World Journal of Fish and Marine Sciences 6 (6): 524-531, 2014
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2014.06.06.86170

Effect of Feed Addition of Distillery Yeast Biomass Protein Hydrolysates in Common Carp (*Cyprinus carpio* L.) Fingerlings Diets

Rajasundari Kandaiah and Murugesan Ramasamy

Department of Agricultural Microbiology, Tamil Nadu Agricultural University,
Coimbatore-641 003, Tamil Nadu, India

Abstract: The effect of protein hydrolysates extracted from distillery yeast biomass (DYBM) as feed additive for common carp fish was documented in this study. Protein hydrolysates from the DYBM were extracted using bacteria producing proteolytic enzymes. Protein hydrolysates were added to the fish feed at 5 and 10%. Each experimental diet containing protein hydrolysates was fed to carp fingerlings (7 weeks old) for 10 weeks and the effects on the growth of fingerlings were monitored. After the experimental duration, it was found that fingerlings fed with protein hydrolysates showed significantly better performance in terms of body weight, protein, ash and dry matter content when compared to the controls fed only with conventional feed. Dietary inclusion of protein hydrolysates levels as high as 10% had no adverse effects on growth and nutrient utilization. It is clear that protein hydrolysates extracted from yeast biomass can be a promising low cost protein source for common carp fingerlings.

Key words: Distillery Yeast Biomass · Protein Hydrolysates · Amino Acids · Feed Additives · Common Carp

INTRODUCTION

Fish is considered as an important protein source in human diet. Over the past decade, the portions of per capita seafood consumption produced by aquaculture have steadily grown to nearly half [1]. Capture fisheries production has declined in recent years and future forecasts for seafood landings to remain constant or decline further. As a result, production of produce from aquaculture sector had to increase to meet the expected future demand for seafood [1]. Recently, an increase in the cost of fish feeds due to decreased marine supplies and increased cost of production in the feed industries has resulted in the search for finding the alternative protein sources for improving the aquaculture feed quality. Protein alternatives sourced ranging from plants to food industries waste are currently being explored in research trails [2].

In aquaculture, proper nutrition has long been recognized as a critical factor in promoting the normal growth and sustaining health of fish. Single cell protein (SCP) comprising micro algae, bacteria and yeast that is rich in protein, B-vitamins, pigments and complex carbohydrates and as the source of alternative and non-conventional protein that can be used as feed ingredient in fish feed [3]. Among SCPs, brewer's yeast is being commonly and most widely in fish feeds [4,5] and is considered as there placement for fishmeal [6]. Alternatively protein hydrolysates on the other hand are rich in amino acids that can be used as feed supplement in aquaculture [7]. Feed supplementation with protein hydrolysates also results in overall improvement of performance in growth and development of the fish, since amino acid rich feed consumption aids in instant absorption of amino acids in to the blood system for the efficient conversion into body biomass and improving fish health.

Dietary supplementation of protein hydrolysates in aquaculture is considered as beneficial in terms of: (1) increasing the chemo-attractive property and nutritional value of aquafeeds with low fish meal inclusion; (2) optimizing efficiency of metabolic transformation in juvenile and sub-adult fishes; (3) suppressing aggressive behaviors and cannibalism; (4) increasing larval stage performance and survival; (5) mediating timing and efficiency of spawning; (6) improving fillet taste and texture; and (7) enhancing immunity and tolerance to environmental stresses. Functional amino acids hold great

Corresponding Author: Rajasundari Kandaiah, Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. Tel.: +61 470151956.

promise for development of balanced aqua feeds to enhance the efficiency and profitability of global aquaculture production [8].

Generally yeast biomass is generated as a waste product in large volumes in sugarcane processing factories and distilleries. Distillery yeast biomassis currently being used for the production of compost. Yeast biomass is a rich source of proteins and also an excellent source of B-complex vitamins, nucleic acids and minerals, including a biologically active form of chromium known as glucose tolerance factor [9, 10]. Because of cheaper cost, easy availability, rich nutrient composition, devoid of toxic components and fermentation inhibitors, cane or beet molasses are widely used as substrate for the production of yeast biomass. Molasses contains 45-55% fermentable sugars including sucrose, glucose, fructose, raffinose, melibiose and galactose [11].

