

Application of yeast active substances in fishmeal substitution

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Fishmeal and Aquafeed

Research progress on fishmeal alternative

**Application of yeast active substances in
fishmeal substitution**

1. Fishmeal and aquafeed

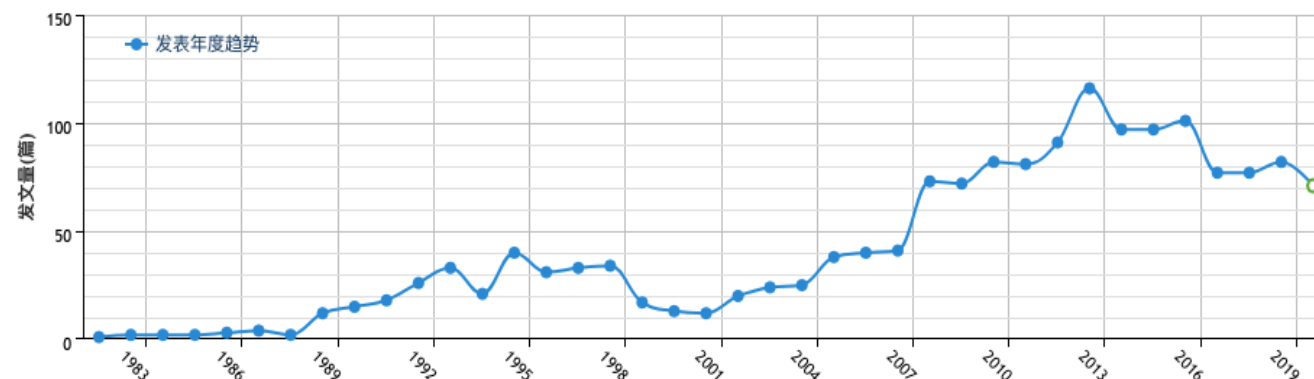
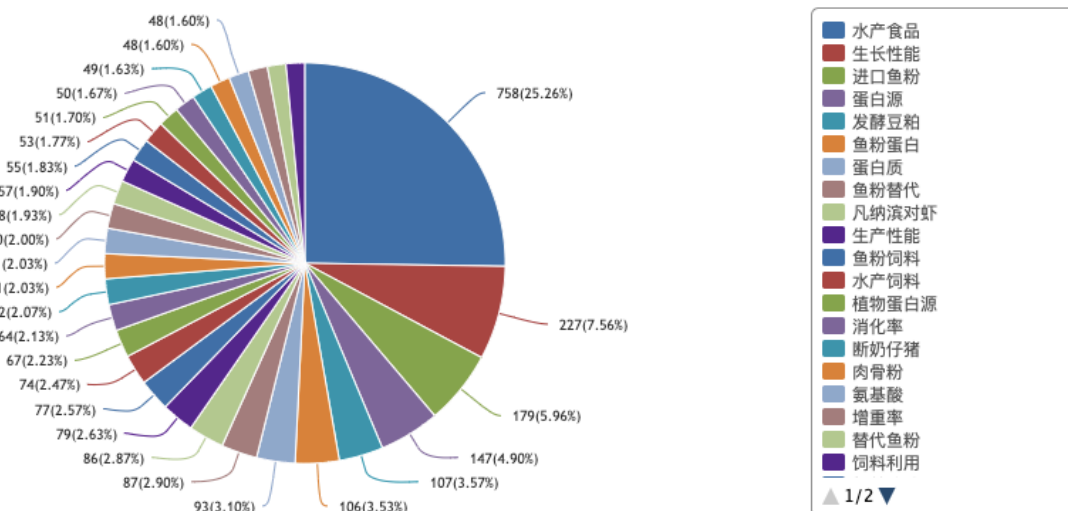
1) Basic nutritional data and role of fishmeal

Serial number	51	China Feed No.	5-13-0044
Name of feed	Fishmeal(CP67%) fish meal		
Feed Description	Imported GB/T 19164-2003,Special grade		
Regular nutrient content		Mineral composition	
Dry matter(%)	92.4	Chlorine(%)	0.71
Crude protein(%)	67	Potassium(%)	0.74
Crude fat(%)	8.4	Copper(mg/kg)	8.4
Crude fiber(%)	0.2	Zinc(mg/kg)	102
Crude Ash(%)	16.4	Sodium(%)	1.04
Nitrogen-free leachate(%)	0.4	Magnesium(%)	0.23
Calcium(%)	4.56	Iron(mg/kg)	337
Total phosphorus(%)	2.88	Manganese(mg/kg)	11
Effective phosphorus(%)	2.88	Selenium(mg/kg)	2.7
Amino acid composition		Vitamin content	
Histidine(%)	2.01	Vitamin B1(mg/kg)	2.8
Leucine(%)	4.94	Pantothenic acid(mg/kg)	9.3
Methionine(%)	1.86	Biotin(mg/kg)	1.3
Phenylalanine(%)	2.61	Choline(mg/kg)	5600
Threonine(%)	2.74	Vitamin B12(μg/kg)	210
Valine(%)	3.11	Vitamin E(mg/kg)	5
Arginine(%)	3.93	Vitamin B2(mg/kg)	5.8
Isoleucine(%)	2.61	Niacin(mg/kg)	82
Lysine(%)	4.97	Folic acid(mg/kg)	0.9
Cystine(%)	0.6	Vitamin B6(mg/kg)	2.3
Tyrosine(%)	1.97		
Tryptophan(%)	0.77		
		Fatty acid content	
		Linoleic acid(%)	0.2

- Fishmeal usually accounts for 20%-60% of protein source in aquafeeds
- High protein content, crude protein up to 67%, domestic 45-55%, protein digestibility up to 90%.
- The amino acid composition is complete and balanced, especially the main amino acids and aquatic animal tissue amino acid composition is basically the same.
- The digestibility of crude fat is about 85%, and the unsaturated fatty acid content is high, and has a fishy smell.
- Among the minerals, the content of calcium, phosphorus, selenium, iodine, zinc and iron is high.
- Rich in vitamin B12, fat-soluble vitamin A, vitamin D and vitamin E.
- Contains unknown growth factors, which can stimulate animal growth and development.

