to determine the most efficient strategies for the active yeast to be ingested by shrimp.

**Keywords:** Diet; nutrition; penaeid; shrimp; yeast (*Source: CAB*).

## RESUMEN

La acuicultura de camarón es una industria que usa harina de pescado en las dietas, el cual es un ingrediente difícil de obtener a bajo costo y es cada vez más escaso. Es importante encontrar estrategias que ayuden a la nutrición del camarón mediante el uso de ingredientes alternativos que reemplacen o complementen a la harina de pescado y ayuden a desarrollar una industria más amigable para el medio ambiente. Las levaduras se pueden añadir a las dietas para los organismos acuáticos y son un producto microbiano generado por sistemas biotecnológicos o un subproducto de las industrias agroalimentarias. Este estudio determinó el uso potencial de levaduras activas (*Candida insectorum*, *C. parapsilosis*, *C. sake*, *C. utilis*, *Debaryomyces hansenii*, *Rhodospiridium paludigenum*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, y *Yarrowia lipolytica*) en camarones juveniles y postlarvas de *Litopenaeus schmitti*, *Fenneropenaeus indicus* y *L. vannamei*.

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Los resultados muestran que las levaduras activas pueden ser empleadas a diferentes dosis como un sustituto parcial de la harina de pescado y/o harina de soya e incorporarse a dietas de camarones juveniles o usarse directamente en dietas para postlarvas. Sin embargo, es necesario realizar estudios que determinen las estrategias más eficientes para que la levadura activa sea ingerida por el camarón.

**Palabras clave:** Camarón; dieta; levadura; nutrición; peneido (*Fuente: CAB*).

## INTRODUCTION

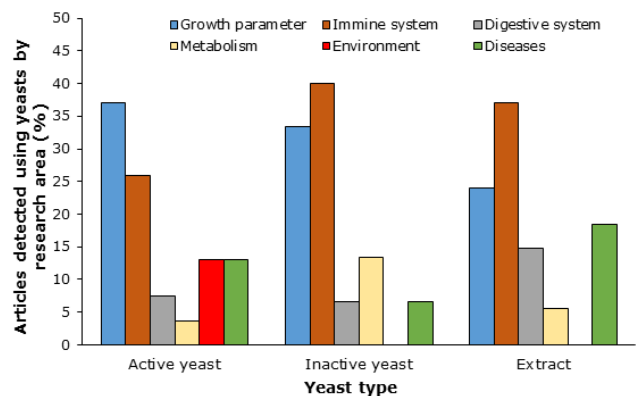
Fishing and aquaculture have had the purpose of supplying food and important products to man, seeking sustainability in both activities (1). In the last century, there was an increase in the consumption of aquatic products due to the taste, nutrient quality, organoleptic qualities, well-being, and health that these products generate in the consumer, which generated an intensification of fishing industry. This increased the production of aquaculture products and the subsequent reduction of the resource due to overfishing and overestimation of the product that could be obtained (2). With the continuous demand for aquaculture products, aquaculture emerges as an alternative that can support the supply of aquatic food obtained by fishing (1,2). Today, aquaculture has become a large-scale and economically important activity in many countries (1,3). However, the development of aquaculture is not immune to problems that reduce production, such as diseases, a decrease in ingredients for the manufacture of acceptable diets for consumption by aquatic organisms, and environmental deterioration.

Commercial shrimp farming is one of the most important products in world aquaculture, especially in Southeast Asia and China (1,3,4). Now, one of the main objectives to develop shrimp farming is the generation of functional and low-cost diets, the growth and health of the organisms, and the reduction of the environmental impact generated by this industry (1). Different researchers want to use alternative ingredients as nutritional supplements or feed additives to replace fish or soybean meal and generate a positive effect on animal growth. It is also sought that these ingredients can help against pathogens to which aquatic organisms are exposed and can reduce the contributions of organic matter to the environment (3,5).

Yeasts are a conventional ingredient that has been used for a long time in animal feed, where the biotechnology industry has worked

with various types of preparations and strains that serve as feed additives to improve shrimp growth, nutrition, feed conversion, and survival (6,7,8). In addition, they have been developed to be able to compete for the intestinal space and change the microbial flora, decrease the presence of non-beneficial bacteria, increase the production of enzymes, and the assimilation of nutrients or digestion of these molecules (9,10,11).

