International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 21-10-2024, Accepted: 20-11-2024, Published: 05-12-2024
Volume 9, Issue 12, 2024, Page No. 89-94

Synergistic effects of yeast and mixed probiotics on protein content and amino acids in *Labeo Rohita*

J Babitha
Department of Zoology, Annai Vailankanni Arts and Science College, Thanjavur, Tamil Nadu, India

Abstract
In *Labeo rohita*, the effects of dietary supplementation with commercial yeast and possible probiotics were assessed. Basal diet (BD) as Group I and 6 experimental diets containing GII- BD with *Saccharomyces cerevisiae* yeast 5mg/kg, G III- BD with mixture of *Saccharomyces sporogene*, *S. acidophilus* and *S. lactis* 5mg/kg, G IV- BD with mixture of *Bacillus subtilis*, *B. licheniformis* and *B. coagulans* 5mg/kg, G V-BD with mixture of *Lactobacillus sporogenes*, *L. acidophilus*, *L. lactis* and *Saccharomyces cerevisiae* yeast 5mg/kg G VI- BD with mixture of *B. subtilis*, *B. licheniformis*, *B. coagulans*, and *S. cerevisiae* yeast 5mg/kg and G VII- BD with mixture of *L. sporogenes*, *L. acidophilus*, *L. lactis*, *B. subtilis*, *B. licheniformis*, *B. coagulans*, *S. cerevisiae* yeast(1x109 cfugl) were fed 5mg/kg, respectively. The results showed that the 30 and 60 day treatment groups had significantly higher total protein content and both essential and non-essential free amino acids compared to the control group. However, fish evolution may have increased the amount of these combined probiotics and yeast. These results suggest that the total protein content and amino acid content in *L. rohita* muscle could be successfully increased by probiotics and yeast supplementation in the diet.

Keywords: *Labeo rohita*, probiotics, yest, protein

Introduction

One of the most promising and fastest growing industries is aquaculture, which produces high quality animal protein, increases nutritional value and creates jobs and income worldwide (FAO, 2010) [8]. Three giant carp (*L. rohita*, *C. catla* and *C. mrigala*) account for 70% of India's aquaculture productivity (Das *et al*., 2004) [5]. *Labeo rohita* is an important freshwater fish species and is widely cultivated throughout Asia, particularly in the Indian subcontinent. Approximately 1 million tons of this important fish are imported from India and are typically farmed in a semi-intensive polyculture system in Pakistan (FGIS, 2007) [9] are viable cell preparations that improve a host's nutritional value, enhance its enzymatic contribution to digestion, suppress microbes, have antimutagenic and anticarcinogenic properties, promote growth and enhance immunity. Dietary supplements in aquaculture and play a role in nutrition, health status and resistance to infectious agents (Gatesoupe *et al*., 1999) [11]. The current trend in aquaculture, also for ecological reasons, is to replace antibiotics with probiotics. Fuller (1992) [10] defined probiotics as live microbial nutritional supplements that have a positive effect on the host by improving the microbial balance in the intestine.

The probiotic bacterium *B. subtilis*, isolated from the gastrointestinal tract of *C. mrigala*, was added to a probiotic-treated diet containing all components of the control diet at a concentration of 108 CFU/g died required for intestinal colonization. (Nayak, 2005) [18]. The influence of the probiotic bacterium *B. subtilis* and vitamin C in the form of ascorbyl polyphosphate on the immune response and disease resistance of Indian large carp was reported by Nayak *et al*. (2007) [19] examined. The bacteria *B. circulans*, *B. subtilis* and *B. pamilus* isolated from the stomach of the Rohu include cellulose, amylase and extracellular protease and are crucial for the nutrition of the young Rohu (Ghosh, 2002) [12]. According to Kurtzman (1998) [15], the commercially available yeast Saccharomyces cerevisiae is a single-celled eukaryotic microbe that is often used in animal nutrition as an excellent source of proteins and vitamins, with B complex vitamins in particular being involved in metabolism.