2. Research progress on fishmeal substitution

Literature search results for fishmeal substitution

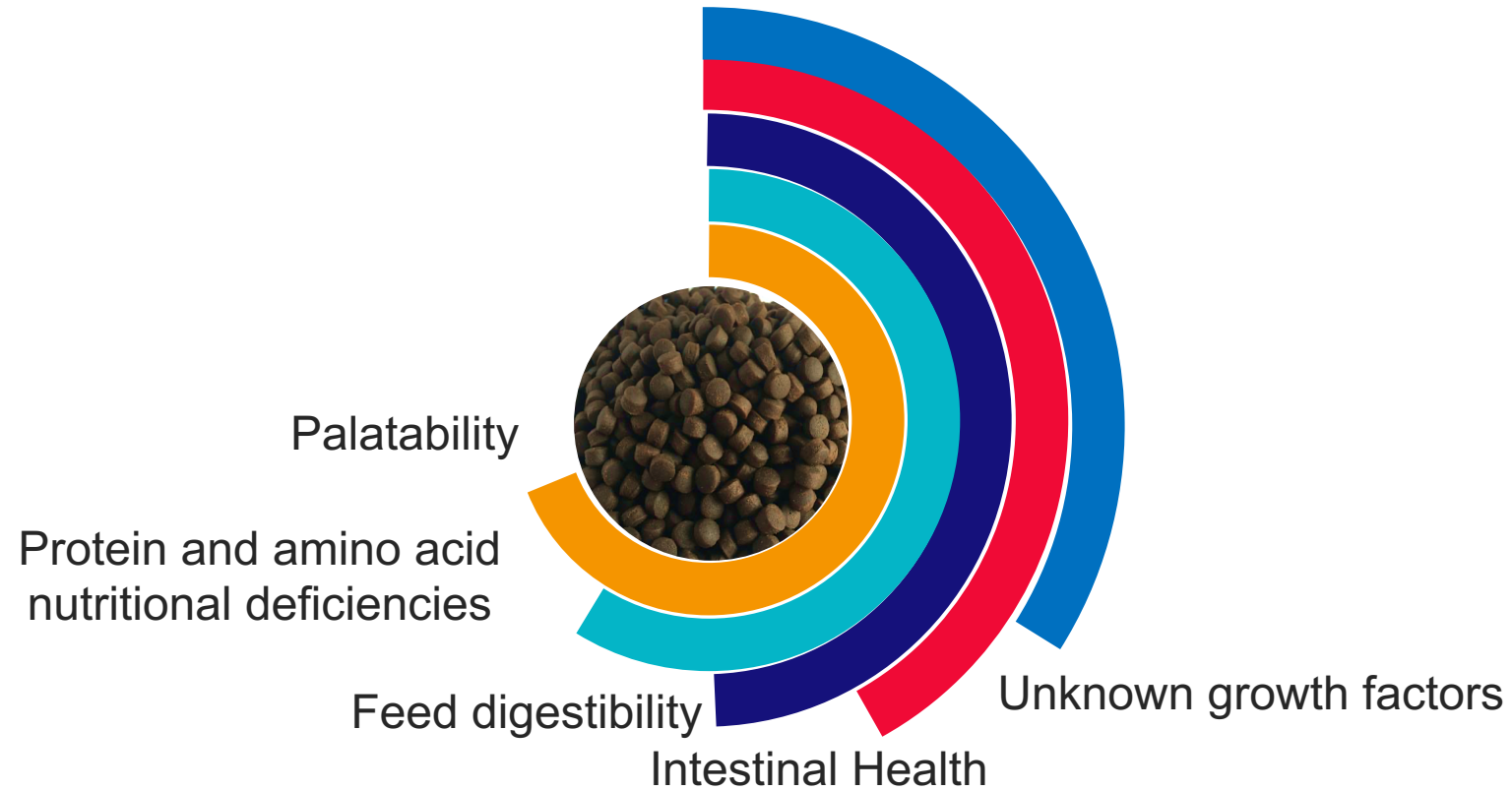


Total number of literature: 1578 articles.

High-frequency keywords: protein raw materials, growth performance, digestibility, breeding representative species

2. Research progress on fishmeal substitution

Reduce the amount of fishmeal, the impact on feed quality



2. Research progress on fishmeal substitution

Technical countermeasures for fishmeal substitution

Explicit substitution

■ Nutritional substitution

Protein balance

Amino acid balance

Fat balance

Implicit substitution

■ Functional alternative

Palatability

Intestinal structure

organismal immune performance

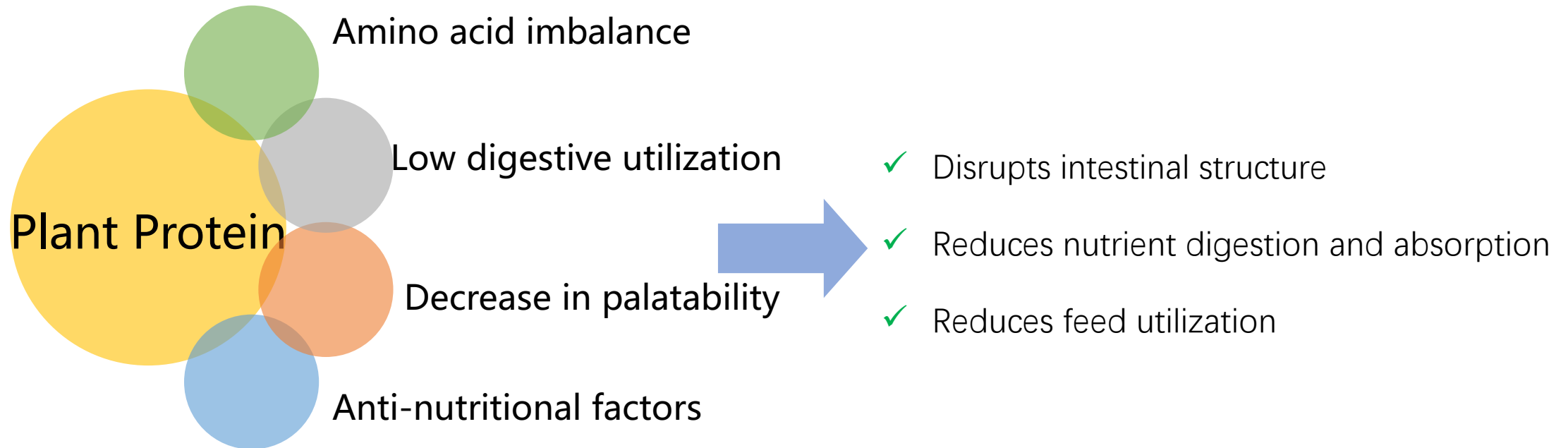
Technical solutions for fishmeal substitution

➤ Choice of raw materials: animal proteins, vegetable proteins, fermented raw materials, single-cell proteins; single application, mixed application

➤ Additive selection: supplement, balance, improve, enhance

➤ Focus: growth performance, feed utilization efficiency, feed intake rate, survival rate, etc.