Brewer's yeast biomass can replace 50% of fishmeal protein with no negative effects in fish growth performance. Moreover, the inclusion of up to 30% brewer's yeast biomass in the fish diet improved feed efficiency [6, 10]. As a proteinsource, brewer's yeast has been included in commercial diet formulations for several fish species, including salmonids [10]. Partially replacement of fish meal with biomass from brewer's yeast showed marked difference in feed conversion in juvenile sea bass [6]. Yeast one of the co-products obtained from bio-ethanol process was tested in series of iso-nitrogenous (38% crude protein) and iso-lipidic (8%) diets for juvenile minor carp (*Cyprinus caprio*) showed better performance over control [12]. In Nile tilapia cultivation, feed addition up to 40 % biomass from *Streptococcus faecium*, *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* added up to 40% in fish diet promoted the growth of Nile tilapia's (*Oreochromis tilapia)*, which suggested that yeast could be used as an appropriate growth-stimulating additive [13]. Dietary supplementation of *S. cerevisiae* at 1-2% has resulted in increased growth performance in Nile tilapia [14].

Apart from direct nutrient source, protein hydrolysates also aids in optimum, absorption and retention of phosphorus in fish (*Pagrus major*) [15,16], Intraperitoneal injection of insoluble polysaccharide namely M-Glucan (β-glucan from specialized cells namely microfold cells) from the cell walls of *S. cerevisiae* was reported to enhance the non-specific disease resistance of Atlantic salmon (*Salmo salar* L.,) [17]. This study is comprised ofthe following objectives, (1) microorganism mediated extraction of protein hydrolysates from yeast biomass, (2) analysis of amino acid content present in the protein hydrolysates and (3) effect of protein hydrolysates as feed additive in the growth and biochemical parameters of common carp.

MATERIALS AND METHODS

Chemicals: All the reagents and chemicals used in this study were of analytical grade, obtained from Sigma Aldrich.

Substrate: Distillery yeast biomass (DYBM) used in the experiment were obtained from, M/S Sakthi Sugars, Appakudal, Bhavani Taluk, Erode District, Tamilnadu, India and Amaravathi Co-Operative Sugar Mills Ltd, Krishnapuram, Udumalpet Taluk, Coimbatore District, Tamilnadu, India. The proximate composition of DYBM is presented in Table 1.

Extraction of Protein Hydrolysates: The microbial extraction of protein hydrolysates from DYBM was carried out in 5-L fermentor with the working volume of 3L. Dried and powdered DYBM (5.0%) was mixed with 3L of water containing glucose (1.0%) followed by sterilization at 121 °C for 20 min. After cooling the fermentor contents, the proteolytic bacterial inoculums (*Bacillus megaterium* -PB4) (10.0%) was transferred into the fermentorun dersterile condition. The fermentorun it was operated at 30±2°C. The drive motor was allowed to rotate continuously for uniform mixing of the medium and better growth of the efficient proteolytic bacterial culture. Complete extraction of protein hydrolysates was achieved in 5 days. Samples were withdrawn periodically and tested for any microbial contaminants.

Table 1: Proximate composition of distillery yeast biomass

Particulars (%)	Yeast biomass
Moisture content**	30.3 ± 0.4
Crude protein	43.2 ± 0.5
Total amino acids	38.4 ± 0.4
Carbohydrate	11.5 ± 0.1
Crude fiber	9.1± 0.1
Ash	6.6 ± 0.1
Nitrogen	7.2 ± 0.1
Phosphorus	1.5 ± 0.02
Potassium	2.1 ± 0.03
Calcium	0.32 ± 0.03
Magnesium	0.15 ± 0.01
Copper	0.14 ± 0.01
Iron	0.4 ± 0.03
Manganese	0.21 ± 0.03
Zinc	0.23 ± 0.01

After the completion of fermentation process, the broth was subjected to high temperature short time pasteurization (72°C for 15 sec). The proteolytic bacterial load in the fermentation broth was eliminated by filtration with 0.45 µm bacteriological filters (Millipore™). The filtrate obtained was termed as DYBM-protein hydrolysates (DYBM-PH).

