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Performance of Brewer's Yeast as a Sustainable Fishmeal Alternative in *Labeo rohita* Nutrition: Effects on Growth, Hematological, Enzyme Activities and Biochemical Indices

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ABSTRACT

Members of the carp family are among the most significant inland aquaculture species worldwide. Rohu and grass carp hold considerable importance in regions of Asia. Nutritional composition of fishes depends upon nutritional values of meal provided to them. For this purpose, aquafeeds industries are trying to prepare complete meal for favorable nutrition. Five experimental diets names as D1, D2, D3, D4 and D5 were prepared in laboratory by replacing 15, 30, 45, 60 and 100% fishmeal by brewer's yeast respectively. Rohu fingerlings were fed with controlled diet for acclimatization for 15 days before starting trials. After acclimatization 30 fingerlings were randomly distributed in each tank. Data was recorded for various growth, hematological, biochemical and digestive enzymes activity attributes. D2 and D3 provided significant results for all growth attributes. D2 caused 27, 34, 14 and 6% increase in final weight, weight gain, specific growth rate (SGR) and survival as compared to fingerlings fed with controlled diet. Red blood cells (RBC) of fishes fed with D2 and D3 were 36% higher than fishes fed with controlled diet. While white blood cells (WBC) highest value was observed in rohu fed with D5. Hemoglobin content was also found highest in fishes fed with D2, which were 16.3% higher than fishes fed with controlled diet. Unlike growth and hematological attributes, biochemical attributes such as glucose, cortisol, alanine amino transferase (ALT) and alkaline phosphatase (ALKP) were significantly reduced in fishes fed with D2 and D3 experimental diet. D4 and D5 caused significant increase in the biochemical attributes. Highest activity of protease and lipase was also observed under D2 diet. From these results, it can be derived that fishmeal 30-45% replacement with brewer's yeast can become a good sustainable alternative diet for *Labeo rohita* culture.

Keywords: Aquaculture, sustainable fishmeal, *Labeo rohita*, Brewer's yeast meal

1. Introduction

In recent decades, there has been a significant and rapid increase in aquaculture activities worldwide. While fish production has consistently grown, the fish processing industry and the domestic fish market have also generated a substantial number of by products (Venmarath *et al.,* 2024). Members of the carp family are among the most significant inland aquaculture species worldwide. Rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*) hold considerable importance in regions like Asia and Africa. Meanwhile, common carp (*Cyprinus carpio*) is the predominant species in Central Europe. Rohu, global production estimated at 1.67 million tons

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annually which is extensively farmed in South Asia (FAO, 2017). Due to its strong market demand, suitability for polyculture, and high consumer acceptance, rohu species is commercially prominent in tropical freshwater regions (Ashaf-Ud-Doulah *et al.,* 2021).

These species of carp are commercially more valuable as farmed fishes, that's why breeders are trying various strategies to make its breeding more efficient. Nutritional composition of fishes depends upon nutritional values of meal provided to them. For this purpose, aquafeeds industries are trying to prepare complete meal for favorable nutrition (Henry *et al.,* 2015). However, there is a huge demand of aqua meals, but the availability of resources is limited which make aquafeeds very expensive. Nowadays used aqua meal is not sustainable for environment. This unsustainability and huge demand of fishmeal, forced to look for sustainable alternative fishmeal protein sources (Hertrampf *et al.,* 2003). Shortage of fishmeal supply cause huge increase in its prices, which adversely affects farmers to maintain sustainable development of their aquacultures. In aquatic animal nutrition research, it became hot topic to replace fishmeal with sustainable alternatives (Weiwei *et al.,* 2016). Development of cost-effective meal for aquaculture is getting a lot of attention of researchers. Partial or complete replacement of aqua meal by alternative sustainable protein sources of animal or plant origin are tested by many researchers (Kamei *et al.,* 2018). To reduce the harmful effects of bioactive compounds and to optimize nutritional composition of plant-based fish meal, various techniques and methods are described in previous studies (Gatlin *et al.,* 2007). Farmers are trying to reduce the use of industrial additives or to replace them with natural additives in aquaculture to reduce the chemical or pharmaceutical residues in food chain, which may result in reduction of environmental pollution.

The Brewers yeast could be a valuable addition to animal and aquafeed because of its beneficial immune stimulants like probiotics, manganese, beta-glucan, nucleic acid and high protein content. for animals and aquaculture, brewer's yeast has the potential to serve as a nutritious feed ingredient (Mussatto, 2014; Conway, 2019). Brewer's yeast (BSY) is promptly available at minimal or no cost whole year as a potential raw material for various applications. Research has shown that in several studies fish species, performed better partially based on brewer's yeast diet as compared to commercially available fishmeal (Levic *et al.,* 2010). Additionally, as these raw materials are being manufactured at an industrial level, they are quite affordable and easily available. Brewer's yeast (*Saccharomyces cerevisiae*) is one of the most favored probiotics, which is rich in dietary protein and could potentially replace the primary protein source in animal feeds (Olvera-Novoa *et al.*, 2002). Yeasts are known for being a valuable source of B vitamins and proteins, making them a prominent option as an additive for animal feed to increase amino acid levels (Egel-Mitani *et al.*, 2000). Additionally, cell wall of brewer's yeast is rich in various bioactive compounds, such as ß-glucans, that bolster the immune system's defenses against viral and bacterial infections. This helps in combating illnesses effectively and enhances overall resistance of fishes (Chang *et al.*, 2003; Lin *et al.*, 2011).