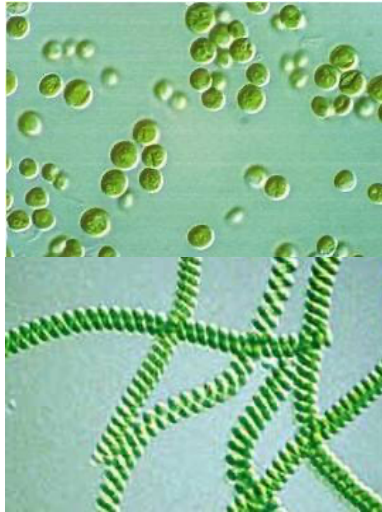
Problems faced by plant protein raw materials



Fermented protein raw materials

- Fermented protein raw materials: the use of microbial growth and reproduction in feed materials and metabolism to accumulate useful bacteria, enzymes and intermediate metabolites, processed protein raw materials. Can be divided into fermented plant protein raw materials (mainly fermented soybean meal), fermented animal protein raw materials (mainly fermented blood meal, feather meal and meat and bone meal), composite fermented protein.
- Advantages of fermented protein raw materials.
 - Degradation of macromolecular proteins into small peptides to improve the quality of protein raw materials
 - Enriched with protease to increase digestive utilization
 - Special fermentation flavor to improve palatability
 - Supplement a large number of active probiotics to improve intestinal health
 - Can effectively remove a variety of anti-nutritional factors
 - Rich in organic acids, active proteins, folic acid and B vitamins and other growth factors

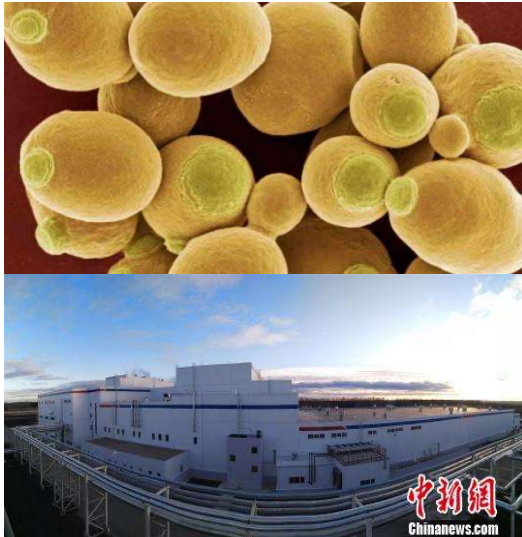
Algae and their processed products



Raw material name	Feature Description	Compulsory marking requirements
Crackpot algae meal	DHA-rich algae powder produced by fermentation, separation and drying processes using Schizochytriumsp.	Crude Fat DHA
Spirulina meal	Spirulina (Spirulinaplatensis) after drying and crushing.	Crude protein crude ash
Microcystis aeruginosa meal	EPA-rich algal powder is produced by culturing, concentrating and drying of Nannochloropsissp.	Crude fat EPA
Microalgae meal	A by-product obtained by drying the fat extracted from the powder of Schizosaccharomyces pombe, Microcystis aeruginosa or Chlorella powder.	Crude protein crude ash
Chlorella meal	Algae meal rich in EPA and DHA is produced from Chlorella (Chlorellasp.) species through the process of cultivation, concentration and drying.	Crude fat EPA DHA

- Microalgae can contain up to 70% protein, 15%-30% carbohydrates, 30%-50% lipids (n-3 and n-6 unsaturated fatty acids) and 1%-14% carotenoids.
- The effects of most microalgae and their bioactive substances on aquatic animals and the related molecular mechanisms and regulatory mechanisms are still not well understood.
- In practical production applications, the research and development of microalgae biotechnology is insufficient, and many technical bottlenecks have not been solved.

Types of single-cell proteins



➤ Single-cell protein: also called microbial protein, bacteriophage protein, is a kind of bacteriophage protein obtained by industrial method of multiplying and culturing single-cell microorganisms. According to the different production raw materials, it can be divided into petroprotein, methanol protein, methane protein, etc.; according to the different types of producing bacteria, it can be divided into bacterial protein, fungal protein, etc. Yeast is the more applied single-cell protein source.

➤ Single-cell protein advantages.

- ① Nutrient-rich and highly bio-efficient.
- ② Using a wide range of raw materials, which can be sourced locally and solved cheaply and in large quantities.
- ③ can provide a stable source of protein.
- ④ simple to use equipment and small footprint.
- ⑤ fast reproduction, short production cycle and high production efficiency
- ⑥ Single-celled organisms are easy to mutate, and easier to improve than animal and plant species.

2. Research progress on fishmeal substitution

Technical countermeasures for fishmeal substitution

Explicit alternative

■ Nutritional substitution

- Protein balance
- Amino acid balance
- Fat balance

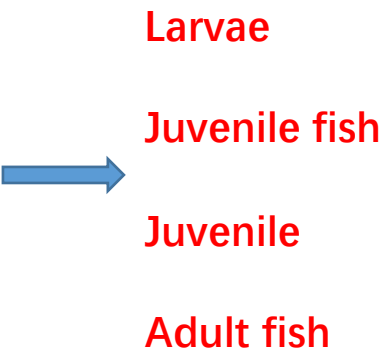
Implicit alternative

■ Functional alternative

- Palatability
- Intestinal structure
- organismal immune performance
-

Technical solutions for fishmeal substitution

- Choice of raw materials: animal proteins, vegetable proteins, fermented raw materials, single-cell proteins; single application, mixed application
- Additive selection: supplement, balance, improve, enhance
- Focus: growth performance, feed utilization efficiency, feed intake rate, survival rate, etc.



Implicit alternative

■ Functional alternative

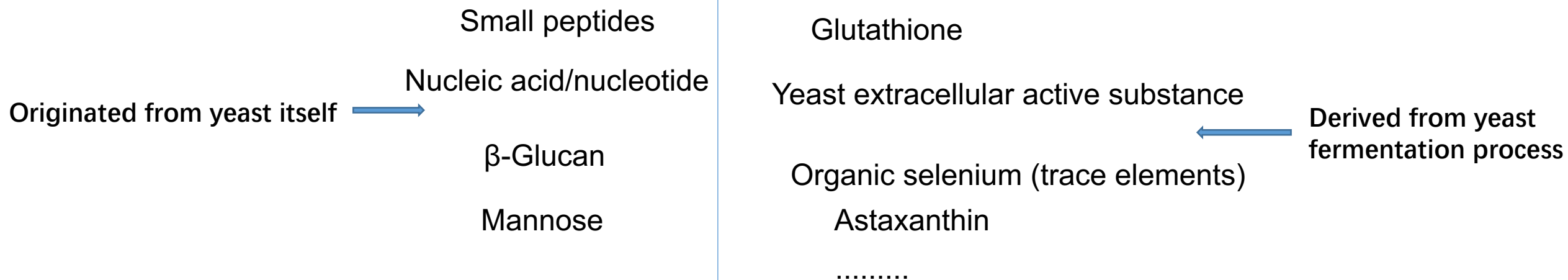
- Palatability: Addition of food attractants, elimination or blunting of anti-nutritional factors in fishmeal substitutes that affect the palatability of aquatic animals
- Intestinal health: addition of nucleotides/nucleic acids, enzymes, acidifiers, microecological agents, etc.
- Liver and gallbladder health: add vitamins (such as B vitamins, VC, VK, etc.) and trace elements, add substances that promote lipid metabolism (lecithin, choline chloride, betaine, taurine, etc.), add antioxidant substances such as yeast selenium
- Immune performance of the body: add immune enhancers (herbs, polysaccharides), etc.