Animals, Experimental Design and Conditions: Six weeks old common carp (*Cyprinus carpio* L.) fingerlings were obtained from local fish farm. Groups of 10 fish were stocked in 25-L glass aquaria equipped with biological filter and aeration. The fishes were acclimatized for 7 days prior to the initiation of the experiment. Ten common carp fingerlings (Approx. 7 weeks old) (Average weight 1.0 g) were randomly selected and released in to 50-L treatment containers. Though no significant feed scrap was observed, the test containers were replenished with 60% fresh water exchange thrice a week to reduce the turbidity and fecal accumulation. Water temperature, dissolved oxygen, pH, ammonia and nitrate were monitored and averaged as 25.2±2°C, 3.1±0.51, 7.5±0.12, 0.11±0.06 and 0.07±0.01 mg L^{-1}, respectively.

Feed Preparation and Feeding: The fingerlings were fed with 2 experimental concentrations of DYBM-PH mixed with commercial feed. The moisture content of the feed was maintained up to 10% permissible level and feeding was done twice a day.

Estimation of Growth Characteristic of Fish: Initial body weight of the fishes was calculated and the body weight was recorded at weekly interval throughout the experimental duration of 10 weeks and the difference in weight was calculated. At the end of the experimental trial all the fishes were weighed and 5 fishes from each treatment were sacrificed by euthanizing them in ice for 15 min. Moisture, protein and ash contents of the fishes were determined according to the standard methods [18].

Analytical Methods: The coloured protein hydrolysates of distillery yeast biomass was decolourized by filtering through activated charcoal followed by centrifugation (Remi R-4C, India) at 10,000 g for 15 min and then the supernatant was filtered through 0.45 µm (Millipore™) membrane filters. The amino acid composition of the protein hydrolysates was analyzed using high performance liquid chromatography (Shimadzu LC20AT Prominence Liquid Chromatograph). The chromatogram was equipped with LC20AT VP pump and manual injector. Mixture of sodium citrate and perchloric acid (pH 3.2) comprised buffer A and buffer B was composed of sodium citrate and boric acid (pH 10.0) was used as the mobile phase. Isocratic separation of amino acids was performed in Welchrom C18, 4.6 × 250 mm, 5µm particle size. UV detection wavelength was 240 nm with the flow rate of 0.5 mL min^{-1} with a total of 70 min run time. The sample injection volume was 20.0 µl. O-phthalaldehyde(OPA) was used as the derivatization agent for detection. The chromatogram peaks and their concentration of the different amino acids present in the sample were identified by comparison with the retention time and peak area of the standard amino acids using Spinchrome software supplied with the instrument.

Statistical Analysis: All the experiments in this study were conducted with 3 replicates unless otherwise stated and reported with the mean values. All the treatments were compared at $p \leq 0.01$ and 0.05 level of significance using the critical difference (CD) test which was performed by Agris Statistical Package as described byPanse and Sukhatme [19]. The analysis of variance (ANOVA), standard error (SE) and *t*test for dependent parameters were tabulated and the level of significance was reported.

RESULTS AND DISCUSSION

Ever growing human population has increased the global demand for food at an alarming rate. The growing competition for land, water and energy has drastically reduced the over all food production all over the world [20]. Sustainable farm intensification is considered as the only economical and viable option for increased food production from the same available area whilst reducing the negative environmental impacts [21]. The method of sustainable agricultural system for increased productivity and optimum resource utilization was flourished in India earlier than reported in China, Rome and Greece [22]. India is one of the largest producers of sugarcane in the world at an average of 270 million tonnes per year [23]. Generally during the processing of sugarcane, by-products such as press mud, bagasse, molasses and sugarcane residues are being produced. Fermentation of molasses, a starting material for the production of alcohol, results in the generation of large quantities of both solid and liquid wastes along with the generation of substantial quantity of yeast biomass. Yeast biomass is the second major by-product obtained from brewing industries. Yeast biomass possesses very high protein content and other nutrients which can have multiple uses as single cell protein and as feed additives [10].