The most expensive food ingredient is often protein. Consequently, feed manufacturers and aquaculture producers can benefit from the use of low-cost alternative protein sources that can promote improved fish development (Davis and Stickney, 1978) [6]. However, due to rising prices and increasing demand for feed proteins from traditional sources, fish farmers in poor countries are forced to use fish as a component of fish feed (Sithara and Kamalaveni, 2008) [21]. Dietary protein becomes part of the amino acid pool and combines the amino acids that are formed during the progressive breakdown of structural and functional proteins from the tissue. Combined with precolumn derivatization of amino acids, HPLC technology has become a crucial method for amino acid analysis. O-phthaldialdehyde (OPA), one of the most commonly used derivatization reagents, guarantees rapid reaction in aqueous solution at ambient temperature and relatively easy derivatization (Hanczko, 2007).

The ability of the fish to use the proteins in its diet to increase its protein content in the body is expressed by protein. The amino acid pool is created when the amino acids from the hydrolysis of the ingested protein mix with those from the regular breakdown of structural and functional proteins from the tissue. According to Anil Kumar (2014) [4], cells supplement amino acids from the common amino acid pool when necessary to integrate them into the cell structure. This study examined the effects of several experimental diets and also used HPLC to determine the total protein content and amino acid composition of *L. rohita*.

Materials and Methods

Bacterial Strains

HiMedia provided the possible probiotic bacterial culture (*L. sporogene, L. acidophilus,* and *L. lactis*) along with the yeasts *S. cerevisiae, B. subtilis, B. licheniformis,* and *B. coagulans*. Bacillus species served as probiotic II, while Lactobacillus species served as probiotic I. These bacterial cultures were maintained and propagated in brain-heart infusion broth at 37°C for an additional 24 h. Cell density was determined using OD (600) values by serial dilution and plating on tryptone soy agar (TSA) and correlated with the number of colony forming units (CFU). When necessary, the measured bacteria were used to prepare feed after maintaining them in suspension at 4 °C.

Diet Preparation

A basal diet was prepared containing 34% peanut oil cake, 40% rice bran, 19% soybean meal, 4% fish meal, and 3% mineral and vitamin mixture. Additionally, a final dose of 5 g/kg of the yeast Saccharomyces cerevisiae and the single and mixed probiotics I and II was added. The bacterial suspension was gradually added to the dough and mixed in a drum mixer to obtain accurate final concentrations of the food. The experimental feedstuffs were allowed to air dry in a drying chamber equipped with an air blower at 38°C until their moisture content was approximately 10%. The feed was allowed to air dry before being pulverized and sieved into appropriately sized pellets and stored at −20°C until use.

Experimental Animals

A fish farm in Thittai village, Thanjavur district, Tamil Nadu, India supplied Rohu fish snares with an average weight of 14 g. Two-thirds of the water energy was exchanged each day while twenty Rohu fry were maintained in plastic tanks with continuous aeration. There were fifteen fish in each of the 200 litter tanks. Equal portions were served at 10 a.m. and 8 p.m. for 30 and 60 days, with a daily food intake of 3% of body weight. The amount of feed consumed was determined by collecting, drying and weighing additional feed daily (Sun *et al*., 2011) [22]. Every ten days, after a 24-hour fast, the daily diet was adjusted by batch weighing. Fresh water was added to each tank daily throughout the experimental period to replace 25% of the water.

Muscle sample preparation for amino acid analysis

Fifteen fish muscles from each experimental treatment were combined to create muscle samples. Then 20 millilitres of 6% TCA was added. After 3 min of homogenization and sonication, they were centrifuged for 15 min at 10,300 rpm and 10°C. After removal of the supernatant, the residue was extracted twice with TCA as mentioned previously. After collecting all the supernatant, adding 30 mL of ether, and shaking for 30 s, the aqueous layer was concentrated to a fairly high viscosity liquid at 400°C in a low-pressure rotary evaporator. For amino acid analysis, the liquid was then diluted with deionized double-distilled water (Dalla via, 1986) [7].