3. Yeast active substances for fishmeal substitution

About Yeast Nutrient Actives

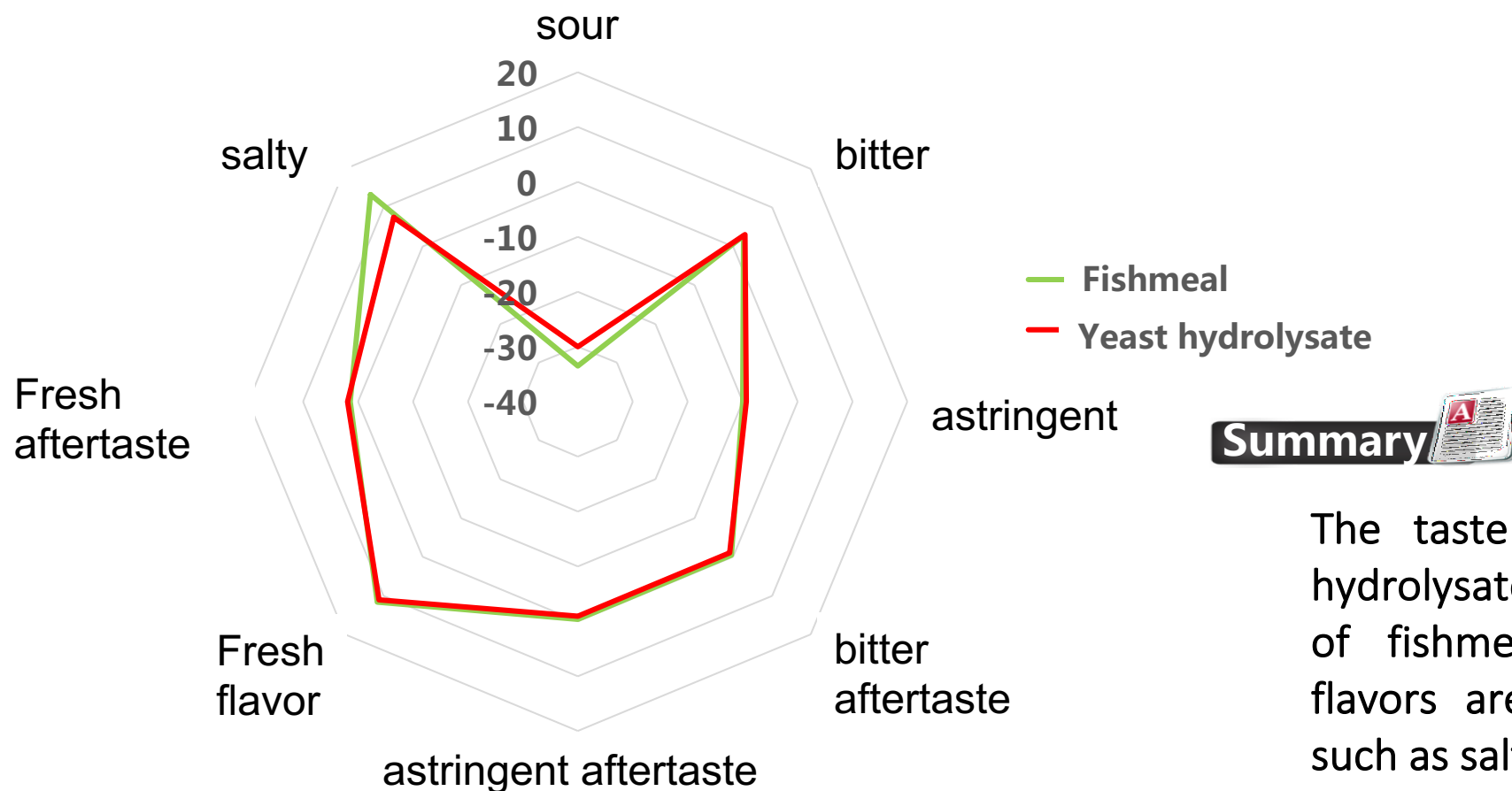
Yeast nutrient active substances

- Trace components naturally present in yeast raw materials or produced by yeast during processing and digestion and metabolism in animals, with special nutritional regulation or health functions such as promoting intestinal health, maintaining ideal immune balance and oxidative balance, and regulating gene expression.



Yeast active substances (aquatic feed application research - feeding inducing effect)

Comparison of yeast hydrolysate and fish meal for taste presentation



Summary

The taste presentation of yeast hydrolysate is comparable to that of fishmeal, and some of the flavors are better than fishmeal, such as salty taste

(Fan Yang, 2017)

Electronic tongue detection test results

Yeast active substance (aquatic feed application research - feeding inducing effect)

Flavor-presenting amino acid content of yeast hydrolysate products and squid by-products

Proportion of taste-presenting amino acids to crude protein (%)	Squid offal powder (Japan, 6.1% moisture)	Squid Offal Powder (Taiwan, 3.7% moisture)	Yeast hydrolysate	
			Powdered sample 1 (6% moisture)	Powdered sample 2 (6% moisture)
Aspartic acid	5.17	4.61	9.44	8.88
Glutamic acid	8.83	7.83	14.17	13.82
Glycine	8.10	8.16	3.67	4.07
Alanine	4.58	5.37	7.17	7.02
Arginine	4.00	3.42	4.61	3.66
Total of 5 taste-presenting amino acids	28.67	29.39	39.06	37.44

