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Health and nutritional performance of young common carp Cyprinus carpio L. feeding diets with added bakery yeast Saccharomyces cerevisiae

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***Abstract*---**The current research was carried out to investigate the health and nutritional efficiency of the using bakery yeast Saccharomyces cerevisiae as a dietary additive at rates of 0.5, 1 and 1.5% and observe the extent of its affect on the growth of common carp Cyprinus carpio fingerlings by following the parameters of growth, feeding efficiency and feed conversion rate. The results showed that T3 in total weight gain ratios of 232.12g/fish was significantly different from other treatments T0, T1 and T2 with no significant difference between them. The daily weight gain of T3 was 3.22g/fish with significant difference from the rest of the treatments which were 2.15, 2.39 and 2.26 for T0, T1 and T2, respectively, T3 by 175.75% with no significant difference between T0, T1 and T2. Growth rate results showed two higher values; 5.27% in T3 and lower in T0 with no significant difference ($0.05<P$) between T0, T1 and T2, respectively. The best result for feed conversion rate was shown by T3 at 1.79 with a significant difference with T0. The higher value of feed conversion efficiency was 55.97% in T3, with no significant difference in all treatments in the amount of protein intake. Higher satiation level was in T3 6.59%, while the lowest was in yeast-free control at 6.10%, with differing values in the remaining treatments, and no significant difference ($p>0.05$) among all treatments. As for digestibility, the results of the statistical analysis showed a significant difference ($P<0.05$) among all four treatments with the highest value of protein digestibility coefficient in T3 95.85, fat 92.19 and

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carbohydrate 90.10 respectively while the values were lower in yeast free T0 control.

***Keywords*---**health, nutrition, bakery yeast, fish feeding, growth, common carp.

Introduction

A healthy and balanced diet is one of the most important components for maintaining fish health and the success of fish farming with positive implications on growth and production (Nekoubin and Sudagar, 2012), so the manufacture of appropriate diets is crucial to achieving a nutritionally balanced and cost-effective nutrition (Hasan and Soto, 2017), especially if cultured in limited places and at high densities (Taher *et al.*, 2022) leading to crowding, competition, lack of oxygen, low water level and poor quality, in a cycle that exposes fish to stress, inhibition of the immune system, increased spread of diseases and inhibition of growth (Gaunt *et al.*, 2010). This needs high quality feed (Singh *et al.*, 2021), by adding certain specific quality food additives that can support growth rates, efficient dietary conversion and protein, improve the digestibility, reduce mortality and increase disease resistance (Kord *et al.*, 2021). Many supplements such as stimulants, growth stimuli and some antibiotics have been used as food additives contributing in improving growth performance and controlling disease outbreaks in fish farming (Lund, 2021).

Attention has now been drawn to the use of safe natural substances as food additives such as probiotics and prebiotics to improve the general health, growth and reduce stress (Hien *et al.*, 2017; Xiong, 2018). Feed additives represent different types of molecules, compounds or organisms that stimulate the intake, absorption and absorption of nutrients (Watts *et al.*, 2020). The use of beneficial living microflora as probiotics can significantly improve the nutritional value and growth rates of fish, develop enzyme efficiency, microbial fermentation, nutrient availability, feed efficiency of raw materials and improve the host's physiological traits including the development of the immune system (Shao *et al.*, 2019; Rahimnejada *et al.*, 2019). Probiotics are microbial preparations or components consisting of a single species or a mixture of living microflora that have beneficial effects on human and animal health through their role in improving the characteristics of intestinal natural microbiota already found in the gut by increasing the number of beneficial bacteria and reducing the number of pathogenic species (Lauzon *et al.*, 2014; Merrifield and Ringo, 2014). By adhesion to the cells lining the gastrointestinal tract and competing with pathologic microbes and preventing their impact on the host's body (Ige, 2013), probiotics and prebiotics could improve the organism's ability to benefit from nutrients, reduce the toxicity of some compounds in feed and increase digestibility of non-digestible nutrients (Talpur *et al.*, 2014 ; Albadran *et al.*, 2018).