In dairy cattle farming, Brewer's yeast has been used to increase the growth and performance of cattles on a large scale (Rossow *et al.*, 2018). It has also been used to enhance poultry production (Dixon *et al.*, 2022), and pigs fattening (van Heugten *et al.*, 2003). Brewer's yeast has also been used in aquaculture of Nile tilapia (Trosvik *et al.*, 2012). But it's use for the fishes of carp family especially rohu aquaculture is limited. Therefore, the aim of this experiment was to examine the fishmeal replacement with brewer's yeast and to analyze the effects of this experimental diet on growth, haematological, biochemical and digestive enzymes activities of *Labeo rohita*.

2. Materials and Methods

2.1. Experimental fish and design

An experiment was performed in 2023 to analyze the effects of brewer's yeast on the performance of Rohu fishes. Fingerlings of *Labeo rohita* were obtained from government fish seed hatchery. Duration of this experiment were 90 days. These fingerlings were fed with controlled diet for acclimatization for 15 days before starting trials. After acclimatization 30 fingerlings were randomly distributed in each tank. Water holding capacity of tank were 250 liters. Temperature of the tank was maintained at 27°C with the help of electric heaters. Dissolved oxygen (DO) and pH were also monitored regularly. 30% water and faecal matter were removed daily. Fishes were fed two times every day.

2.2. Brewer's spent yeast (BSY)

Breweries industry, yeast slurry was purchased Murree Brewery and were brought to laboratory in plastic containers. This slurry was dried in oven at 50°C for uniform weight. Then this slurry was centrifuged for 5 minutes at 5000 rpm, supernatant was deserted. After washing with distilled water, solid residue was immediately filtered by using a filter paper. Then this solid residue was oven dried for 16 hours at 50°C. This oven dried solid residue is known as BSY which will be used in feed preparations. Oven drying of yeast cause autolysis of yeast cells, which leads to release of α-amino nitrogen (Tan- guler and Erten, 2008).

2.3. Experimental Diet

Five experimental diets names as D1, D2, D3, D4 and D5 were prepared in laboratory by replacing 15, 30, 45, 60 and 100% fishmeal by brewer's yeast (BSY) respectively. While D0 was used as a controlled diet which had 0% replacement of fishmeal by BSY. In the preparation of experimental diets, BSY was the chief component. Nutrient composition of these experimental diets were analyzed at fish nutrition laboratory following standard methods (Samantaray and Mohanty 1997; AOAC, 2005) as shown in Table1. In an electric grinder, required quantities of ingredients were blended to form a dough. Then small pellets were prepared from dough and were stored in containers at room temperature.

Table 1: Ingredients composition in controlled and experimental diets

Ingredients	D0	D1	D2	D3	D4	D5
Wheat flour	28.140	28.140	28.140	28.140	28.140	28.140
Rice bran	33.140	33.140	33.140	33.140	33.140	33.140
Groundnut oil	15.86	15.86	15.86	15.86	15.86	15.86
Fishmeal	15.86	13.481	11.102	08.723	06.324	00.00
Brewer's yeast	00.00	02.379	04.758	07.137	09.516	15.86
Vegetable oil	02.00	02.00	02.00	02.00	02.00	02.00
Fish oil	02.00	02.00	02.00	02.00	02.00	02.00
CMC	01.00	01.00	01.00	01.00	01.00	01.00
Vitamins and Minerals	02.00	02.00	02.00	02.00	02.00	02.00

2.4. Growth Attributes and Feed Utilization

Growth parameters like initial weight, final weight and weight gain were measured in laboratory with the help of weight balance. While other growth attributes like specific growth rate (SGR), feed conversion ratio (FCR) and survival rate were calculated by the help of following formulas:

SGR % d-1 = (log Final weight – log Initial weight) × 100 / Experimental period
FCR = Quantity of feed consumed / Weight gain
Survival = Final count of fish × 100 / Initial count of fish

2.5. Blood and Serum Sample Collection

For blood samples collection, fishes were anaesthetized and from caudal peduncle blood was collected with the help of syringe rinsed in EDTA anti-coagulant. Then blood was stored in vials having 0.5 mg anti-coagulant. For serum collection, blood was centrifuged for 20 minutes at 10000 rpm. When serum was separated, it was stored at − 20 °C for further analysis.

2.6. Haematological parameters

Blood was diluted in Hay-men's fluid and Turk's fluid to count total number of red blood cells (RBC's) and white blood cells (WBC's) respectively (Wintrobe, 1967; Shah and Altindağ, 2005).

Cyanmethemoglobin method was used to estimate hemoglobin (Kapoor *et al.,* 2002). While PCV (packed cell volume) or hematocrit was measured by the centrifugation of hematocrit capillary tube (Jain, 1986).

2.7. Serum Biochemical Analysis

With the help of automated blood biochemistry analyzer, ALKP (alkaline phosphatase), ALT (alanine amino transferase), albumin and total protein content were estimated. With the help of formula: total protein – albumin, globulin level was measured. While commercially available ELISA kit was used to estimate cortisol and glucose content in serum.

2.8. Sample Collection for Digestive Enzyme Analysis

After 24 hours starvation, fishes were anaesthetized with tricaine methane sulfonate. Then whole digestive tract was removed after dissection and was washed with cold Tris-HCl buffer. Then in Tris-HCL buffer using a homogenizer, these samples were homogenized with glass beads and centrifuged for 15 minutes at 5000 rpm at 4°C. With the help of amylase kit, amylase activity was measured. Ability to hydrolyze 10 mg substrate in 30 minutes at 37°C is known as one activity unit of amylase. With the help of lipase kit, lipase activity was measured. Ability to hydrolyze 1µmol in 30 minutes at 37°C is known as one activity unit of lipase. While nonspecific assay was to measure total protease (Walter, 1984).