Hydrolysis of protein from protein rich agro wastes is considered as the simple and cost effective technique for the conversion of protein into free amino acids and short chain peptides. The hydrolysed protein is more hydrophilic than the substrate by itself and has a stable composition [24]. Chemical and biological hydrolysis of protein is the most widely practised techniques. Chemical protein hydrolysis can be further classified as acidic and alkaline hydrolysis. However both acidic and alkaline hydrolysis involves in the use of acids such as hydrochloric acid, sulphuric acid, strong alkalis including alkaline sodium chloride. As a result several deleterious reactions and toxic wastes are generated during chemical hydrolysis, which in turn and also affects the quality of the protein hydrolysates [25]. Biological deproteinization on the other hand can be carried out with the help of proteolytic microorganisms and commercial protease enzyme to extract proteins from these substrates in the form of protein hydrolysates which was proved to be effective and environmental friendly [26]. Microbes capable of producing protein hydrolysing enzyme called protease has been used for the deproteinization studies. Enzyme mediated protein hydrolysis is considered as the viable option for recovery of valuable amino acids from by-products obtained during alcohol distillation. Application of enzymes in the proteolysis of various sources has been previously described by several authors [27]. Hence, the present investigation aimed at isolating efficient proteolytic microbial strains for the deproteinization of distillery yeast biomass through the process of fermentation. Studies have been carried out for the optimization of fermentation process for the maximum deproteinization efficiency in pilot scale processes.

The proximate compositions such as moisture content, crude protein, total amino acids, carbohydrate, crude fiber, total nitrogen, phosphorus, potassium, calcium, magnesium and micronutrients of yeast biomass were analyzed and presented in Table 1. The moisture content in the wet samples of distillery yeast biomass was 30.3±0.4%. The samples were dried and analyzed for other constituents. Crude protein and total amino acid content of yeast biomass were found to be 43.2±0.5 and 38.4±0.4%, respectively. The total carbohydrate content of yeast biomass was 11.5±0.1%. In terms of crude fiber, distillery yeast biomass recorded 9.1±0.1%, respectively, whereas ash content registered 6.6±0.1% in yeast biomass. Total nitrogen in yeast biomass was (7.2±0.1%). Yeast biomass phosphorus and potassium content were 1.53±0.02% and 2.13±0.03% respectively. The chemical composition, nutritional and functional properties of *Saccharomyces cerevisiae* were examined [28,29] and found out that the whole yeast cell contains 39.6 - 47.2% of protein, 4.6 - 8.6% of ash, 13.0-31.4% of total fibre, 10.5 - 21.5% of carbohydrate and other major and minor nutrients and amino acids. Duarte *et al*. [30] studied the composition of hemicellulosic hydrolysate of yeast biomass produced in brewery and also had similar proximate compositional factors. As yeast biomass is one of the major by-products from brewing industries, Fierreira *et al*. [10] study pointed out that apart from high protein content, yeast biomass was also rich in B-complex vitamins, nucleic acids and minerals. The proximate compositional analysis of yeast biomass of this research also supports the literature evidences on yeast and yeast biomass.

DYBM-PH was analyzed for amino acid composition in an amino acid analyzer. The analysis revealed the protein hydrolysates was found to be rich in 7 essential aminoacids namely leucine, lysine, valine, threonine, isoleucine, phenylalanine and arginine and 4 non-essential aminoacids like glutamine, histidine, tyrosine and alanine. Among the essential amino acids, leucine was found to be the highest in yeast biomass protein hydrolysate (2.25 g) followed by lysine, valine, threonine, isoleucine, phenylalanine and arginine. On the other hand, glutamine (1.92 g) was found to be the highest in non-essential amino acids category followed by histidine, tyrosine and alanine was found to be the least which recorded 0.02 g 100 mL^{-1} of protein hydrolysate (Table 2).

Yeast biomass was hydrolysed using neutral protease producing *Bacillus megaterium* PB4. At the end of hydrolysis, the protein hydrolysates were analysed for its amino acid contents. Amino acid profile of the protein hydrolysates revealed that it contained totally 11 amino acids, of which 7 were essential amino acids namely leucine, lysine, valine, threonine, isoleucine, phenylalanine and arginine and remaining 4 were non-essential amino acids like glutamine, histidine, tyrosine,

Table 2: Amino acids content of the protein hydrolysates

Amino acids	Concentration (g 100 mL^{-1} protein hydrolysate)
Leucine*	2.25
Lysine*	2.01
Glutamine**	1.92
Valine*	1.91
Threonine*	1.44
Isoleucine*	1.42
Phenylalanine*	1.38
Arginine*	1.34
Histidine**	0.91
Tyrosine**	0.07
Alanine**	0.02