The mechanism of probiotics in the digestive system includes altering the metabolism of pathologenic bacteria by changing their pathways as well as stimulating the body's cellular immunity and disease resistance (Goran *et al.*, 2017). Yeasts have been considered as vital boosters that contribute to the health of fish and dry yeast *Saccharomycess cerevisiae* is the most common strain among commercially produced yeasts as a source of single cell protein, which has immune boosting properties as it contains glycoproteins and beta-glucan (Feldmann, 2012). Dry yeast is used in fish feeds either as an added dietary component or as an alternative to protease (Agboola *et al.*, 2021), where yeast is described as probiotics with an immune-enhancing effect and supports the mechanism of symbiotic coexistence in the intestinal environment (Ke *et al.*, 2021), as well as its role in stimulating the action of lactic acid bacteria (Adel *et al.*, 2016) generating short bioactive amino and fatty acids and artificially fermenting sugars such as glucose and maltose into ethanol and carbon dioxide (Al-Fragy, 2006). The current research aimed to investigate the effect of adding *Saccharomyces cerevisiae* bakery yeast in different proportions in the nutrition of common carp on the growth criteria for feed fish, as a probiotic and single cell protein.

Materials and Methods

A total of 150 common carp fish were brought from Aquaculture Unit, College of Agriculture, University of Basrah with an average weight of 13.5 g and an average length of 8.61 cm. Feed materials available in local markets were used as the main raw materials in the manufacture of experimental diets, namely fish meal, soybean meal, maize, wheat bran, wheat flour, vitamin, mineral and edible oil mixture obtained from the Feed Manufacturing Laboratory at the College of Agriculture, University of Basra, in addition to using dry yeast *S. cerevisiae* (Turkish origin) obtained from the local markets of Basrah.

Culture system

Upon arriving to Aquaculture laboratory in the Department of Fisheries and Marine Resources, College of Agriculture, fish were disinfected with 3% saline solution for one minute to get rid of bacteria and external parasites (Muhaysin, 1983), after which the fish were cultured in 30-liter glass aquariums pre-sterile with sodium hypochlorite solution (200 ppm) for one hour (Herwing *et al.*, 1979). Perforated covers were fitted to aquariums to prevent fish jumping and facilitate ventilation with submersible heating devices to stabilize water temperature. The experiment was designed with four treatments and three replicates for each (12 aquariums) and fish were distributed randomly at the beginning of the experiment with 10 fish/ aquarium. Fish were acclimated for 10 days before experiment during which they were fed on the standard diet.

Feed preparation

After determining the proportions of primary feed materials used to manufacture fish diet under study described in table 1, feed materials were well milled and passed through a sieve with openings of 2 mm and then mixed with the calculated ratios well to homogenize, after which approximately 100 ml of boiling water was added per 250 g of mixture and after mixing with increasing the temperature of the mixture to ° C. After cooling, vitamins and minerals were added to the mixture to prepare the four experimental diet as follows:

T1: First treatment / added yeast *S. cerevisiae* by 0.5 %
T2: Second treatment / added yeast *S. cerevisiae* by 1 %
T3: Third treatment / added yeast *S. cerevisiae* by 1.5 %
C: Control diet without added yeast *S. cerevisiae* 0%

After mixing with the proportions shown of bakery yeast, mixtures were transferred to the incubator and leave for 4 hours at 28°C, then formed the diets as pellets using meat grinder with meshes of 4 mm then left to dry antenna at the laboratory temperature for 48 hours with continuous flipping to get rid of moisture and make sure to dry completely after which the feed was packaged into plastic containers 2 kg and placed in the refrigerator until use.

Table 1
Ratios of feed materials and bakery yeast *S. cerevisiae* used in the manufacture of diets

	Ingredients	Control T0 (0) %	T1 0.5 %	T2 1 %	T3 1.5 %
1	Fish meal	23	23	23	23
2	Soybean meal	20	20	20	20
3	Yellow corn	15	15	15	15
4	Wheat bran	20	19.5	19	18.5
5	Wheat flour	18	18	18	18
6	Vitamins and minerals	2	2	2	2
7	Vegetable oil	2	2	2	2
8	Dry yeast	0	0.5	1	1.5
9		100	100	100	100