2.9. Statistical Analysis

This experiment was performed according to CRD by using three replicates of each diet. Data was recorded for growth, hematological, hemato-biochemical and digestive enzymes activities parameters. Recorded data was analyzed by ANOVA with the help of Statistix 8.1 software. Then Tukey's HSD test was applied to analyze the multiple comparisons of means of all diets.

3- Results

3.1. Growth Attributes

In all tanks water quality was unformally maintained to minimize the effects of water quality on diet performance. Growth parameters of fingerlings on various levels of BSY diet are represented in Table-2. Analysis of Table-2 revealed that D2 significantly effected on final weight of fingerlings and provided highest final weight (13.37g) followed by D3 (12.21g). D2 also showed the highest weight gain percentage, at 561.88%.

Except D2 and D3, controlled diet performed better than D1, D4 and D5. Specific growth rate was also affected by BSY experimental diets. Table-2 shows that D2 significantly effected on SGR of fingerlings and provided highest SGR (0.91%) followed by D3 (0.87%). Feed conversion ratio (FCR) was lowest at D2 (1.632) followed by D3, D4, D1 and D5. FCR of controlled was found highest (1.916). Survival rate was also found highest (96.8%) under D2 diet. From the results of growth attributes, it can be derived that fishmeal 30-45% replacement with BSY can become a good sustainable alternative diet for Labeo rohita culture.

Table 2: Mean values ± SE of growth attributes of *Labeo rohita* fed with various BSY experimental diets

Diets	Initial weight	Final weight	Weight Gain (%)	SGR	FCR	Survival
D0	2.02 ± 0.03	10.52 ± 0.08	420.80 ± 4.11	0.8 ± 0.03	1.916 ± 0.03	91.7 ± 3.02
D1	2.02 ± 0.03	10.09 ± 0.09	399.50 ± 5.27	0.78 ± 0.03	1.897 ± 0.02	94.5 ± 2.77
D2	2.02 ± 0.03	13.37 ± 0.1	561.88 ± 7.14	0.91 ± 0.05	1.632 ± 0.02	96.8 ± 2.91
D3	2.02 ± 0.03	12.21 ± 0.07	504.45 ± 8.27	0.87 ± 0.04	1.757 ± 0.02	95.4 ± 1.96
D4	2.02 ± 0.03	9.77 ± 0.06	383.66 ± 4.21	0.76 ± 0.02	1.859 ± 0.04	93.1 ± 2.39
D5	2.02 ± 0.03	9.68 ± 0.09	379.21 ± 4.97	0.77 ± 0.02	1.898 ± 0.03	92.6 ± 2.09

Figure1 shows the percentage effect of various BSY diets on growth attributes of rohu. All five BSY experimental diets performed better than commercially available controlled diet, but D2 and D3 performed more prominently as compared to all diets. D2 caused 27, 34, 14 and 6% increase in final weight, weight gain, SGR and survival as compared to fingerlings fed with controlled diet. FCR of D2 was lowest, which is also benificial. Performance of D2 was followed by D3, which also caused significant percentage increase in all growth attributes.

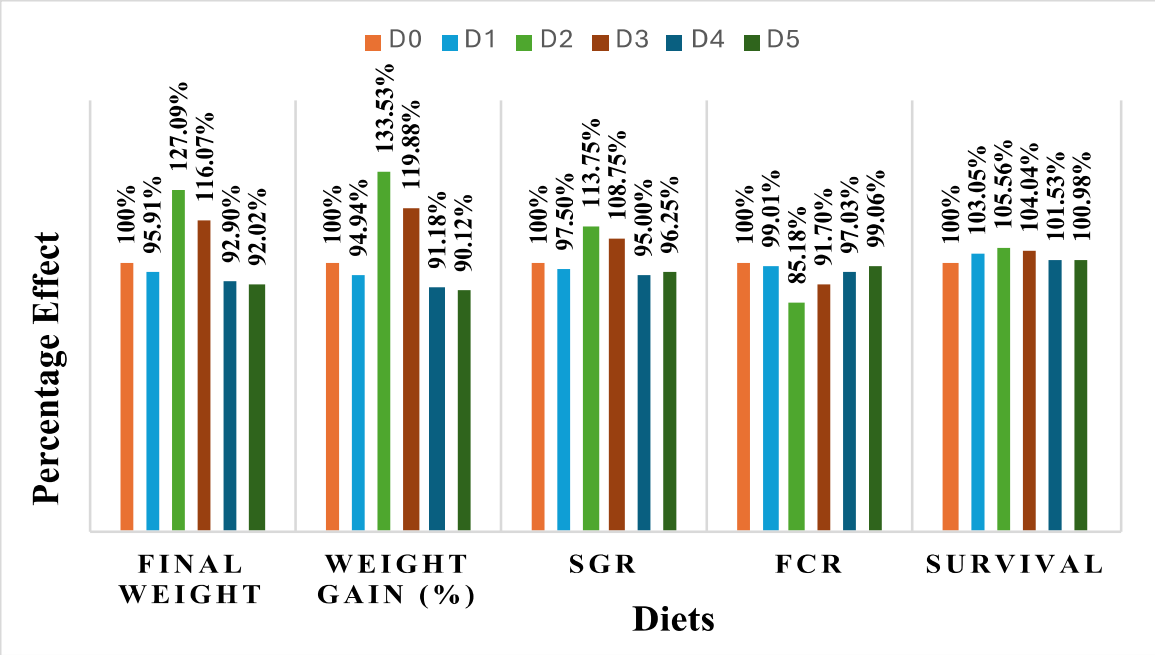

Fig.1: Graphical representation of percentage effect of Brewer's yeast experimental diets as compared to controlled diet