Amino acid analysis through HPLC

To carry out the amino acid analysis, the procedure described in the manufacturer's instruction manual was followed according to the methodology of Dalla *et al.* (1986) [7] used. An equivalent volume of sample dilution buffer 10 mM, adjusted to pH 6.4, was added to 100 microliters of sample. A high-performance liquid chromatography system was then filled with half of the mixture. Four phases of a lithium citrate buffer system were used to elute the HPLC column at a temperature range of 34 - 72°C, a flow rate of 1 mL, and a wavelength of 254 nm. Finally, ninhydrin was used as a post column reactant at a flow rate of 10 mL/h.

Result and Discussion

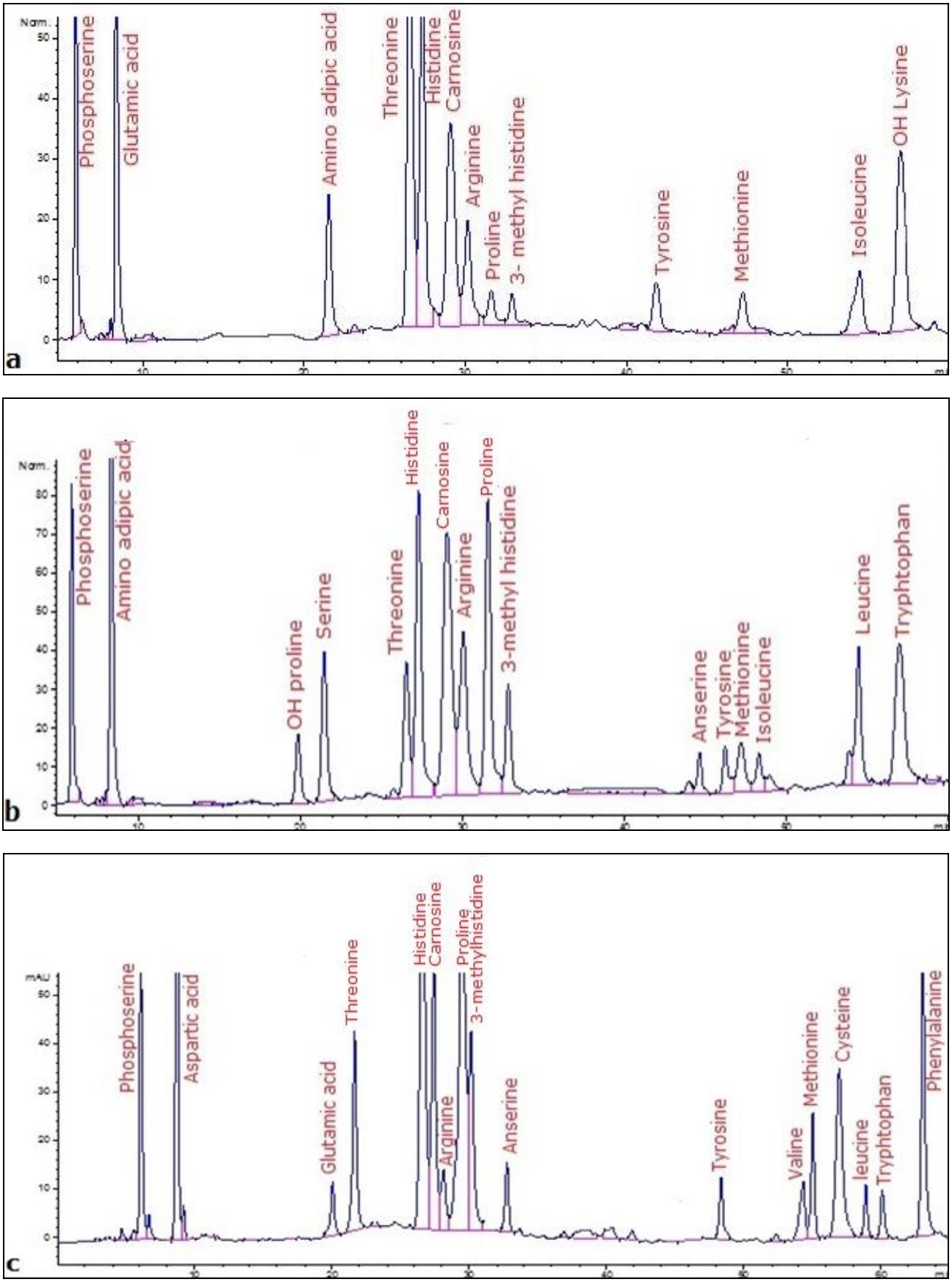
The biochemical composition of the selected feeds was identified and displayed. To provide both value-added meals in 30 and 60 days with comparable nutrient contents, these probiotics were prepared according to the crude protein requirements of *L. rohita*. Compared to standard feed, which has a crude protein content of 3.5 mg/100 mg, according to an analysis of the immediate composition of the probiotics, the highest crude protein concentration (45.63 and 45.93%) was found after 30 and 60 days of treatment (Nalawade, 2014) [17]. The protein content of the control group hardly fluctuated over time. The graph showed the actual value. In the mixed diet G-VII, raw body meat had the maximum crude protein storage (45.93 ± 2.05). The results are consistent with those of Asad (2005) [3], who found that beef meal had the highest digestibility. However, occasionally these results do not agree with those of other studies due to possible problems in feed preparation or variations in feeding method (Noreen, 2008) [20]. According to another study, animals are high in protein. The high mineral content of animal protein sources could be the reason for this (Abid, 2009) [1].