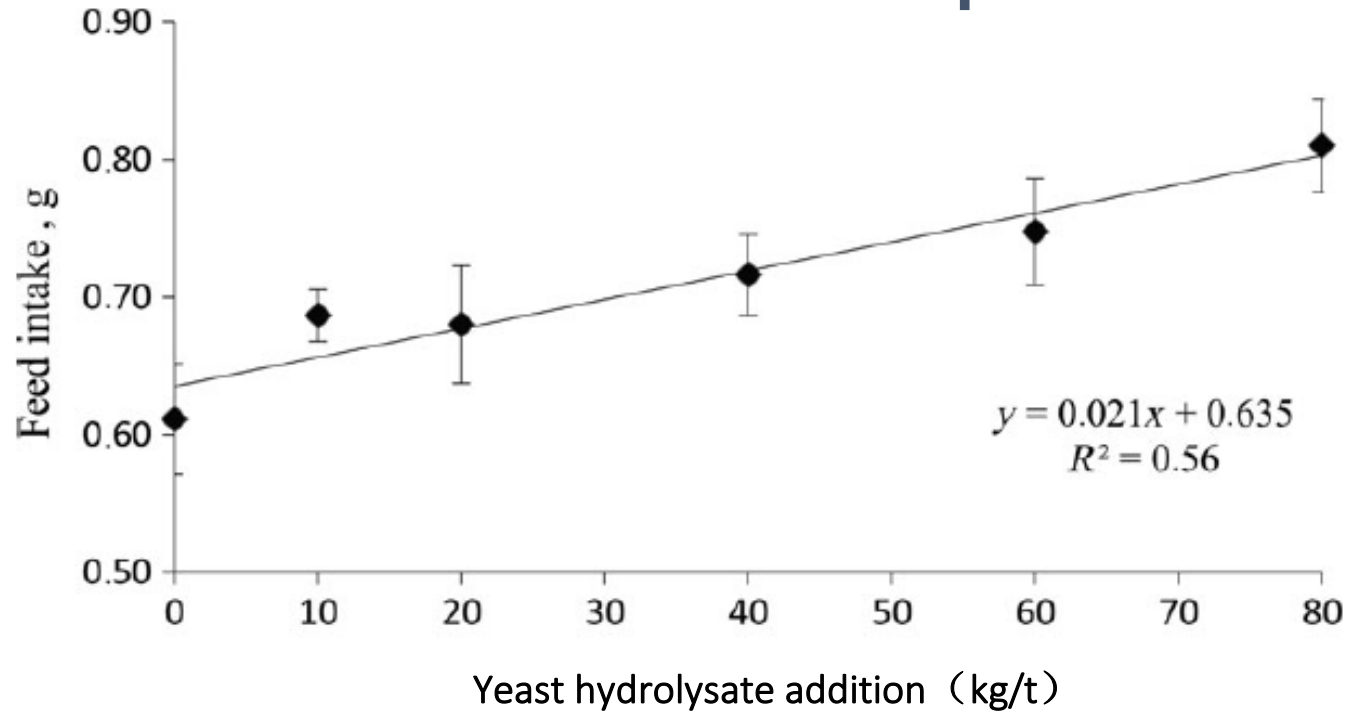
(Qin Nie, 2014)

Summary

Yeast hydrolysates contain significantly more taste-presenting amino acids than squid by-products

Yeast active substance (aquatic feed application research - feeding inducing effect)

Evaluation of yeast hydrolysate on the feed intake of Nile tilapia



Test subject: Nile tilapia, 2.63±0.63 g
Test period: 75d
(Ricardo da Silva Berto et al., 2015)

Summary

With the addition of more yeast hydrolysate, feed intake increased significantly

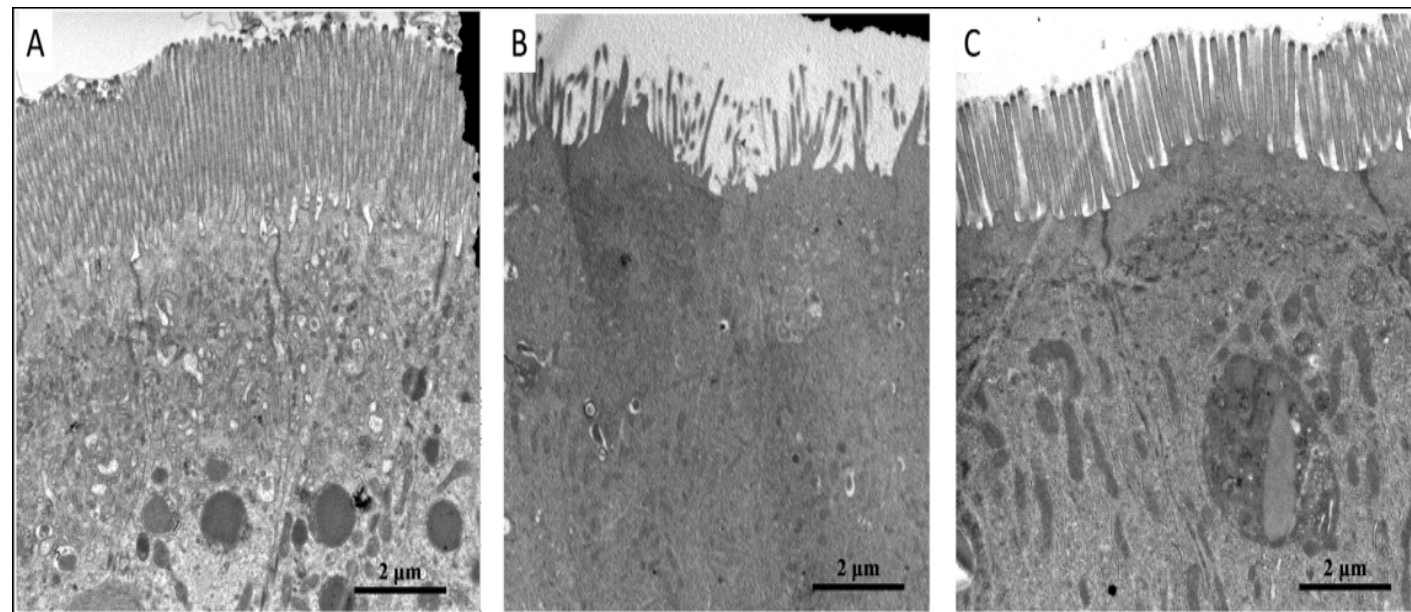
Yeast Active Substances (Aquatic Feed Application Research - Gut Health 1)



MOS helps improve symptoms of enteritis caused by plant-based protein substitution for fish meal

- High levels of added soybean meal can cause soybean meal-type intestinal inflammation in fish, poor growth performance and impaired intestinal health (shorter and fewer intestinal microvilli, changes in intestinal native colonies). Adding 0.2% MOS can reduce the symptoms of soybean meal-type enteritis (Aquaculture, 2017)

	A Fishmeal	B Soybean meal	C Soybean meal + MOS
Initial weight	7.6	7.7	7.6
End weight	34.2c	25.9a	29.3b
SGR	2.69c	2.17a	2.41b
FER	1.43c	1.3a	1.38b
Microfleece length(um)	2.84c	1.78a	2.33b
Microfleece density	16.4c	11.1a	14.3b