3.2. Hematological Parameters

Significant variations were found in hematological attributes of rohu fed with different BSY experimental diets as shown in Figure-2. Results of hematological attributes revealed that D2 and D3 also significantly affected on hematological parameters and provided highest mean values. Mean values of total RBC in rohu fishes were almost similar under D2 (1.92 × 106 mm^{-3}) and D3 (1.93 × 106 mm^{-3}) followed by D4, D5 and D1. Lowest values of RBC were found in rohu fed with controlled diet. RBC of fishes fed with D2 and D3 were 36% higher than fishes fed with controlled diet. While in case of WBC highest value was observed in rohu fed with D5 followed by D4. While D2 and D3 fed rohu showed lowest number of WBC count.

Hemoglobin content was also found highest in fishes fed with D2 (8.27 g dL^{-1}) and D3 (8.04 g dL^{-1}). These values were 16.3% and 13.1% higher than hemoglobin content of rohu fed with controlled diet. Packed cell volume (PCV) were also significantly effected by experimental diets. Mean values of PCV ranged from 23.62 to 28.37 as shown in Figure-2d. D2 fed fishes showed highest values of PCV (28.37) followed by D3. From the results of hematological attributes except WBC's, it can be derived that fishmeal 30-45% replacement with BSY can become a good sustainable alternative diet for Labeo rohita culture.

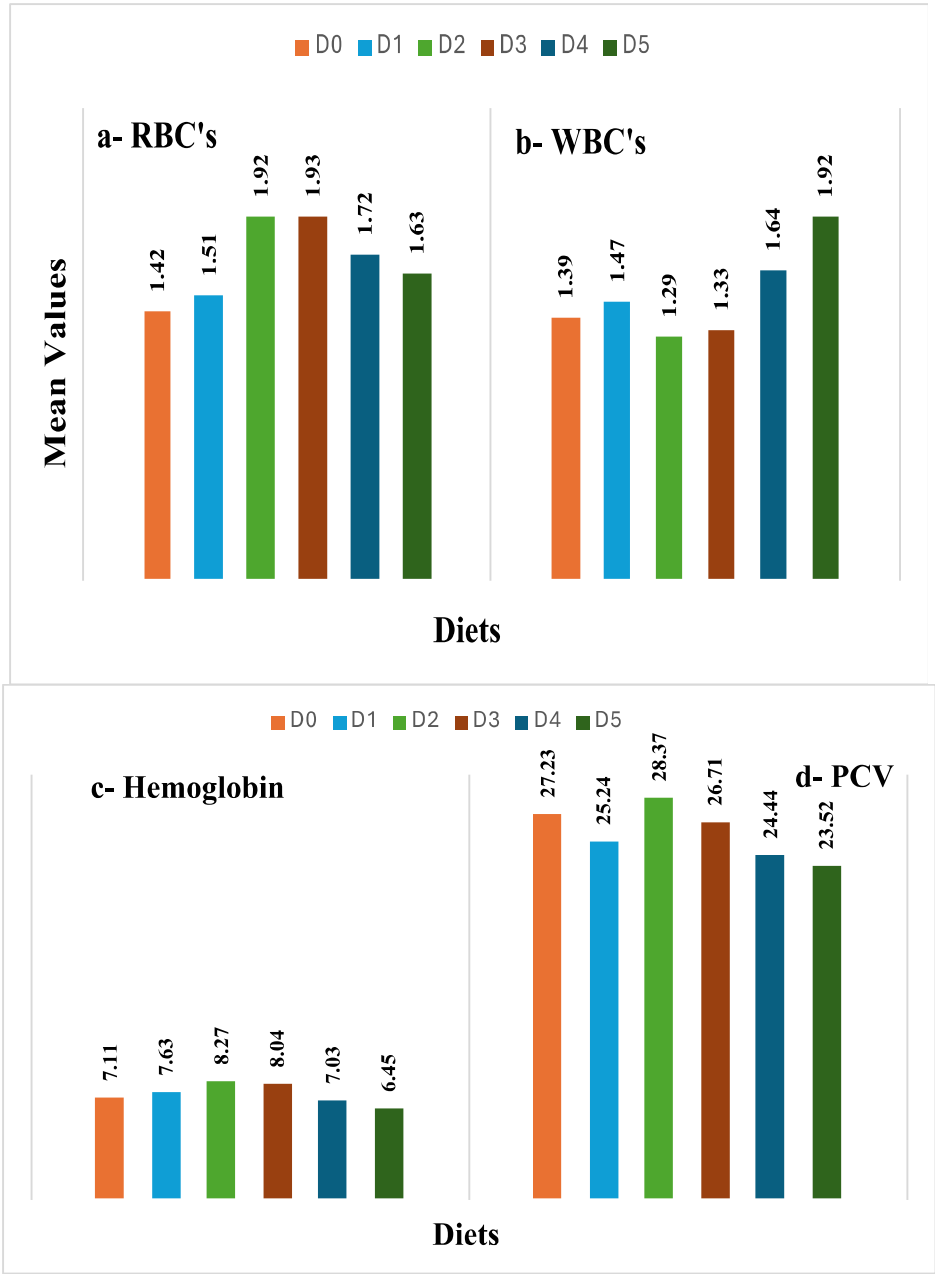

Fig. 2: Graphical representation of mean values of hematological attributes of *Labeo rohita* fed with various Brewer's yeast experimental diets