Figure 1 shows that yeast-related res (2000 to 2022) associated with aquaculture shrimp and focused on the use of active or inactive yeast, and extracts or parts of yeast in topics such as growth parameters, immune system, digestive system, metabolism, environment and disease control. This figure also shows that active yeast is used mainly for growth and immune system parameters (37 and 26%, respectively), a phenomenon that is reversed when using inactive yeast or extracts (24-33 and 37-40%, respectively). Topics such as metabolism, environment, digestive system, and diseases are less studied (0-18%). This work shows the current information on the use of active yeasts, the type of yeasts used, the dose, and the effect generated on the growth parameters of shrimp under culture.



**Figure 1.** Research detected (%) from 2000 to 2022 that use yeast or parts of them in aquaculture shrimp and their main topic areas.

## Yeast used in shrimp

Yeasts are unicellular ascomycetes and basidiomycetes with budding or fission reproduction. About 2-5% of all yeast species are known worldwide (12), which have shown their potential to produce many bioactive substances, such as enzymes, phytase, glucans, glutathione, toxins, and vitamins with applications in aquaculture, food, pharmaceutical, environmental, and chemical (13). Yeasts are a popular product for use in aquaculture as a source of amino acids, proteins, and vitamins (mainly B complex) with a positive effect on shrimp growth and immunity (14,15). Another use of yeasts in aquaculture is in the control of diseases (bacteria or viruses), which could reduce the use of antibiotics and other chemical products that affect the resistance or pathogenicity of microorganisms (16,17). Yeasts can be used as ingredients or feed additives in diets to improve their nutritional value, they can also improve crustacean health as a source of anticoagulant proteins, agglutinins, antimicrobial peptides, or AMP (defensins and chemokines) and bacteriocins. They have also been used as a source of free radicals, support in the formation of humoral components, phenoloxidase enzyme, lysozymes, nodules, proteases, hydrogen peroxide, polymyxin, siderophores, thyrothricin, and promoters of phagocytosis (17,18,19,20,21,22,23,24).

Marine yeasts are microorganisms normally found in the natural environment of farmed shrimp where their role in health and nutrition has been documented (25). These microorganisms can be obtained as by-products of some industries or produced specifically to increase the contribution of a particular nutrient or for their bioactive products (9,17,26,27,28). The main yeasts used in shrimp nutrition are shown below.

**Candida.** Yeasts known as *Candida* are from the Saccharomycetes class, Ascomycota division, they are widely distributed in nature since they can grow in different environments and temperatures, and at least 100 species have been described. Yeast cells are globose, ellipsoidal, cylindrical, or elongated in shape and occasionally ogival and triangular during asexual reproduction (29). The *Candida* yeasts that have been used in the last two decades as an ingredient in research works associated with the growth parameters of aquaculture shrimp (postlarvae of *Litopenaeus schmitti*, *Fenneropenaeus indicus*, and *L. vannamei*) are: *Candida insectorum*, *C.*

*parapsilosis*, *C. sake* and *C. utilis* (14,30,31,32) (Table 1). García-Galano & Carrillo-Farnés (30) and Sarlin & Philip (33) used active yeasts *C. sake* and *C. utilis* as supplements in diets for shrimp postlarvae (*L. schmitti* and *F. indicus*, respectively) showing that the use of the yeasts increased growth and survival in shrimp compared to other strains, this confirms that marine yeasts can be used as possible food supplements in shrimp postlarvae whose diet is at the microparticle level (33). Gamboa-Delgado et al (14) formulated diets with fishmeal and *C. utilis*, where the mixture of these ingredients increased the growth rate of shrimp (*L. vannamei*), compared to diets containing only *C. utilis*. It was also shown that a diet with 85/15% fishmeal/yeast provided a significantly higher final weight and growth rate result than the other diets (with or without yeast). This suggests that the supply of this yeast improves the amino acid profile of fishmeal when this ingredient is reduced in diets. On the other hand, Apún-Molina et al (31) used *C. parapsilosis* that was applied externally to a commercial shrimp diet (32%, Purina, Ciudad Obregón, Mexico) at a dose of  $5 \times 10^5$  CFU g<sup>-1</sup>. Juvenile *L. vannamei* shrimp (2.5±0.06 g) fed the yeast diet had significantly higher final weight, growth rate, and feed conversion ratio compared to organisms fed the control diet. However, this effect was reduced when the food was stored and used later, suggesting that shelf life may affect yeast viability.