Feeding experiment
Growth

The fish were fed on processed diet to which S. *Cerevisiae* yeast was added, as well as controlling the feeding of fish by 3% of living weight throughout feeding experiment, where it was provided on the first meal at 9 a.m. and the second meal at 2 p.m. Rations were change biweekly after fish weighing. Siphoning was used to clean aquariums of waste and uneaten feed daily with changing 70 % of volume by dechlorinated fresh water per week to maintain water quality. Total Weight Gain (TWG) and Daily Weight Gain (DWG) were calculated according to Sevier *et al.*, (2000). Relative growth rate (RGR) and Specific Growth Rate (SGR) were calculated based on Jobling (1993). Feed conversion rate, feed conversion efficiency, protein intake (Pl) and protein efficiency ratio (PER) were calculated based on Tacon (1990) according to the following equations:

Total weight gain (g/fish) = final weight rate (g) - primary weight rate (g)
Daily weight gain (g/day) = weight gain (g) / duration of this increase (day)
Relative growth rate (%) = weight gain (g) / primary weight (g) ×100
Specific growth rate (% /day) = Ln final weight (g) – Ln initial weight (g) / period in days × 100
Feed conversion ratio = weight of provided feed (g) / weight gain for fish (g)

Food conversion efficiency (%) = wet weight gain for fish / weight of feed provided ×100
Protein intake (g/fish) = weight of feed intake (g) × protein content in the diet
Protein efficiency ratio (%) = wet weight gain for fish / protein intake (g) ×100

Digestibility experiment

For the purpose of measuring total apparent digestibility coefficients and apparent digestibility coefficients of nutrients in the diet, the indirect method shown by Talbot (1985) was adopted, where Cr_2O_3 chromium oxide was added as an indicator at 1 % of weight of the components of the diet. Fish were fed on the diet containing chromium oxide daily at 9.00 am until the end of the day and after an hour of feeding the uneaten feed was withdrawn and feces were collected by siphoning at intervals of no more than 15-20 minutes (Schmit *et al .,* 1983). Collected feces are then filtered and washed with distilled water and left to dry completely at room temperature and collected in tightly sealed plastic vials and kept in the refrigerator. Chromium oxide contents in dried feces was estimated according to the spectrophotometric method of Olvera-Novoa *et al.*, (1994) at a wavelength of 350 nm. Absorptance values has been converted into chromium oxide concentration using the following equation:

0032/0.2089. y - 0×X=1/4

Where: X = amount of chromium oxide (g) in the sample, Y = absorption
The figures indicate the constants of the curve measuring the values of chromium oxide information (standard values). After completing the process of estimating the percentage of chromium oxide in waste, by total total apparent digestibility coefficient TADC and virtual digestion factors nutrients nutrient apparent digestibility coefficient NADC using the following equations:
Chromium oxide ratio = (chromium oxide weight ÷ sample weight) × 100
Virtual total digestion coefficient % = 100- (100× chromium oxide in food ÷% chromium oxide in waste %).
Virtual digestion of nutrients % = 100 (- 100 [(× percentage of chromium oxide in the diet % ÷ percentage of chromium oxide in waste %) × (percentage of the nutrient in waste % ÷ percentage of the food element in the diet) %))]

Satiety level

The satiety experiment was lasted for ten days during which the fish fed on a known amount of weight of feed by7 % in one meal per day at 9.00 am, and after an hour of feeding time uneaten feed was collected by siphoning on a filter paper and air dried then weighed to calculate the amount of consumed feed by fish to estimate the level of satiation as a percentage of fish body weight according to the equation provided by Tamimi (2007).

Satiation level (%) = (feed intake g \ body weight g) × 100

Results and Discussion

Table (2) shows the rates of initial and final weights, the rate of weight gain of fish and the total increase of various treatments. The highest final weight value in the T3 treatment was 380.15 g and the lowest value of the yeast-free T0, which was 299.28 g and the final weight gain for T1 and T2 treatments ranged from 299.28 g. 326.83 and 305.78, respectively. The highest total weight gain value in the diet containing 1.5% dry yeast T3 was 242.12 g/fish and the results of the statistical analysis indicated that the weight gain of the T3 treatment differed significantly (P<0.05) for the rest of the treatments, which amounted to a weight gain of 161.44, 179.66 and 169.56 for each of the yeast-free treatment T0 and T1 and T2, in the same order, where statistical analysis proved that there were no significant differences (p>0.05) between T0 treatments, T1 and T2. The daily weight gain of the T3 found to be 3.22 g/fish, which is significantly different from (P<0.05) from the rest of the treatments, in which the daily weight gain was 2.15, 2.39 and 2.26 per T0 and T1. And T2 in the same order and without significant differences (p>0.05) between them.