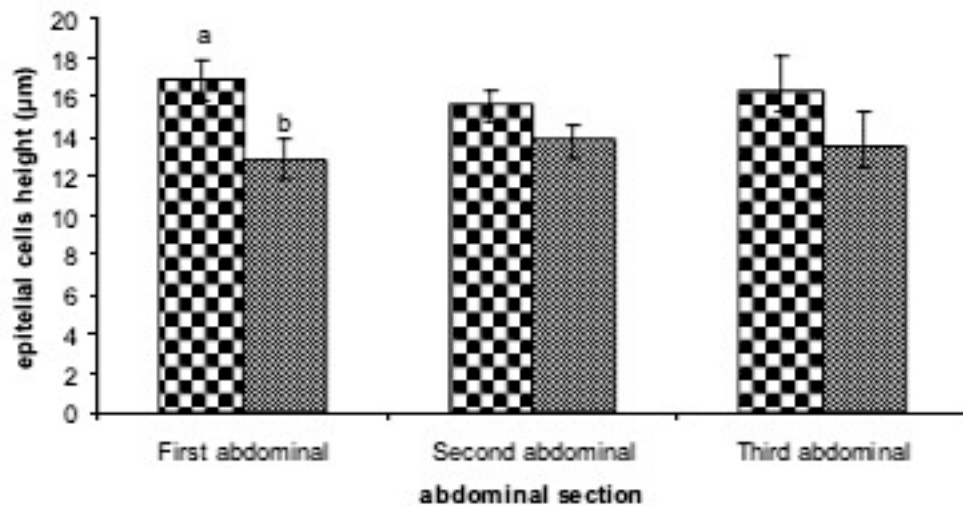


Remarks: turbot (initial weight 7.6g) fed isonitrogenous and isolipid feed for 8 weeks, 3 treatment groups: FM group, fish meal as the main protein source; SBM group, 40% soybean meal instead of fish meal; SBMMOS group, 0.2% MOS added to SBM group; 100 tails per group, 3 repetitions Product: yeast MOS product
Institution: Nan Bai, Shandong University 2017

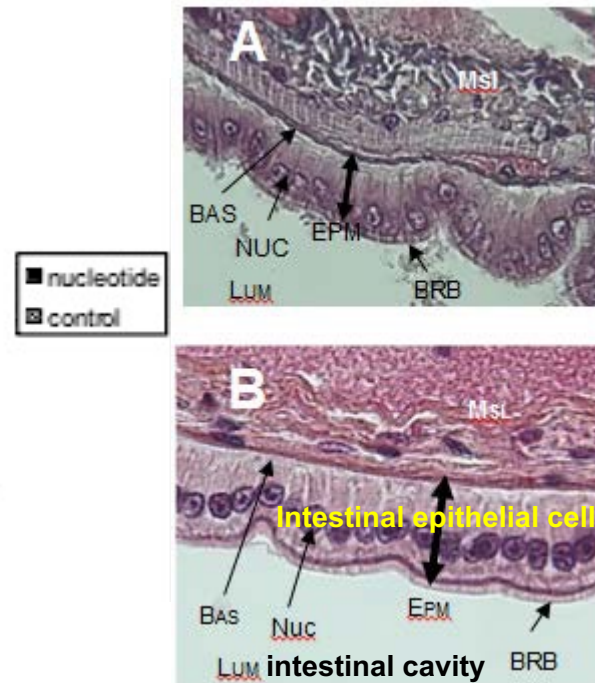
Yeast Active Substances (Aquatic Feed Application Research - Gut Health 2)

Nucleotides contribute to intestinal epithelial cell development

■ Addition of 0.2% nucleotides significantly increased the height of foregut epithelial cells and repaired the brush border membrane of epithelial cells



Effect of nucleotides on the height of epithelial cells in different parts of the intestine of the South American white shrimp



Cross-sectional tissue structure of the midgut of South American white shrimp (×100)

Remarks: South American white shrimp (initial weight $3.20 \pm 0.02g$), control group A, experimental group B (added 0.2% complex nucleotides)
(Abdolmohammad Abedian-Kenari, 2013)

Yeast active substance (shrimp feed application study - intestinal health)

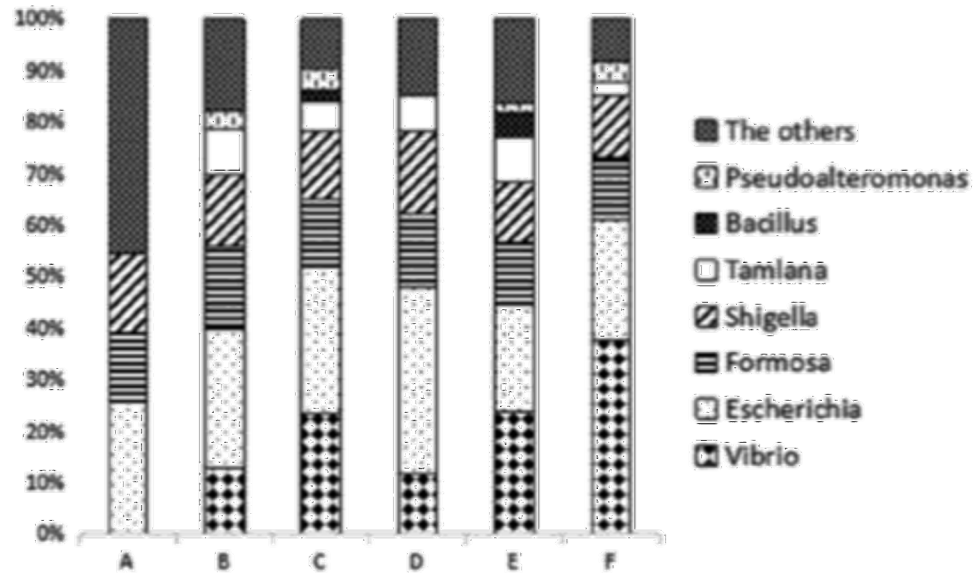


图7 凡纳滨对虾肠道各类菌群的丰度

Fig. 7 Relative abundance of bacteria of shrimp's intestine

注: A组为在实验饲料中添加0.6%的破壁酵母;B组为在实验饲料中添加0.3%的中草药提取物;C组为在实验饲料中添加0.3%的柠檬酸;D组为在实验饲料中添加 10^9 cfu/kg的益生菌;E组为在实验饲料中添加15mg/kg的氟苯尼考;F组为实验用基础饲料。

Note: A: adding 0.6% broken yeast to the basal diet; B: adding 0.3% herbal extract to the basal diet; C: adding 0.3% citric acid to the basal diet; D: adding 10^9 cfu/kg probiotics to the basal diet; E: adding 15mg/kg florfenicol to the basal diet; F: the basal diet.