**Debaryomyces.** This yeast is a Saccharomycetes class, Ascomycota division, and is commonly found in the environment and used in the food industry. At least 30 species have been described and their cells are globose, ovoid, or lenticular in shape during asexual reproduction, which is of the multilateral germination type (42). This yeast is found in the intestinal microflora and sediments in the culture areas of marine organisms such as shrimp (13). *Debaryomyces hansenii* is the main species observed in shrimp studies, and it has been used as an ingredient that promotes weight gain, growth, and feed conversion in both postlarvae and juveniles (Table 1). Sarlin & Philip (32) used live *D. hansenii* as a supplement in diets for shrimp postlarvae (*F. indicus*) at different doses, showing that it favored growth and survival in shrimp. Similar results were shown by Vidya et al (35) when used in *L. vannamei* at the same dose ( $10^6$  CFU g<sup>-1</sup> of diet). Campa-Córdova et al (34) provided it with *C. insectorum* to feed *L. vannamei* (0.14±0.02 g), showing significant results in all its growth parameters compared to a commercial control diet (Table 1).

**Table 1.** Effect of active yeast on growth parameters of aquaculture shrimp.

Yeast	Shrimp	Dose	Application	Reference
<i>Candida utilis</i>	<i>Litopenaeus schmitti</i> (PL)	5-30%	Significantly increases the growth of postlarvae when used in the diet between 15-25%.	30
<i>C. parapsilosis</i>	<i>L. vannamei</i> (2.5±0.06 g)	5×10 <sup>5</sup> CFU g <sup>-1</sup>	Increase of final weight gain, growth rate, and feed conversion rate.	31
<i>C. utilis</i>	<i>L. vannamei</i> (0.58±0.08 g)	60%	Similar growth and survival when used as a substitute for fishmeal (≤ 60%).	14
<i>C. sakei</i> , <i>C. utilis</i> , <i>Debaryomyces hansenii</i>	<i>Fenneropenaeus indicus</i> (PL 20-30 mg)	20%	Weight gain compared to control diet	32
<i>C. insectorum</i> + <i>D. hansenii</i>	<i>L. vannamei</i> (0.14±0.02 g)	10 <sup>6</sup> CFU mL <sup>-1</sup>	Significant gain in final weight, feed intake, feed conversion rate, and survival compared to the control diet.	34
<i>D. hansenii</i>	<i>L. vannamei</i> (PL15)	10 <sup>6</sup> CFU mL <sup>-1</sup>	Weight gain, specific growth rate, feed conversion rate, and survival when used in diets in commercial shrimp ponds (PL15).	35
<i>Rhodospiridium paludigenum</i>	<i>L. vannamei</i> (1.0 ± 0.06 g)	1% (10 <sup>8</sup> cel g <sup>-1</sup> diet)	Specific growth gain and survival when fed orally compared to the control diet.	36
	<i>L. vannamei</i> (0.05±0.00 g)	1-1.5 g kg <sup>-1</sup>	Weight gain, feed conversion rate, and survival.	20
	<i>L. vannamei</i> (0.40±0.01 g)	300 g kg <sup>-1</sup>	Growth gain, feed conversion rate, and survival were like the control, suggesting the use of the ingredient to partially replace fishmeal and soybean meal.	37
	<i>L. vannamei</i> (3.05 ± 0.22 g)	50-150 g kg <sup>-1</sup>	Final weight, feed conversion rate, and survival like the control, suggesting that this ingredient can be used in diets for juvenile shrimp.	38
	<i>F. indicus</i> (PL 0.02±0.018 g)	50%	It is possible to use it as a substitute for fishmeal and soybean meal in doses <50%.	39
<i>Saccharomyces cerevisiae</i>	<i>L. vannamei</i> (1.78±0.03 g)	10-40 g kg <sup>-1</sup>	No effects were detected in the final biomass, final weight, percentage of weight gain, feed conversion, and protein efficiency ratio compared to the control diet.	40
	<i>L. vannamei</i> (0.89 g)	1%	Increase in the final weight, survival, percentage of specific growth, and feed conversion.	5
	<i>L. vannamei</i> (12 g)	30%	Shows a digestibility of 84-85%	10
	<i>L. vannamei</i> (0.35±0.002 g)	1%	Weight gain, percentage of specific growth, survival, and feed conversion rate than the control diet	7
	<i>L. vannamei</i> (1.38±0.01 g)	1.5 × 10 <sup>10</sup> CFU g <sup>-2</sup>	Specific growth gain, weight gain, condition factor, and survival compared to the control diet	11
<i>Schizosaccharomyces pombe</i>	<i>L. vannamei</i> (0.35±0.002 g)	< 40 g kg <sup>-1</sup>	This yeast generates a positive impact on growth, feed conversion, and protein retention when combined with fishmeal.	41
<i>Yarrowia lipolytica</i>	<i>L. vannamei</i> (0.95±0.11 g)	2% (8000 cel mL <sup>-1</sup> )	Weight gain and higher specific growth rate by improving shrimp digestibility.	15