With regard to the relative and specific growth rates, the results in table (3) showed that fish which fed on the control and yeast added diets recorded the highest values for the relative growth rate of T3 fish 175.75% and this treatment varies significantly (P<0.05) from the rest of the treatments. Additionally, the results showed a lower value of the relative growth rate of 117.47% in the yeast-free T0 control while values in T1 and T2 treatments varied to 134.49 and 124.65% respectively, with no significant differences (p>0.05) between treatments T0, T1 and T2. Specific growth rates varied between 4.9% and 4.81%/day for T1 and T2 treatments, respectively, while the lowest was in yeast-free T0 4.73%/day. Highest qualitative growth rate was recorded in T3 at 5.27%/day, which represents diet with 1.5% of yeast and T3 differed significantly (P<0.05) from the rest of treatments with no significant differences (p>0.05) between the other three treatments.

Table 2
Initial and final weights and weight gain for fish in different treatments

Treatments	Primary weight (g)	Final weight (g)	Total weight gain (g)	Daily weight gain (g)
T0 (0) %	137.83a±4.48	299.28±13.59	161.44 d ± 17.80	2.15 d ± 0.237
T1 (0.5) %	133.95a±8.87	326.83±11.71	179.66 d ± 6.32	2.39 d ± 0.084
T2 (1) %	136.22a±3.55	305.78±13.66	169.56 d ± 15.64	2.26 d ± 0.208
T3 (1.5) %	138.02a±3.63	380.15±17.19	242.12 a ± 20.64	3.22 a ± 0.275

*The different letters within the single column indicate significant differences at the level of (p<0.05).

Table 3
Relative and specific growth rates of fish in different treatments

Treatments	Relative growth rate (%)	Specific growth rate (%/day)
T0 (0) %	117.47 d ± 16.51	4.73 d ± 0.173
T1 (0.5) %	134.49 d ± 9.728	4.9 d ± 0.04
T2 (1) %	124.65 d ± 13.877	4.81d ± 0.12
T3 (1.5) %	175.75 a ± 19.506	5.27 a ± 0.11

*The different letters within the single column indicate significant differences at the level of ($p<0.05$)

Table (4) shows the rate and efficiency of feed conversion, protein intake, and the proportion and efficiency of protein for fish fed on diets with addition of dry yeast in different proportions as well as control. Results indicated that the best result of the feed conversion ratio was for the T3 1.79 with significant difference ($P<0.05$) compared to other treatments with 2.88, 2.3 and 2.54 feed conversion ratios for T0, T1 and T2, respectively and significant differences between T3 , T2 and T0 treatments. The results also showed that the values of the feed conversion efficiency in T3 was 55.97%, followed by T1 containing 0.5% of added yeast where it was 45.28% compared to the control T0 34.93% and the T2 39.49% and with a significant difference ($P<0.05$) between T3 and the rest of treatments. The amount of protein intake showed no significant differences ($p>0.05$) among all treatments with values of 14,008.68, 12,557.59, 13,056.63 and 13,224.17 for T0,T1, T2 and T3, respectively. It was 1.83 while the lowest ratio was in T0 with the rest ranging from 1.49 to 1.29 for T1 and T2 in the same order with significant differences ($P<0.05$) between T3, T0, T2 and T1 treatments.

Table 4
Feed conversion ratio, feed conversion efficiency, protein intake and protein efficiency of fish in different treatments

Treatments	Feed conversion ratio	Feed conversion efficiency	Protein intake	Protein Efficiency ratio
T0 (0) %	2.88 dcf±2.29	34.93 dcf±0.67	14008.68 a±576.79	1.15 dbh±0.12
T1 (0.5) %	2.3 dcfg±0.53	45.28 dcfg±12.10	12557.59 a±2886.63	1.49 dbhc±0.39
T2 (1) %	2.54 dcf±0.17	39.49 dcf±2.70	13056.63 a±760.20	1.29 dbh±0.08
T3 (1.5) %	1.79 acg±0.12	55.97 acg±3.95	13224.17 a±640.90	1.83 abc±0.12

*The different letters within the single column indicate significant differences at the level of ($p<0.05$).