* Essential aminoacid ** Non-essential aminoacid

serine, alanine and glycine. Kurozawa *et al*. [31] hydrolysed chicken breast meat using commercial protease resulted in protein hydrolysates which was rich in essential amino acids like histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and non-essential amino acids like alanine, arginine, aspartic acid, cysteine, glutamine, glycine, proline and serine. The protein hydrolysates produced by the hydrolysis of shrimp protein using protease was also found to contain 17 amino acids with higher lysine content [32]. Sasikala [33] deproteinized the silkworm pupal bio-waste using protease producing *Bacillus* sp. PB1 and its protein hydrolysates was found to contain eight amino acids with threonine (1238.8 mg $100mL^{-1}$) as the highest of all the remaining amino acids. Though the amino acid content of the protein hydrolysates were equally rich in non-essential amino acids, the critical roles of these non-essential amino acids should not be under estimated because under certain conditions endogenous synthesis cannot satisfy the immediate requirements of the body system.

DYBM-PH in the present study was found to have higher quantity of amino acids when compared to the whole cell composition of *Candida utilis* which recorded lysine (2g), leucine (2.1g), phenylalanine (0.8g), histidine (0.4g) and arginine (1g) 100 mL^{-1}, respectively [34]. *Kluyveromyces fragilis* biomass reported to have rich in amino acids like threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine and tryptophan using whey as the substrate [35]. Brewer's yeast (*S.cerevisiae*) was found to contain 18 different amino acids of which highest being glutamine (60.4 mg g^{-1}) and deficient in sulphur containing amino acids like methionine and cysteine [36]. The study was in agreement with the present study since DYBM-PH was also found to have devoid of sulphur containing amino acids. There were several studies that recorded the importance of methionine in the diet for the improvement of fish growth. However, there was no methionine content in the amino acid composition of both the hydrolysates. The absence of methionine in the hydrolysates was justified with reports of Teles and Goncalves [6] and Kaushik and Luquet [37] who observed that the sulphur containing amino acids, methionine and cysteine in the fish feed supplementation did not have any positive influence in the growth performance in rainbow trout.

On the other hand, yeast protein concentrate prepared by the alkaline phosphorylation and precipitation of protein at acidic pH that were known to contain high concentration of sulphur containing amino acids [29]. The main problem in the phosphorylation was high alkaline and acidic content of waste materials that make them difficult to discharge into the environment. This was overcome by the use of proteolytic bacterial culture for effective extraction of protein hydrolysates in the present study. With respect to the supplementation of protein hydrolysates with fish feed, it was found that the growth and body weight of the common carp (*Cyprinus carpio*) was increased with increase in supplemented concentration of protein hydrolysates. Of the two concentrations tested, 10% protein hydrolysate feed supplementation performed comparatively well. As discussed earlier, DYBM-PH was rich in both essential and non-essential amino acids, which rendered DYBM-PH as an excellent feed supplement for common carp.

The growth performance of common carp in terms of body weight gain, dry matter, ash and protein content were assessed in 5 and 10% of DYBM-PH feed supplementation. The initial body weight was recorded in all the treatments such that there was no significant difference in the initial weight among the treatments. Difference in weight was observed from the first week onwards. Both the feed supplement concentrations were found to have positively influenced the body weight (Fig.1). A gradual increase was noticed over 10 weeks of experimental duration that showed DYBM-PH performed comparatively well when compared to the control group fed with commercial fish feed alone. Variations in the body weight of the fish were observed from starting of the experiment till tenth week among all the treatments. The results revealed that body weight varied significantly among the treatments at each week. At the end of the tenth week, the feed supplement with 10% protein hydrolysate feed supplementation recorded higher body weight of 9.63 ± 0.22 g which was 68% increase over control. This was followed by 5% protein hydrolysate feed supplementation which registered 44.6% (8.3±1.95 g) increase over control. The untreated control recorded the lowest body weight of 5.74±0.36 g at the end of the tenth week.

The dry matter percentage, ash and protein content of the each treatment were analyzed (Table 3). Both the dry matter and ash content was found to non-significant

Table 3: Effect of protein hydrolysates on muscle composition of common carp

	Growth parameters (%)		
Treatment	Dry matter	Ash	Protein
T_1 – DYBM-PH 5%	74.3 ± 2.6[a]	3.47 ± 3.3[a]	15.8 ± 7.5[a]
T_2 - DYBM-PH 10%	75.3 ± 2.2[a]	3.51 ± 1.4[a]	16.3 ± 2.8[b]
T_3-Control	73.2 ± 5.1[a]	3.52 ± 3.9[a]	15.5 ± 6.2[c]

* Different letter indicates a significant difference (1-way ANOVA) followed by T-test, $p< 0.05$).