(Dongdong Wang, Ocean University of China. 2018)

Yeast hydrolysate in the yeast hydrolysate helps to optimize the intestinal flora structure of South American white shrimp

The addition of yeast hydrolysate reduced the content of *Vibrio intestinalis* in shrimp relative to other groups.

Analysis: Yeast cell wall is rich in mannan oligosaccharide and β -glucan, and the acid produced by the selective fermentation of mannan oligosaccharide by animal digestive tract flora will cause the pH value of the whole intestine to drop, which can inhibit the growth of harmful bacteria, and at the same time, mannan oligosaccharide as a "pathogen scavenger" can greatly affect the immune system of animals and interfere with the colonization of intestinal pathogens. It is a powerful modifier of intestinal microflora.

Shrimp feed application research

Application on shrimp



Experimental design

- Amount of yeast hydrolysate added: 1%
- Initial body weight of Vannamei shrimp: 0.88 ± 0.01 g
- Experimental period: 8 weeks
- Examine content: growth performance, mTOR signaling pathway gene expression level (Jiang , 2019)

Feed formulation composition

Ingredients and proximate composition of the experimental diets (air-dry basis, %).

Ingredients	Diet		
	Control diet	Yeast hydrolysate	Brewer's yeast
Fish meal	25.00	25.00	25.00
Soybean meal	25.00	25.00	25.00
Peanut meal	7.50	7.50	7.50
Shrimp meal	6.00	6.00	6.00
Wheat flour	27.75	27.75	27.75
Fish oil	1.00	1.00	1.00
Soybean oil	1.00	1.00	1.00
Soybean lecithin oil	2.00	2.00	2.00
Ca(H ₂ PO ₄) ₂	1.50	1.50	1.50
Vitamin premix ¹	1.00	1.00	1.00
Mineral premix ¹	1.00	1.00	1.00
Choline chloride	0.25	0.25	0.25
Yeast hydrolysate	0.00	1.00	0.00
Brewer's yeast	0.00	0.00	1.00
Cellulose	1.00	0.00	0.00
Total	100.00	100.00	100.00
Nutrient levels ²			
Dry matter	89.59	89.87	89.97
Crude protein	42.37	42.51	42.89
Crude lipid	7.93	7.89	7.92
Ash	12.59	12.63	12.34

Shrimp feed application research

Growth performance, feed utilization and morphological indicators of South American white shrimp fed the experimental diets

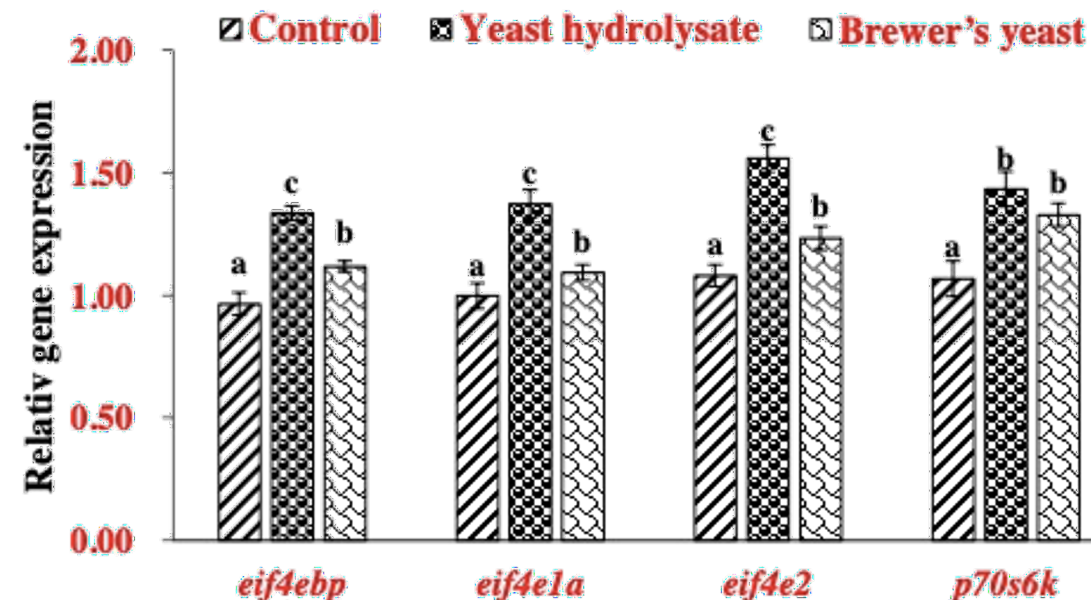
Growth performance, feed utilization and morphologic index of *L. vannamei* fed the experimental diets

Parameters	Control diet	Yeast hydrolysate	Brewer's yeast
IBW ^a /g	0.89±0.00	0.88±0.01	0.89±0.01
WG ^b /%	1107.28±7.52 ^a	1229.02±28.85 ^b	1156.34±22.71 ^{ab}
Survival/%	93.33±8.16	97.50±3.19	100.00±0.00
SGR ^c /% day ⁻¹	4.31±0.07 ^a	4.56±0.02 ^b	4.46±0.04 ^{ab}
FCR ^d	1.59±0.03 ^a	1.42±0.04 ^b	1.52±0.04 ^{ab}
HSI ^e /%	3.55±0.14	3.28±0.40	3.20±0.18
CF ^f /g·cm ⁻³	0.60±0.01	0.59±0.02	0.59±0.01

Summary

- The addition of 1% yeast hydrolysate or brewer's yeast powder to the diet improved the growth performance and increased the expression level of mTOR signaling pathway in South American white shrimp.
- The addition of 1% yeast hydrolysate to the ration was more effective than brewer's yeast powder for South American white shrimp.

Expression levels of genes in the intestinal mTOR signaling pathway in South American white shrimp fed different diets.