Amino acid levels in *L. rohita* muscle treated with various probiotics are summarized in Figure 1. The figure shows the total amount of free amino acids. Essential and nonessential amino acids included isoleucine, leucine, lysine, tryptophan, methionine, phenylalanine, threonine, valine, arginine, tyrosine, and histidine. Other amino acids were phosphoserine, phosphoenolamine, aminoadipic acid and (-aminobutyric acid). In the successful therapy of G-VII, anserine, leucine (3.77 µm/ml), threonine (4.83 µm/ml), and arginine (2.84 µm/ml) were relatively larger than other components. Over 27% of the FAA pool consisted of glycine. Arginine and valine levels increased rapidly in response to an increase in threonine and leucine concentrations, while levels of OH-lysine (0.69 µm/ml), tryptophan (0.61 µm/ml), and phenylalanine (0.40 µm/ml) increased. In the brain, tyrosine (0.52 µm/ml) and methionine (0.31 µm/ml), precursors of the neurotransmitter serotonin, which have a significant influence on how animals eat (Mullen and Mortin, 1992) [16], were reduced. Compared to the control, there was an increase in the levels of the non-essential amino acids arginine (2.38 µm/ml) and histidine (2 µm/ml).

These crucial components for controlling osmotic pressure are followed by smaller amounts of isoleucine, leucine, tryptophan, OH-lysine, threonine, phenylalanine and tyrosine. The standard table and the measured peak amino acid values were compared and examined. These growth therapy groups have been successful. Valine is believed to be necessary for protein synthesis and healthy development and is involved in several metabolic processes (Wilson, 2002) [24].

In addition to being a necessary amino acid, histidine is involved in other metabolic processes, such as the synthesis

of histamines, which contribute to inflammatory and allergic reactions. Not only is it associated with energy production or utilized in other metabolic pathways under certain harsh conditions and crises, but it is also crucial for maintaining the osmoregulation process (Abe and Ohmama, 1987) [2]. This implies that adult fish have different physiologies and sex differences. The total amount of these amino acids indicates that the fish grew well in the wild (Fig. 2).