3.3. Biochemical Parameters

Hemato-biochemical attributes of rohu fishes were also significantly affected by BSY experimental diets. Unlike growth and hematological attributes, biochemical attributes such as glucose, cortisol, ALT and ALKP were significantly reduced in fishes fed with D2 and D3 experimental diet as shown in Figure-3. Highest value (56.44 µg dL^{-1}) of glucose level were found in fishes fed with controlled diet followed by D5 (54.91 µg dL^{-1}) and D1 (54.67 µg dL^{-1}). While D2 fed rohu showed lowest value of glucose level (38.74 µg dL^{-1}) (Figure-3a). Similarly cortisol level was also found lowest in fishes fed with D2 followed by D3. While D5 fed fishes showed highest levels of cortisol followed by controlled diet (Figure-3b). BSY experimental diets effect on rohu showed similar trends on ALT level just like glucose and cortisol. D2 and D3 caused significant reduction in ALT level as compared to controlled diet. While D4 and D5 caused significant increase in ALT level

(Figure-3c). These diets caused 15% and 4% increase in ALT level of rohu fishes as compared to fishes fed with controlled diet. Just like all above mentioned biochemical attrributes ALKP activity was also reduced in fishes fed with D2 and D3 diets. ALKP activity in fishes fed with D2 and D3 was also less than ALKP activity in fishes fed with controlled diet. D5 fed fishes showed highest activity of ALKP enzymes, which was 40% higher than ALKP activity in fishes fed with controlled diet (Figure-3d).

Biochemical traits like globulin, albumin and total proteins showed opposite trends to all above mentioned biochemical attributes. In these cases D2 and D3 fed fishes performed better and showed highest values. Highest values of total protein were observed in fishes fed with D2. Which were 19% higher as compared to fishes fed with controlled diet (Figure-3e). Similarly, highest values of albumin and globulin were also observed in fishes fed with D2 experimental diet (Figure-3f,g).

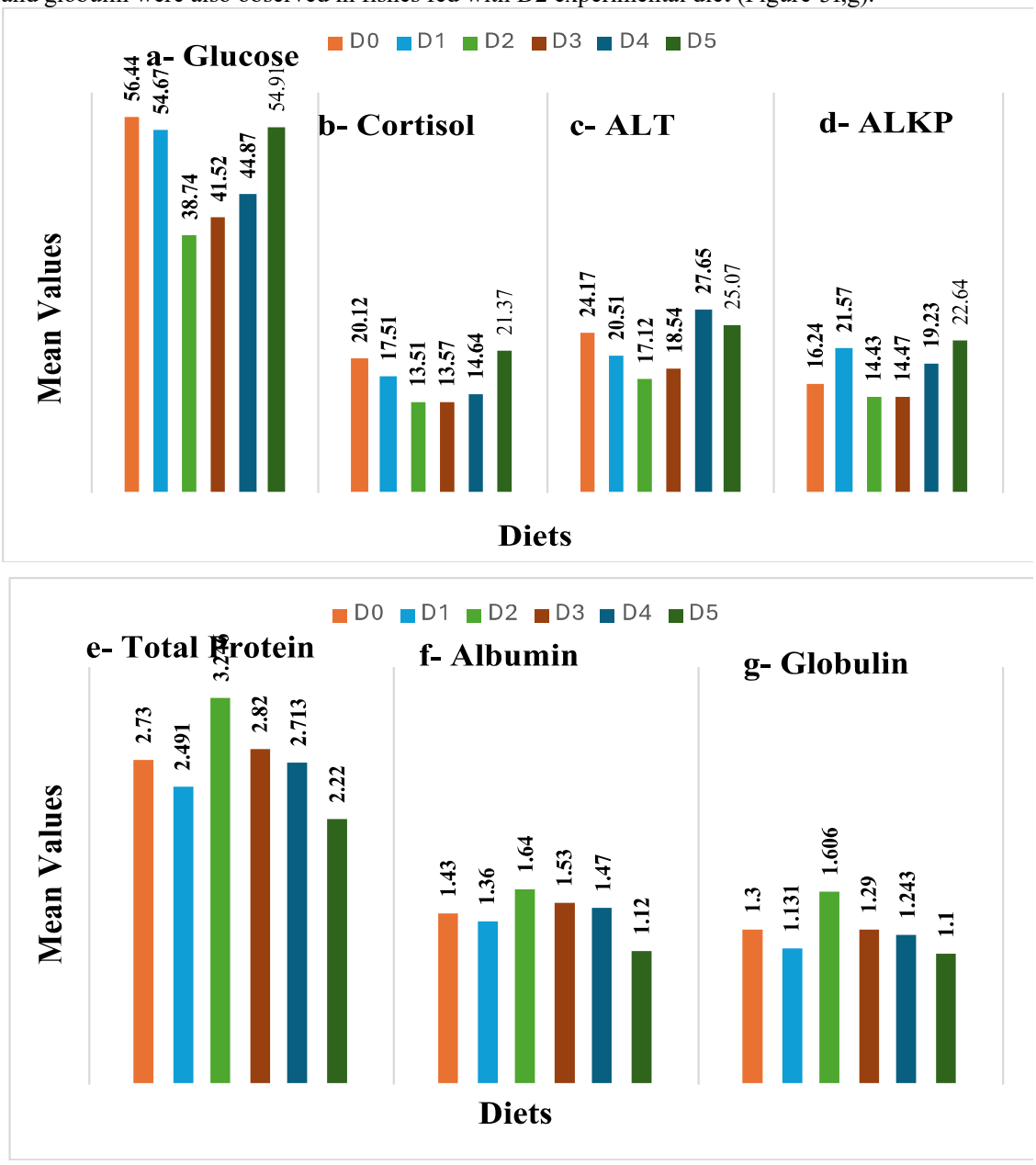

Fig. 3: Graphical representation of mean values of serum biochemical attributes of *Labeo rohita* fed with Brewer's Yeast Experimental Diets