The results of the current study showed that the use of different proportions of dry yeast *S.cerevisiae* in the manufacture of fish diet resulted in a significant variation in the growth parameters of experimental fish. Hepher (1988) showed weight gain and growth rate as one of the most important scientific and practical parameters used to assess the quality and protein content of the diet. The increase in daily growth rates and weight gain in the three treatments containing different proportions of *S.cerevisiae* yeast is due to the chemical composition of yeasts and to the presence of sugars, amino acids and other nutrients that have positive effects on growth and weight gain with increasing yeast addition ratios (Mageney *et al.*, 2017). Yeast acts to enhance immunity and raise the health level of fish as well as reduce stress, in a cycle that positively affects weight gain and growth (Cooney, 1998). This is in line with T3 contained 1.5% of yeast in terms of weight gain and daily growth rates in comparison to yeast free T0 control.

The probable cause of these results is the high concentrations of prebiotics which contain large amounts of active substances as well as their role in providing fermented substances that change microflora of intestinal to support the growth of beneficial bacteria (Djauhari *et al.,* 2017). These results were consistent with El.Haroun *et al.*, (2006) who found that the addition of probiotics to Nile tilapia *Orechromis niloticus* feed also resulted in an increase in growth and weight gain. The same was shown by Ghosh *et al.*, (2005) when adding different concentrations of yeast in fish feed leadind to an increase in growth rates and a reduction in feed conversion ratios as well as increased specific growth rate and protein efficiency as increasing and improving growth and digestion rates of nutrients in carp fingerlings fed on yeast. These results were consistent with Al-Janabi *et al.*, (2021) who found that the addition of probiotics Thepax yeast to *Cyprinus Carpio* feed also resulted in an increase in growth and weight gain.

The best assessment of weight gain is the criteria for relative and qualitative growth because they reduce the impact of the variation in primary weight between fish where the results of the statistical analysis showed an increase in the values of relative growth rate, specific growth rate and more valuable in the treatment of T3 and significant difference with other treatments, and the improvement in growth criteria in the fish of the experiment was attributable, especially in the treatment T3, to stimulate the targeted microorganisms of growth and reproduction as the presence of yeast in the gut increases the representation of fatty acids as well as their secretion of digesting enzymes for carbohydrates, protein and lipids (Guerreiro *et al.*, 2018), resulting in an increase in the conversion of complex nutrients into simpler substances (Djauhari *et al.*, 2017). The results of the present study were agreed with the Guerreiro *et al.* (2017) study who found an improvement in fish health and an increase in growth rates for fed fish on additions of pro and prebiotics due to increased use of nutrients for feed taken due to the increased effectiveness of protease and amylase in fish intestine (Anguiano *et al.*, 2013 ; Azevedo *et al.*, 2015).

An important economic indicator is the rate of feed conversion, which indicates the efficiency of fish in converting feed into weight gain while maintaining its health status (Al-Qaoud, 2003). It was observed through the results that there is an improvement in the rate of feed conversion in addition treatments compared to the control as the best values were in the treatment of T3 by a percentage and this is due to the use of probiotics that improve the rate of feed conversion due to the fact that the microflora found in the probiotic leads to an increase in natural flora beneficial to the intestines and the speed of their adhesion on the surface of the mucous layer where they are suitable for their growth and reproduction and these results agreed with the findings of Al-Dubakel *et al.*, (2015) when using a probiotic in feeding fish leding to an increase in the rate of feed conversion and improved the nutritional value of the diet causing an increase in fish appetite. The results also agreed with Mathew (2001) who noted that yeast in diet stimulates growth, immune system and body resistance to diseases due to its cellular wall components consisting of oligosaccharide mannan.

It secretes the enzyme inulinase and breaks the beta bonds found in inulin and turns it into a carbon source where it shows its superiority over harmful bacteria. Naji, (2011) arrived to same conclusions with the use of probiotics in fish feeding, and confirmed by Mazurkiewicz *et al.*, (2005) when adding probiotics in carp feeding and noted an increase in the growth and improvement of the feed conversion ratio due to the addition of *S.cerevisiae* yeast, which is used as a protein source as well as to bind some foods. Other workers ascribed same results to bacteria and yeast that act by producing enzymes and increasing digestibility, absorption and utilization of nutrients using the same mechanisms (Fritts, 2003). Results in table 5 showed the level of satiation of fish with the highest level for T3 fish 6.59%, while the lowest yeast-free control was 6.10%, and values varied in the remaining treatments at 6.35 and 6.48 per T1 and T2, respectively, with no significant differences (p>0.05) between all treatments.