**Rhodospiridium.** This yeast is a Ustilaginomycetes class, Basidiomycota division, their cells are globose, ovoid, and elongated during asexual reproduction, with multilateral and polar germination. It has a reddish pigmentation due to the carotenoids it contains (43). The identification of species requires biochemical, physiological, and phylogenetic studies, and 9 species are known (*Rhodospiridium azoricum*, *R. babjevae*,

*R. diobovatum*, *R. fluviale*, *R. kratochvilovae*, *R. lusitaniae*, *R. paludigenum*, *R. tutoroides*, and *R. sphaerocarpum*). Yang et al (36) fed *L. vannamei* shrimp (1.0±0.06 g) with a diet containing 1% *R. paludigenum* (10<sup>8</sup> cel g<sup>-1</sup> diet), detecting an increase of the specific growth and survival of the shrimp. It would be of great interest to also study the effect that the carotenoids of this yeast have on aquatic organisms such as shrimp.

**Saccharomyces.** *Saccharomyces cerevisiae* is the yeast most used in shrimp for nutritional purposes (Table 1), it is the yeast most used in different processes worldwide, being used in the production of beer, wine, and bread, and is the first eukaryotic cell, whose genome was sequenced (44). Is a yeast of the Saccharomycetes class, Ascomycota division, which reproduces by budding, presenting a haploid and diploid life cycle. Genetic studies indicate the existence of eight well-defined species (*S. arboricolo*, *S. cerevisiae*, *S. eudayanus*, *S. jurei*, *S. kudjiazzevii*, *S. mikatae*, *S. paradonus*, and *S. uvarum*) (44). Yeast can be obtained as a by-product from different food and alcohol industries, being an interesting ingredient that can be used in agricultural and aquatic organism feed, it can already be used as active, non-active (dead), or extracts (28). Table 1 shows that *S. cerevisiae* has been used mainly in diets for *L. vannamei* shrimp from postlarvae (0.05 g) to juveniles (0.4-3 g) and with *F. indicus* postlarvae (0.02 g). The results show that this ingredient increases shrimp weight gain, feed conversion ratio, survival, and protein efficiency ratio (PER) like the control diet containing fishmeal and/or soybean. This suggests that this yeast can partially replace the previous ingredients (fish and/or soybean meal). Sharawy et al (39) show similar results and suggest the use of yeast at a dose <50%. Qiu et al (10) reported that the use of *S. cerevisiae* at a dose of 30% in diets for *L. vannamei* (12 g), showing that the ingredient has a digestibility of 84-85% in shrimp, which increases its use. Yeast is interesting in diets for shrimp larvae since the cell size of the microorganism increases its digestion and use by the shrimp digestive system. It is important to show that juvenile shrimp, when stocked in farms, have an initial diet associated with the natural productivity of culture ponds that increases their growth (45), where the use of yeast is a natural ingredient for them compared to larger juveniles that feed mainly on formulated diets (8,20,37,38).

**Schizosaccharomyces.** This yeast is a Schizosaccharomycetes class, Ascomycota division, which shows reproduction by binary fission, its cells have a rod shape measuring 3-4  $\mu$  in diameter and 7-14  $\mu$  in length. Five species are known (*Schizosaccharomyces cryophilus*, *Sc. japonicus*, *Sc. pombe*, *Sc. Octosporus*, and *Sc. osmophilus*) (46). Solorzano-Reyes et al (47) showed that Schizosaccharomyces is a marine yeast that is part of the microbial community of coastal and mangrove environments, contributing

to the balance of the ecosystem and is part of the natural diet of fish, crustaceans, and mollusks by feeding on the particles in suspension or adhered to the substrates where these yeasts were present. Research on the use of *Sc. pombe* active as a nutritional ingredient in shrimp is scarce, Qiu (41) used this yeast in diets for juvenile shrimp *L. vannamei* (0.35 g) at a dose of 40 g kg<sup>-1</sup>, showing that this microorganism had a positive effect on growth, feed conversion, and protein retention when combined with fishmeal in the diet (Table 1).