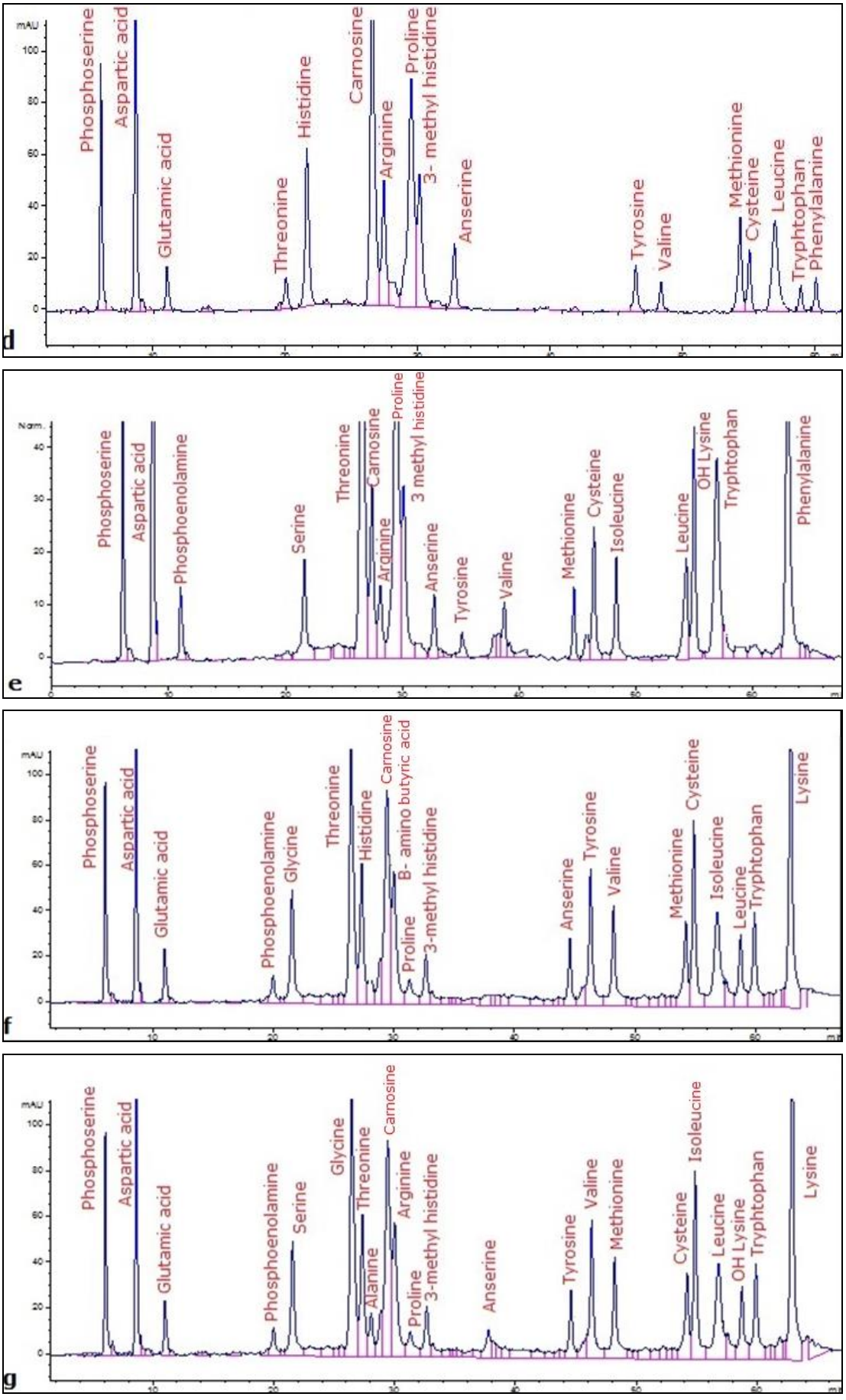
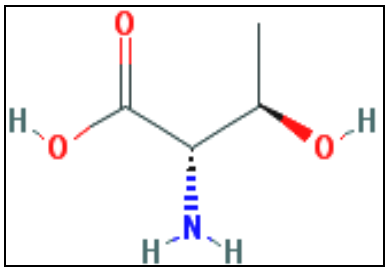
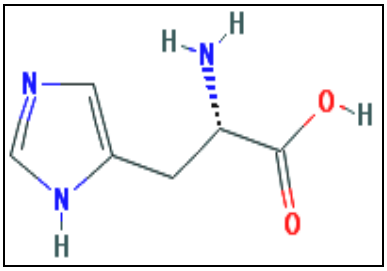
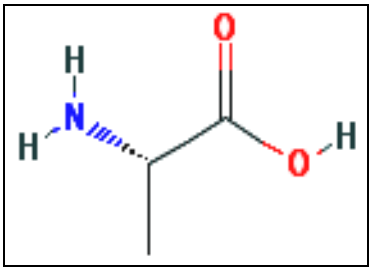
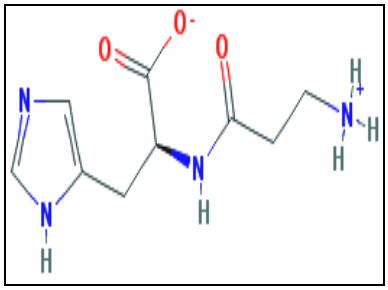