Carnivorous fish application research - low fishmeal application



Effect of yeast hydrolysate on growth performance, immunity and antioxidant capacity of California bass

Ingredient (%)	Y0	Y1.5	Y3.0	Y4.5
Fishmeal ^a	35.00	35.00	35.00	35.00
Soybean meal	18.00	18.00	18.00	18.00
Casein	16.14	15.26	14.38	13.46
Wheat flour	8.00	8.00	8.00	8.00
Tapioca	11.90	11.90	11.90	11.90
Yeast hydrolysate ^b	0.00	1.50	3.00	4.50
Fish oil	4.54	4.54	4.54	4.54
Kelp meal	2.00	2.00	2.00	2.00
Vitamin premix ^c	0.10	0.10	0.10	0.10
Mineral premix ^d	0.15	0.15	0.15	0.15
Monocalcium phosphate	2.00	2.00	2.00	2.00
Choline chloride (50%)	0.20	0.20	0.20	0.20
Methionine	0.15	0.15	0.15	0.15
Cellulose	1.82	1.20	0.58	0.00
<i>Proximate composition</i>				
Moisture (%)	9.24	10.17	10.39	10.67
Crude protein (% DM)	45.70	46.18	46.01	46.01
Crude lipid (% DM)	5.99	6.21	5.93	6.00
Ash (% DM)	9.89	10.17	10.35	10.39

- Addition of yeast hydrolysate: 0, 1.5%, 3.0% and 4.5%, respectively.
- Body weight of California perch: 7.71 ± 0.02 g
- Test period: 8 weeks.
- Indexes to be examined: growth performance, blood immunity index, antioxidant capacity

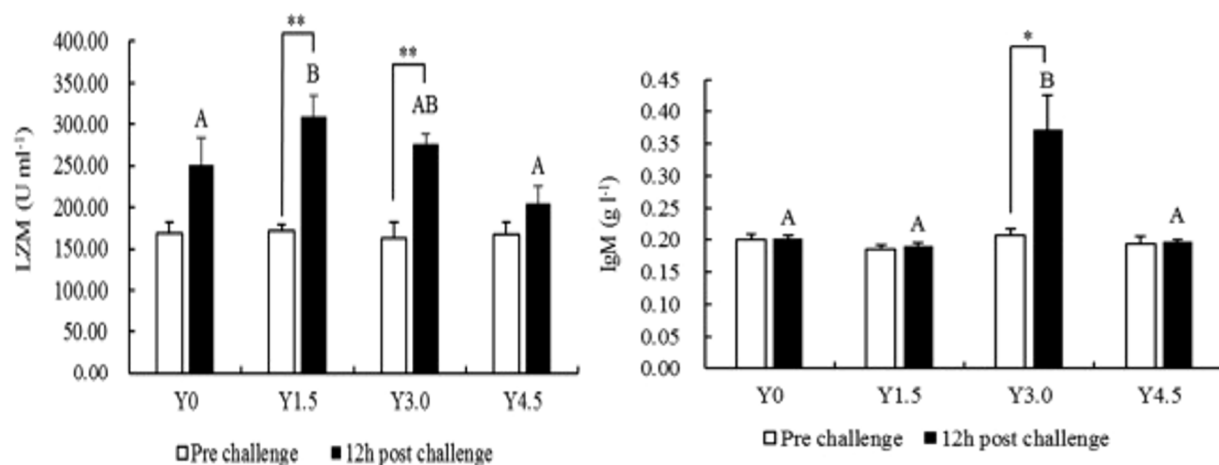
(Gong , 2019)

Effect of enzyme hydrolysate addition to feed on the growth performance of California bass

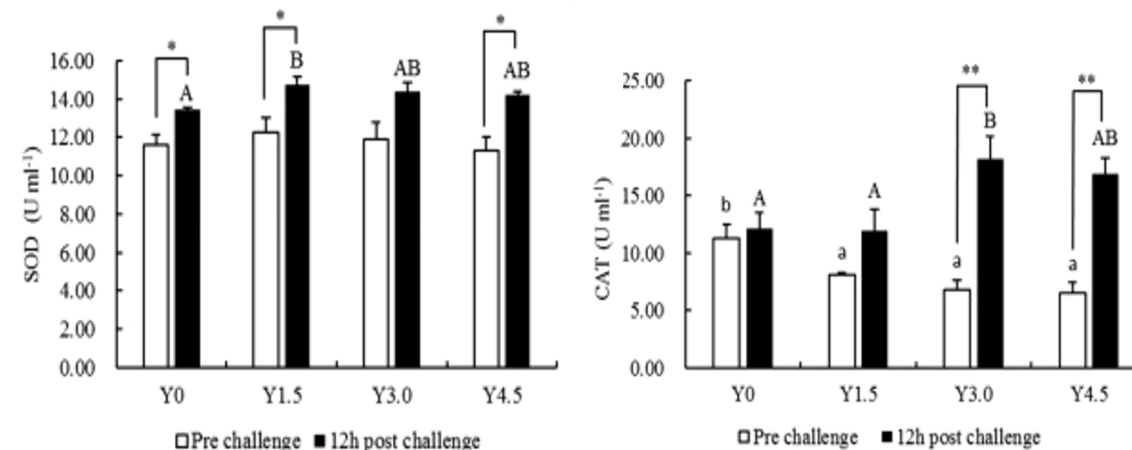
Effects of dietary yeast hydrolysate on the growth performance of largemouth bass (Means \pm SEM).

	Y0	Y1.5	Y3.0	Y4.5
IBW ^a , g	7.77 \pm 0.02	7.72 \pm 0.03	7.69 \pm 0.06	7.68 \pm 0.02
FBW ^b , g	30.58 \pm 1.67 ^a	34.88 \pm 0.92 ^b	33.03 \pm 0.61 ^{ab}	31.54 \pm 0.62 ^{ab}
WGR ^c , %	294.2 \pm 21.80 ^a	352.13 \pm 13.41 ^b	329.71 \pm 10.91 ^{ab}	310.75 \pm 7.17 ^{ab}
FCR ^d , %	96.84 \pm 4.40 ^a	92.22 \pm 2.09 ^a	100.43 \pm 2.35 ^{ab}	108.91 \pm 1.74 ^b

Immunological indices of California bass plasma



Plasma antioxidant index of California bass



Conclusion: The addition of moderate amount (1.5%-3%) of yeast hydrolysate to the feed can improve the growth performance and enhance the immunity and antioxidant capacity of California perch.

Ideas for the application of yeast active substances in fishmeal-free/low fishmeal feed formulations

Application ideas	Target Customers	Products	Usage and Dosage
Increased feed intake	Special aquatic animal hatchery stage, intensive enticement needs	Nucleotidylated yeast hydrolysate Bonilla Pro	Feed formulation using 5-10kg/ton
Reduce costs and fishmeal	Adult stage of special aquatic products (scaleless fish, carnivorous fish, marine fish, crustaceans)	Normal yeast hydrolysate Bonilla B	Feed formulation using 1.0-3.0% (with animal and plant protein ingredients to reduce the amount of fish meal)
Intestinal Health	Hatchery stage, intensive intestinal health needs	Nucleotidylated yeast hydrolysate Bonilla Pro + Yeast cell wall	Feed formulation using 15kg/ton + 3kg/ton
Improved survival rate of fish and shrimp	Nursery period, special period	Yeast cell wall	Additional 4kg/ton of feed
Anti-oxidation capacity enhancement	Full period of breeding	Yeast Selenium + Glutathione Yeast Hydrolysate Bonilla G	Additional 100g+3kg/ton of feed



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