**Yarrowia.** This yeast is a Saccharomycetes class, Ascomycota division that has asexual growth by multilateral budding. There is only the species *Yarrowia lipolytica*, its cells have a spheroidal, ellipsoidal, or elongated shape, found alone, in pairs or small groups. Also, have dimorphic characteristics, pseudomycelium and branching can be observed (48). Patsios et al (49) show that *Y. lipolytica* is detected in food products such as meat, fish, dairy products, etc., and can grow in different agricultural by-products with high levels of fatty acids. This yeast is used in terrestrial and aquatic organisms generating positive effects on their nutrition since it stores essential fatty acids intracellularly ( $\leq 20\%$ ) and has a low content of nucleic acids that increases the palatability of diets. It also secretes heterologous proteins, has a high lysine content, and generates enzymes that increase animal digestion (esterases, lipases, phosphatases, and proteases) (49,50). Álvarez-Sánchez et al (15) used this active yeast mixed in the diet for juvenile shrimp (0.95 g) at a dose of 2%, showing a higher weight gain and growth rate in shrimp fed with diet compared to the control diet, suggesting this result to the higher digestibility detected *in vitro* tests of the diets (Table 1).

## Use of yeast in the nutrition of farmed shrimp

Shrimp farming is an activity that changes as knowledge of the techniques and knowledge about farmed organisms and their physiology, environment and biotic-abiotic interactions, nutritional requirements, and production processes. Yeasts found in the environment and shrimp can be a tool to increase the growth and health of shrimp, in addition to improving the environment for this industry. However, knowledge about microorganisms is limited and yeasts are no exception. Different species of yeast, like other microorganisms, do not



function in a similar way. There are differences between species or strains used, culture medium where the yeasts were produced, product, by-product or parts of the cells obtained, method of extraction and purification of these by-products or cell parts, and specific application considered.

This study shows that yeasts play an interesting role in shrimp farming and have different applications due to their versatility. Yeasts have been used in shrimp in an active, inactive, or extract (cell wall, glucans, nucleotides, etc.) (Fig. 1). In active yeast, its use in nutrition is important (Table 1), followed by the immune system, disease control and for environmental sustainability (5,8,17). In inactive yeast or cell extracts, it is used mainly to stimulate the immune system, followed by components that increase nutrition, disease control, digestive system support, or shrimp metabolism (8,10,15,16,23,31).

Initially, the yeast strains used in aquaculture were by-products obtained from other industries, supplied at different levels (1-60%). Currently, yeasts obtained by biotechnology can be used with specific applications in animal nutrition (proteins, lipids, vitamins, minerals, etc.) and the presence of bioactive compounds with an effect on growth, survival, digestive system, intestinal structure, and interaction of shrimp microflora (10,13,25). The results presented in Table 1 show the importance of knowing the characteristics of the different species or strains of yeast to define their application in the shrimp diet. Active yeast can be used as a partial substitute for fish and soybean meal in balanced feed or additives with an effect on growth parameters (7,8,14).

In shrimp, the balance between the level of protein, digestion, and absorption of amino acids from diets generally represents higher growth, survival, and a lower feed conversion rate in cultured organisms, as suggested by Ceseña et al (8) and Mendez-Martínez et al (51). Active yeasts have been used in recent decades as part of shrimp diets as a protein source, even when they show a low level of some essential amino acids and their high content of nucleic acids (6,16,52). Active yeasts have shown their potential to be used as ingredients in shrimp diets (postlarvae - juveniles). This strategy can increase the assimilation of nutrients from the diets, decrease the cost of these, and increase the growth of cultured organisms.

In future research with active cells, it is important to show the different interaction routes between yeasts and the organism that ingests them, for example, yeasts can be associated as a nutrient (protein level, amino acid profile, microelements, fatty acids, vitamins, etc.) and as stimulators or enhancers of the immune system, space, or nutrient competitors against pathogens. Active yeast can also interact as a stimulator of the digestive system with the production of extracellular enzymes, reduce the impact on the environment or stress in the biotic and abiotic interactions of cultivated organisms, etc.

It is possible that molecular, proteomic, and isotopic studies, etc., may generate new information that increases the use of different yeast species and strains in diets for aquaculture shrimp, and the specific functioning of these active cells in organisms and their around.

### **Conflict of interests**

The authors declare that there is no conflict of interests regarding of the publication of this manuscript.

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