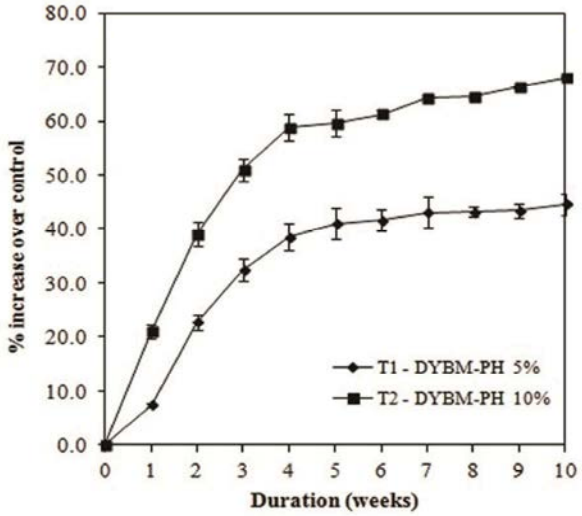

Fig. 1: Effect of DYBM-PH as feed additive at 5% and 10% mixing ratio in common carp fingerlings diet

among the treatments. The highest protein content observed in 10% DYBM-PH which recorded 16.3±2.8% whereas 5% feed supplementation produced slightly lower protein content (15.8±7.5%). The control group fed only with commercial feed recorded less protein content (15.5±6.2%). African sharp tooth cat fish (*Clarias gariepinus*) fed with probiotics such as *Lactobacillus* and *Bifidobacterium* were found to improve the fish health [38]. On the other hand brewer's yeast (*S. cerevisiae*) replacement of fish meal with upto 50% which led to good growth performance and improved feed efficiency of sea bass [6]. Common carp diet added with the mixture of *S. cerevisiae* and *Bacillus subtilis* was found to have improved the hematological and biochemical parameters as the indicator of higher health values [39]. Feeding salmon with yeast cell wall protein resulted in highest level of disease resistance [17]. The effect of size fractionated fish protein hydrolysates on the feed utilization and growth of turbot (Flat fish) was previously reported [40]. The hydrolysates fed upto 3.7% resulted in improved feed utilization and good overall growth of juveniles and the total antioxidant capacity of the fish meat were also increased. Several research works have shown that protein hydrolysates of fish wastes at low level of substitution in fish diets were beneficial [41]. Plant based diets rich in amino acids like lysine, methionine, threonine and glycine were known to improve the protein retention efficiency and muscle ratio in rainbow trout [42]. The presence of lysine, threonine and glycine in the protein hydrolysates of the two substrates might be the reason for the increase in body weight of common carp fish. Nile tilapia fed with 6% shrimp protein hydrolysates, which was rich in essential amino acids, recorded the increased protein content and reduced ash content [43].

CONCLUSION

In summary, the present study has demonstrated that the addition of protein hydrolysates extracted from distillery by-product namely yeast biomass have significant positive effects on the physiological and biochemical parameters of common carp (*C. carpio*). Factors such as level of feed supplementation, amino acid utilization and disease resistance will shed new lights in the use of protein hydrolysates in aquaculture. Apart from this further studies in this area will not only facilitate the effective utilization of waste by-products but also our ability in formulating protein-rich fish feed for sustainable fish farming.

REFERENCES

1. Hardy, R.W. and C.S. Lee, 2010. Aquaculture feed and seafood quality. Bull. Fish. Res. Agen., 31: 43-50.
2. Enami, H.R., 2011. A review of using canola/rape seed meal in aquaculture feeding. J. Fisheries Aquatic Sci., 6: 22-36.
3. Sanderson, G.W. and S.O. Jolly, 1994. The value of phaffia yeast as a feed ingredient for salmonid fish. Aquac., 124: 193-200.
4. Tacon, A.G.J., 1994. Feed ingredients for carnivorous fish species: alternatives to fishmeal and other fishery resources. FAO Fisheries Circular, pp: 881. FAO, Rome.
5. Nayar, S., S. Hegde, P.S. Rao and P. Sudha, 1998. Live organisms as feed in aquaculture. Infofish Int., 4: 36-39.
6. Teles, A.O. and P. Goncalves, 2001. Partial replacement of fishmeal by brewer's yeast (*Saccharomyces cerevisiae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. Aquac., 202: 269-278.
7. Wu, H.C., H.M. Chen and C.Y. Shiau, 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomberaustriasicus*). Food Res. Int., 36: 949-957.