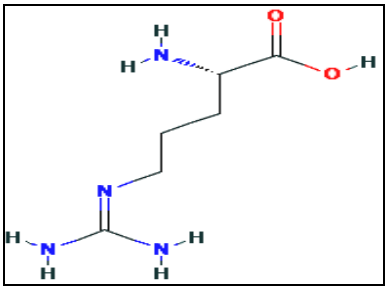
Fig 1: Amino acid analysis by HPLC: a) G-I (Basel diet); b) G-II (*S. cerevisiae* yeast); c) G-III (*L. sporogene* + *L. acidophilus* + *L. lactis*; d) G-IV (*B. subtilis* + *B. licheniformis* + *B. coagulans*); e) G-V (*L. sporogene* + *L. acidophilus* + *L. lactis* +*S. cerevisiae* yeast); f) G-VI (*B. subtilis* + *B. licheniformis* + *B. coagulans* +*S. cerevisiae* yeast); e) G-VII (*L. sporogene* + *L. acidophilus* + *L. lactis* +*B. subtilis* + *B. licheniformis* + *B. coagulans* + *S. cerevisiae* yeast.

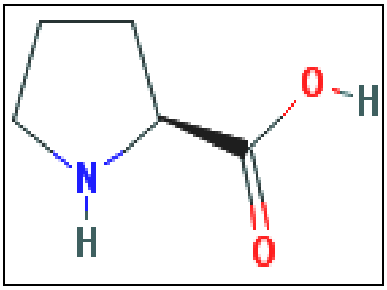

Threonine

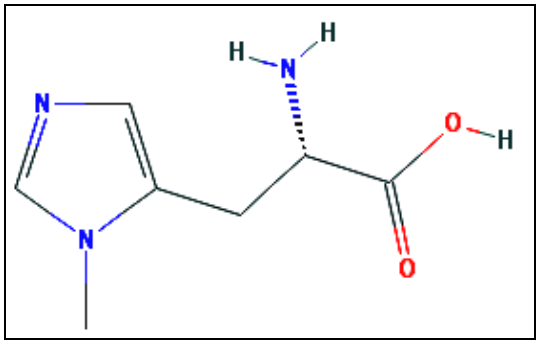

Histidine

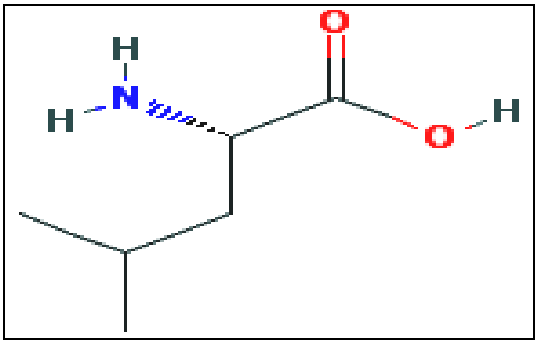

Alanine


Carnosine


Arginine


Proline


3-methylhistidine


Leucine

Conclusion

The current study showed that *L. rohita* benefited from the supplementation of the yeasts *L. sporogene*, *L. acidophilus*, *L. lactis*, *B. subtilis*, *B. licheniformis*, *B. coagulans* and *S. cerevisiae*. This type of probiotic is intended to support the building of proteins. To support this approach and expand knowledge on the role of probiotics in identifying a different health management approach for the growth of aquaculture production, further research in this area is required. When fish are given the right nutrition, they may respond positively and encouragingly. Based on the evidence we collected, we concluded that the developed diet improved fish development and nutritional content.