Table 5
Level of satiation % of body weight for common carp during the experiment

Treatments	Satisfaction level%
T0 (0) %	6.1 a ± 0.5
T1 (0.5) %	6.35 a ± 0.7
T2 (1) %	6.48 a ± 0.5
T3 (1.5) %	6.59 a ± 0.2

The different letters within the single column indicate significant differences at the level of (p<0.05)

The satiation is expressed as the point of access to the state of satiation by fish when it stops feeding (Alejos *et al.*, 2019). The results showed a high level of satiation in fish fed on the T3 feed due to the fact that increasing the amount of feed consumed may improve growth rates with high efficiency by improving the palatability of feed. Hancz (2011) confirmed that fish are highly palatable to animal protein higher than plant-protein feeds, which explains the current results due to increased palatability and acceptance of food with increasing added yeast ratios (EFAS, 2012). Bakery yeast is an important factor in growth experiments and fish feed efficiency, where it has a significant effect in improving body weight, immunity and feed conversion efficiency, reducing stress and acting to increase feed palatability due to their production of vitamins and factors enhancing and stimulating fish growth (Bajagai *et al.*, 2016).

Table (6) shows the results of apparent digestibility of feed components of containing different ratios of yeast with significant difference of (P<0.05) among all four treatments. Highest value of protein digestibility coefficients in T3 was 95.85 followed by T2.2 94.40 while the values of the protein digestibility in T1 91.20 which was different from T0 without adding dry yeast. The value increased to 91.91 and 92.19 per 1% and 1.5% of dry yeast, respectively, compared to the lowest values of the yeast-free control 0 % 87.88% with significant differences in all treatments. With regard to carbohydrate digestibility, statistical results showed significant differences between all treatments, with 82.77 for T0 yeast-free control while values has increased with increasing yeast content to 87.70 and 9.10 in T2 and T3, respectively.

Table 6
Apparent digestibility of feed components by experimental fish

Chemical component	Apparent digestibility of feed			
	T0 (0) %	T1 0.5 %	T2 1 %	T3 1.5 %
Protein	89.19 d±0.02	91.20 c±0.16	94.40 b±0.39	95.85 a±0.02
Fat	87.88 d±0.05	89.90 c±0.05	91.91b±0.02	92.19 a±0.07
Carbohydrates	82.77 d±0.16	85.24 c±0.00	87.70 b±0.07	90.10 a±0.08

*Different letters within the same row indicate significant differences at the level of ($p<0.05$).

The high digestibility of feed ingredients was evident with the use of dry yeast in fish dies compared to yeast-free control. The results of protein and fat digestibility were higher in T3 and T2, confirming that the presence of probiotics, including yeast in the diet, leads to an increase in the digestion of nutrients. Al-habeeb and Al- basam (2011) indicated that the improvement in nutrient digestibility is due to an increase in the digestion of protein and carbohydrates in diet, due to the role of dry yeast by increasing the digestion and breakdown of undigested substances in the intestines by producing many digesting enzymes for carbohydrate, protein and fatty substances and increasing their absorption as well as their role by stopping anti-digestion factors (EL-Haroun *et al.*, 2006 ; Imran *et al.*, 2019). Mohammed and Al-Safao (2013) pointed out that improved digestion processes lead to improved growth and survival rates which were also consistent with the Welker, Lim study (2011) who found that the use of yeast in fish diets improves digestion and performance of growth by stimulating nutrient digestion enzymes. Tovar-Ramirez (2002) confirmed that the use of yeasts in fish feeding led to stimulating secretion of amylase enzyme and enhances membrane activity to infuse enzymes in the intestines and this improved growth performance and food utilization. The current results were also consistent with Mohapatra *et al.*, (2012) who found an increase in the growth rate and performance of fish using yeast *S.cerevisiae* ascribing this to its content of some growth-stimulating vitamins and enzyme production which has a vital role to play in increasing digestion and feed utilization in fish.

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