8. Li, X., O. Xu, M. Chen, X. Song, Q. Yong and S. Yu, 2009. Optimization of culture conditions for production of yeast biomass using bamboo waste water by response surface methodology. Biores. Technol., 100: 3613-3617.
9. Cefalu, W.T. and F.B. Hu, 2004. Role of chromium in human health and in diabetes. Diabetes Care, 27: 2741-2751.
10. Ferreira, I.M.P.L.V.O., O. Pinho, E. Vieira and J.G. Tavarela, 2010. Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. Trends Food Sci. Technol., 21: 77-84.
11. Skountzou, P., M. Soupioni, A. Bekatorou, M. Kanellaki, A.A. Koutinas, R. Marchant and M. Banat, 2003. Lead uptake during baker's yeast production by aerobic fermentation of molasses. Proc. Biochem., 38: 1479-1482.
12. Omar, S., S. Daniel, L. Merrifield, H.K. Peter, E.V. Williams and S.J. Davies, 2011. Biofuel derived yeast protein concentrate (YPC) as a novel feed ingredient in carp diets. Aquac., 333: 54-62.
13. Flores, M.L., A. Miguel, O.N. Beatriz, E.G. Mendez and W.L. Madrid, 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquac., 216: 193-201.
14. Rad, M.A., M. Zakeri, V. Yavari and S.M. Mosavi, 2013. Effect of different levels of dietary supplementation of *Saccharomyces cerevisiae* on growth performance, feed utilization and body biochemical composition of Nile tilapia (*Oreochromis niloticus*) fingerlings. World J. Fish. Marine Sci., 5(1): 88-95.
15. Ashmead, H.D., 2001. The absorption and metabolism of iron amino acid chelate. Arch. Latinoam. Nutr., 5: 13-21.
16. Sarker, S.A., S. Satoh and V. Kiron, 2007. Inclusion of citric acid and amino acid chelated trace elements in alternate plant protein source diets affects growth and excretion of nitrogen and phosphorus in red sea bream *Pagrus major*. Aquac, 262: 436-443.
17. Robertsen, B., G. Rorstad, R. Engstad and J. Raa, 1990. Enhancement of non-specific disease resistance in atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. J. Fish Diseases, 13: 391-400.
18. AOAC, 1990. Official methods of analysis of the Association of Official Analytical Chemists. (14th Ed.). Washington, D.C., pp: 1094.
19. Panse, V.G. and P.V. Sukhatme, 1976. Statistical methods for Agricultural workers. Indian Council of Agricultural Research, New Delhi, pp: 180.
20. Charles, H., J. Godfray, J.R. Beddington, I.R. Crute, L. Haddad, D. Lawrence, J.F. Muir, J.P. Retty, S. Robinson, S.M. Thomas and C. Toulmin, 2010. Food Security: The Challenge of Feeding Nine Billion people. Sci., 327: 812-818.
21. Hobbs, P.R., K. Sayre and R. Gupta, 2008. The role of conservation agriculture in sustainable agriculture. Phil. Trans. R. Soc. B. Biol. Sci., 363: 543-555.
22. Balasubramanian, A.V., T.D. Nirmala Devi and M.M. Franco, 2009. (Ed) Use of animal products in traditional agriculture -a pilot project in Southern India, Centre for Indian Knowledge Systems, Chennai.
23. Shweta, R., B. Kumar, L. Singh and V. Deepshikha, 2010. Integrating microbial composting and vermicomposting for effective utilization of by-products of sugarcane processing Industries. Bioremed. J., 14: 158-167.
24. Cumby, N., Y. Zhong, M. Naczk and F. Shahidi, 2008. Antioxidant activity and water-holding capacity of canola protein hydrolysates. Food Chemistry, 109(1): 144-148.
25. Kristinsson, H.G. and B.A. Rasco, 2000. Fish protein hydrolysates: production, biochemical and functional properties. Critical reviews in food science and nutrition, 40(1): 43-81.
26. Kumar, C.G. and H. Takagi, 1999. Microbial alkaline proteases: from a bio industrial viewpoint. Biotechnol. Adv., 17: 561-594.
27. Petreus, T., C.E. Cotrutz, P.D. Sirbu, M. Neamtu, A.M. Filioreanu, L. Badescu and A. Neamtu, 2011. Bioinformatic lookup for metalloproteinases and their natural inhibitors. Structural relationships. Annals of the roman society for cell biology, 16(1): 175-180.
28. Cordoba, G.M.C. and V.C. Sgarbieri, 2000. Nutritional and toxicological evaluation of yeast (*Saccharomyces cerevisiae*) biomass and a yeast protein concentrate. J. Sci. Food Agric., 80: 341-351.
29. Yamada, E.A. and V.C. Sgarbieri, 2005. Yeast (*Saccharomyces cerevisiae*) protein concentrate: preparation, chemical composition and nutritional and functional properties. J. Agrl. Food Chem., 53: 3931-3936.