3.4. Digestive Enzymes Activities

Figure4 represents the activities of digestive enzymes present in intestine. BSY experimental diets significantly effected on activities of amylase, protease and lipase enzymes. Highest activity of amylase enzyme were recorded in fishes fed with D1 followed by D2. D1 and D2 caused 16% and 9% increase in amylase activity as compared to amylase activity of fishes fed with controlled diet. D3, D4 and D5 didn't cause much increase in amylase activity as shown in Figure-4a. All BSY experimental diets caused significant increase in protease activity of enzymes in fishes as compared to enzyme activity in fishes fed with controlled diet. But among experimental diets effects on protease activity, no significant differences were observed. All these diets caused almost 50% increase in the activity of protease activity as shown in Figure-4b. Highest activity of lipase enzyme was observed in fishes fed with D2 followed by D1. While lowest activity of lipase enzyme was observed in fishes fed with D5 as shown in Figure-4c.

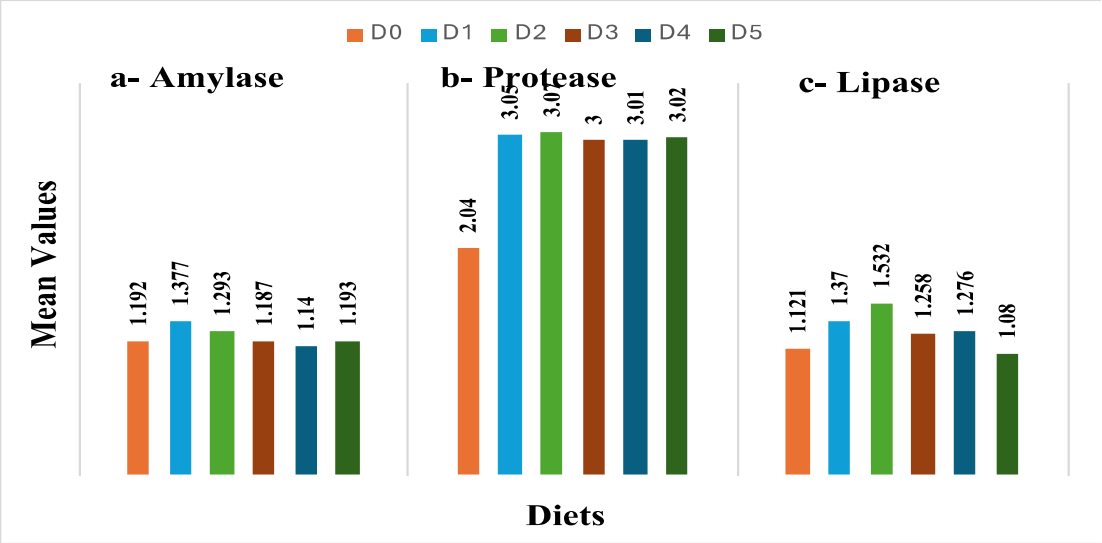

Fig. 4: Graphical representation of mean values of digestive enzymes activities of *Labeo rohita* fed with Brewer's yeast experimental diets

4. Discussions

In aquatic animal nutrition research, it became hot topic to replace fishmeal with sustainable alternatives. Development of cost-effective meal for aquaculture is getting a lot of attention of researchers. In this study fishmeal was substituted with brewers yeast in feed of rohu. Our results showed that 30-45% replacement cause significant increase in all growth attributes. Our these findings were in accordance to the findings of (Zhang *et al.,* 2020). Who found that BSY replacement cause 7% increase in weight gain of gibel carp in 63 days trial. More changes in the composition of fish diets can cause stress in fishes, which can be examined by measuring changes in number of blood cells and changes in concentrations of plasma constituents (Ahmed and Jaffar, 2013).

By monitoring hematological changes, dietary stress can be assessed (Seibel *et al.*, 2021). Our findings showed a slight increase in hemoglobin levels under D2 and D3 diets. This slight increase suggests that fishmeal replecement with BSY didn't cause any stress or harmful effects on the health of fishes. Decrease in numbers of leukocytes and increase in erythrocytes numbers under diet D2 and D3 also support above statement that BSY replacent didn't cause any stress or harmful effects on the health of fishes. These findings were in correspondance with (Bozorgnia *et al.,* 2011). Increase in number of WBC or leukokocytes indicates stress in fishes (Roberts, 2012). Decrease in WBC numbers under D2 and D3 suggests that 30-45% fishmeal replacement with BSY had benificaial effects on Labeo rohita.

Glucose level in blood is also considered a stress indicator in fishes (Swain *et al.,* 2022; Kumar *et al.,* 2022). In previous studies, it is reported that cortisol release from kidneys is increased under stress conditions which leads to increase in glucose level to mitigate stress (Nakano and Tomlinson, 1967).