References

1. Abid M, Ahmed MS. Growth responses of *Labeo rohita* fingerlings fed with different feeding regimes under intensive rearing. *The Journal of Animal & Plant Sciences*,2009:19(1):45–49. ISSN:1018-7081.
2. Abe H, Ohmama S. Effect of starvation, and seawater acclimation on the concentration and free *L-histidine* and related dipeptides in the muscle of eel, rainbow trout, and Japanese dace. Comparative Biochemistry and Physiology,*1*987:88B:507–511.
3. Asad F, Salim M, Shahzad K, Noreen U. Estimation of apparent digestibility coefficient of guar, canola, and meat meal for *Labeo rohita*. International Journal of Agriculture and Biology,*2*005:7:816–819.
4. Anil Kumar P. Biochemical effects on protein and free amino acid metabolism in *Catla catla* and *Labeo rohita* due to *Pallisentis nagpurensis* infection. American International Journal of Research in Formal, Applied and Natural Sciences,2014:6(1):82–85.
5. Das PC, Ayyappan S, Das BK, Jena JK. Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*. Comparative Biochemistry and Physiology, Part C,2004:138:3–10.
6. Davis AT, Stickney RR. Growth response of *Tilapia aurea* to directly protein quality and quantity. *Fish Soc Trans,1*978:107:479–483.
7. Dalla Via GJ. Salinity responses of the juvenile penaeid shrimp *Penaeus japonicus*. II. Free amino acids. Aquaculture,1986:55:307–316.
8. FAO. The state of world fisheries and aquaculture. Rome, 2010. ISBN: 1:106675, 978-25-25.
9. FGIS. Fisheries global information system. Rome: FOA, 2007.
10. Fuller R. Probiotics: The Scientific Basis. London: Chapman and Hall, 1992.
11. Gatesoupe FJ. The use of probiotics in aquaculture. *Aquaculture,1*999:180:147–165.
12. Ghosh K, Sen SK, Ray AK. Characterization of *Bacillus* isolated from the gut of rohu, *Labeo rohita*, fingerlings and significance in digestion. *Journal of Applied Aquaculture,2*002:12:33–42.

13. Hanczkó R, Jámbor A, Perl A, Molnár-Perl I. Advances in the *o-phthalaldehyde* derivatizations: Comeback to the *o-phthalaldehyde-ethanethiol* reagent. *J Chromatogr A,*2007:1163(1-2):25–42.
14. Khan MA, Jafri AK, Chadha NK. Growth and body composition of rohu, *Labeo rohita* (Hamilton), fed compound diet: Winter feeding and rearing to marketable size. *J Appl Ichthyol,*2004:20:265–270.
15. Kurtzman CP, Fell JW. Yeast systematics and phylogeny—implications of molecular identification methods for studies in ecology. In: Rosa CA, Peter G, editors. The Yeast Handbook. Springer-Verlag Berlin Heidelberg, 1998, 11–30.
16. Mullen BJ, Martin RJ. The effect of dietary fat on diet selection may involve central serotonin. American Journal of Physiology Relation in Integration Comparative Physiology,1992:263:R559–566.
17. Nalawade VB, Bhilave MP, Kulkarni JJ. Protein conversion efficiency in muscle of freshwater fish *Labeo rohita* fed on formulated feed. Biological Forum – An International Journal,2014:6(1):79–83.
18. Nayak SK. Role of gastrointestinal flora of Indian major carps against bacterial infections. [Ph.D. Thesis]. Utkal University, Bhubaneswar, Orissa, India, 2005.
19. Nayak SK, Swain P, Mukherjee SC. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Labeo rohita* (Ham.). Fish and Shellfish Immunology,2007:23:892–896.
20. Noreen U, Salim M. Determination of nutrient digestibility and amino acid availability of various feed ingredients for *Labeo* rohita. International Journal of Agriculture and Biology,*2*008:10:551–555.
21. Sithara K, Kamaladevi. Formulation of low-cost feed using *Azolla* as a protein source. Aquaculture,2008.
22. Sun YZ, Yang HL, Ma RL, Zhang CX, Lin WY. Effect of dietary administration of *Psychrobacter* sp. on the growth, feed utilization, digestive enzymes, and immune responses of grouper *Epinephelus coioides*. Aquacult Nutr,2011:17:733–740.
23. Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Review,2000:64:655–671.
24. Wilson RP. Amino acids and protein. In: Halver JE, Hardy RW, editors. Fish Nutrition. San Diego: Academic Press, 2002, 143–179.