30. Duarte, L.C., F. Carvalheiro, S. Lopes, I. Neves and F.M. Girio, 2008. Yeast biomass production in brewery's spent grains hemicellulosich ydrolysates. Appl. Biochem. Biotechnol., 148: 119-129.
31. Kurozawa, L.E., K.J. Park and M.D. Hubinger, 2008. Optimization of the enzymatic hydrolysis of chicken meat using response surface methodology. J. Food Sci., 73: 405-412.
32. Dey, S.S. and K.C. Dora, 2011. Optimization of the production of shrimp waste protein hydrolysate using microbial proteases adopting response surface methodology. J. Food Sci. Technol., 10: 1-9.
33. Sasikala, G., 2010. Microbial studies on optimization and scale up of value added products from silkworm pupae biowaste. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.
34. Zheng, S., M. Yang and Z. Yang, 2005. Biomass production of yeast isolate from salad oil manufacturing wastewater. Biores. Technol., 96: 1183-1187.
35. Paul, D., R. Mukhopadhyay, B.P. Chatterjee and A.K. Guha, 2002. Nutritional profile of food yeast *Kluyveromyces fragilis* biomass grown on whey. Appl. Biochem. Biotechnol., 97: 209-218.
36. Suhajda, A., J. Hegoczki, B. Janzso, I. Pais and G. Vereczkey, 2000. Biotransformation of vegetable and fruit processing wastes into yeast biomass enriched with selenium. Biores. Technol., 96: 747-751.
37. Kaushik, S.J. and P. Luquet, 1980. Influence of bacterial protein incorporation and of sulphur amino acid supplementation to such diets on growth of rainbow trout, *Salmo gairdnerii* Richardson. Aquac., 19: 163-175.
38. Ayoola, S.O., E.K. Ajani and O.F. Fashae, 2013. Effect of probiotics (*Lactobacillus* and *Bifidobacterium*) on growth performance and hematological profile of *Clarias gariepinus* juveniles. World Journal of Fish and Marine Sciences, 5(1): 01-08.
39. Ghodratizadeh, S., S. Ghodratizadeh, M. Farhoudi and R. Habibian, 2011. Effects of addition of *Saccharomyces cerevisiae* and *Bacillus subtilis* in diet on hematological and biochemical parameters in Common carp (*Cyprinus carpio*). World J. Fish. Marine Sci., 3(1): 96-99.
40. Zheng, K., M. Liang, H. Yao, J. Wang and Q. Chang, 2012. Effect of size-fractionated fish protein hydrolysate on growth and feed utilization of turbot (*Scophthalmus maximus* L.). Research. Aquac., 261(1): 305-317.
41. Hevroy, E.M., M. Espe, R. Waagbo, K. Sandnes, M. Ruud and G.I. Hemre, 2005. Nutrient utilization in Atlantic salmon (*Salmo salar* L.) fed increased levels of fish protein hydrolysate during a period of fast growth. Aquac. Nutr., 11: 301-313.
42. Gaylord, T.G. and F.T. Barrows, 2009. Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. Aquacul., 287: 180-184.
43. Leal, A.L.G., P.F. De Castro, J.P.V. Lima, E.S. Correia and R.S. Bezerra, 2010. Use of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus* L.) feeds. Aquac. Int., 18: 635-646.