Jatropha platyphylla kernel meal enhanced glucose level in Nile tilapia (Akinleye *et al.,* 2012). Fishmeal replaced by 20% Jatropha concentrate caused higher glucose level in rohu as compared to other diets (Shamna *et al.,* 2017). In our experiment, glucose and cortisol levels were found lower in fishes fed with D2 and D3 as compared to fishes fed with controlled diet while glucose and cortisol were higher in fishes fed with D4 and D5. Which shows that higher replacement of fishmeal by BSY increases nucleic acid level which cause stress in rohu fishes. ALT enzyme activities were found significantly lower in fishes fed with D2 and D3 experimental diet. Which shows that D2 and D3 didn't cause any stress in fishes and metabolism in liver cause sparing of proteins, which are used as energy source (Jurss, 1981). These results suggests that plasma membrane of liver were protected from damage in fishes fed with D2 and D3. Similarly, ALKP enzyme activities were also found lower in fishes fed with D2 and D3. While diets D1, D4 and D5 caused significant increase in ALKP concentration, this may be due to osteoblastic activity (Labarrère *et al.,* 2013).

Different fish species have varying feed habits, which depends on presence of digestive enzymes present in their intestine. Before forming any diet compositions, it is important to understand the digestive capabilities of that fish specie to be reared (Genovese *et al.,* 1995). Digestive enzymes play important role in absorption of nutrients required for proper growth (Furne *et al.,* 2005). Some fishes have the ability to adapt according to the ingredients of diet by regulating their metabolism and enzymes secretion. Digestive enzymes are required for hydrolysis of lipid, protein and carbohydrates. In our findings no significance differences were observed for protease activity under all experimental diets. Level of protease activity determines how efficiently fish utilize protein (Bindhumol and Kumaraswamy, 2013). Secretion of protease enzyme depends on the protein percentage in composition of diet (Rønnestad et al., 2008). Pangasianodon showed better activity of protease in intestine fed with alternatively 25 and 35% crude protein as compared to fed with 35% crude protein continuously (Tok *et al.,* 2017). In giant cat fish amylase activity was found highest at optimum temperature of 25-30°C (Tongsiri *et al.,* 2010). Tok *et al.* (2017) found that amylase activity was positively correlated with carbohydrates in diet. While some previous studies found that carbohydrate level didn't have any influence on amylase activity (Lopez-Lopez *et al.,* 2005) . In our findings highest amylase activity was found at 15% BSY replacement, but carbohydrate level was constant in all diets. So our findings were in correspondance to some previous studies. Lipase enzyme activity depends on lipid content in diet (Mukhopadhyay and Rout, 1996). Debnath *et al.* (2007) found less variations in lipase activity in rohu fishes fed with different protein sources while keeping lipids level constant. In our study slightly higher lipas activity was observed in BSY 15 and 30% replacement, while lipids level was constant for all BSY diets which may be due to the different levels of inclusion of BSY.

5. Conclusions

From results and discussions of this study it can be concluded that D2 (30% fishmeal replacement by brewer's yeast) provided significant higher weight gain, specific growth rate, survival rate and its nutrients were efficiently utilized by rohu fishes as compared to controlled diet. Higher RBCs, hemoglobin and digestive enzymes activities were also observed under D2 diet. So, it can be concluded that fishmeal 30-45% replacement with brewer's yeast can become a good sustainable alternative diet for *Labeo rohita* culture.

Abbreviations:

BSY: Brewer's Spent Yeast
SGR: Specific Growth Rate
FCR: Feed Conversion Ratio
RBC: Red Blood Cells
WBC: White Blood Cells
PCV: Packed Cell Volume
ALT: Alanine Amino Transferase
ALKP: Alkaline Phosphatase

References

Ahmed, S. M. and R. S. Jaffar. 2013. Effect of salt stress on energy usage and growth in grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) and common carp (*Cyprinus carpio* L.juveniles). Iraqi J Aquacult., 10(1): 1–24.

Akinleye, A. O., V. Kumar, H. P. S. Makkar, M. A. Angulo-Escalante & K. Becker, 2012. Jatropha platyphylla kernel meal as feed ingredient for Nile tilapia (*Oreochromis niloticus* L.): growth, nutrient utilization and blood parameters. Journal of animal physiology and animal nutrition, 96(1): 119-129.

AOAC. 2005. Official methods of analysis of AOAC international. Asso- ciation of Official Analytical Chemists, Washington.

Ashaf-Ud-Doulah, M., S.M. Islam, M. M. Zahangir, M. S. Islam, C. Brown & M. Shahjahan, 2021. Increased water temperature interrupts embryonic and larval development of Indian major carp rohu *Labeo rohita*. Aquaculture International, 29: 711-722.

Bindhumol, V., & A. Kumaraswamy, 2013. Effect of dietary protein levels on the proteolytic enzyme activity of common carp (*Cyprinus carpio*). International Journal of Current Trends in Research, 2: 66-68.

Bozorgnia, A., R. Alimohammadi & M. Hosseinifard. 2011. Acute effects of different temperature in the blood parameters of common carp (*Cyprinus carpio*). In 2nd International Conference on Environmental Science and Technology IPCBEE (Vol. 6), 52-55.

Chang, C. F., M. S. Su, H. Y. Chen & I. C. Liao. 2003. Dietary β-1, 3-glucan effectively improves immunity and survival of Penaeus monodon challenged with white spot syndrome virus. Fish & shellfish immunology, 15(4): 297-310.

Conway, J. 2019. Beer production worldwide from 1998 to 2018.

Debnath, D., A. K. Pal, N. P. Sahu, S. Yengkokpam, K. Baruah, D. Choudhury & G. Venkateshwarlu. 2007. Digestive enzymes and metabolic profile of *Labeo rohita* fingerlings fed diets with different crude protein levels. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 146(1): 107-114.

Dixon, B., A. Kilonzo-Nthenge, M. Nzomo, S. Bhogoju & S. Nahashon. 2022. Evaluation of selected bacteria and yeast for probiotic potential in poultry production. Microorganisms, 10(4): 676.

Egel-Mitani, M., A. S. Andersen, I. Diers, M. Hach, L. Thim, S. Hastrup & K. Vad. 2000. Yield improvement of heterologous peptides expressed in yps1-disrupted *Saccharomyces cerevisiae* strains. Enzyme and microbial technology, 26(9): 671-677.

FAO, 2017. Cultured Aquatic Species Information Programme: *Labeo rohita*, Ham-ilton,1822.

Furne, M., M. C. Hidalgo, A. Lopez, M. Garcia-Gallego, A. E. Morales, A. Domezain & A. Sanz. 2005. Digestive enzyme activities in Adriatic sturgeon Acipenser naccarii and rainbow trout *Oncorhynchus mykiss*. A comparative study. Aquaculture, 250(1-2): 391-398.

Gatlin III, D. M., F. T. Barrows, P. Brown, K. Dabrowski, T. G. Gaylord, R. W. Hardy & E. Wurtele. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture research, 38(6): 551-579.

Genovese, L., F. Patti & G. Caruso. 1995. Studies of the digestive cycle in the amberjack (*Seriola dumerilii*) (Risso, 1810) in intensive rearing. Oebalia, 21: 5-15.

Henry, M., L. Gasco, G. Piccolo & E. Fountoulaki. 2015. Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology, 203: 1-22.

Hertrampf, J. W., & F. Piedad-Pascual. 2012. Handbook on ingredients for aquaculture feeds. Springer Science & Business Media.

Jurss, K. A. R. L. 1981. Influence of temperature and ratio of lipid to protein in diets on aminotransferase activity in the liver and white muscle of rainbow trout (*Salmo gairdneri* Richardson). Comp. Biochem. Physiol., 68: 527-533.

Kamei, M., B. Sahu, S. Raman, S. Nanda, D. Choudhury & M. S. Dorothy. 2018. Use of fish silage based blended protein source for replacement of fish meal in thai-pangas diet. Int. J. Curr. Microbiol. App. Sci, 7(10): 2949-2961.

Kapoor, S. K., U. Kapil, S. N. Dwivedi, K. Anand, P. Pathak & P. Singh. 2002. Comparison of HemoCue method with cyanmethemoglobin method for estimation of hemoglobin. Indian pediatrics, 39(8): 743-746.

Kumar, V., H. S. Swain, B. K. Das, S. Roy, A. Upadhyay, M. H. Ramteke & H. Banerjee. 2022. Assessment of the effect of sub-lethal acute toxicity of Emamectin benzoate in *Labeo rohita* using multiple biomarker approach. Toxicology Reports, 9: 102-110.

Labarrère, C. R., P. M. C. D. Faria, E. D. A. Teixeira & M. M. Melo. 2013. Blood chemistry profile of Surubim hybrid fish (*Pseudoplatystoma reticulatum* X P. corruscans) raised in different stocking densities. Ciência e Agrotecnologia, 37: 251-258.

Levic, J., O. Djuragic & S. Sredanovic. 2010. Use of new feed from brewery by-products for breeding layers. Romanian Biotechnological Letters, 15(5): 5559-5565.

Lin, T., J. Xing, J. Jiang, X. Tang & W. Zhan. 2011. β-glucan-stimulated activation of six enzymes in the haemocytes of the scallop Chlamys farreri at different water temperatures. Aquaculture, 315(3-4): 213-221.

Lopez-Lopez, S., H. Nolasco, H. Villarreal-Colmenares & R. Civera-Cerecedo. 2005. Digestive enzyme response to supplemental ingredients in practical diets for juvenile freshwater crayfish *Cherax quadricarinatus*. Aquaculture nutrition, 11(2): 79-85.

Mukhopadhyay, P. K., & S. K. Rout, 1996. Effects of different dietary lipids on growth and tissue fatty acid changes in fry of the carp *Catla catla* (Hamilton). Aquaculture research, 27(8): 623-630.

Mussatto, S. I., 2014. Brewer's spent grain: a valuable feedstock for industrial applications. Journal of the Science of Food and Agriculture, 94(7): 1264-1275.

Nakano, T., & N. Tomlinson. 1967. Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. Journal of the Fisheries Board of Canada, 24(8): 1701-1715.

Jain, N.C., 1986. Schalm's veterinary hematology. Lea and Febiger, Philadelphia, 1221.

Olvera-Novoa, M.A., C. A. Martínez-Palacios & L. Olivera-Castillo, 2002. Utilization of torula yeast (*Candida utilis*) as a protein source in diets for tilapia (*Oreochromis mossambicus* Peters) fry. Aquaculture Nutrition, 8(4): 257-264.

Roberts, R. J., 2012. Fish pathology: fourth edition. New York (NY): John Wiley and Sons.

Rønnestad, I., S. Morais, R. N. Finn & B. G. Kapoor. 2008. Digestion in fish larval physiology, Science Publishers, Enfield, 201–262.

Rossow, H. A., T. Riordan & A. Riordan, 2018. Effects of addition of a live yeast product on dairy cattle performance. Journal of Applied Animal Research, 46(1): 159-163.

Samantaray, K., & S. S. Mohanty, 1997. Interactions of dietary levels of protein and energy on fingerling snakehead, *Channa striata*. Aquaculture, 156(3-4): 241-249.

Seibel, H., B. Baßmann & A. Rebl. 2021. Blood will tell: what haematological analyses can reveal about fish welfare. Front Vet Sci., 8: 616955.

Shah, S. L., & A. Altindağ, 2005. Alterations in the immunological parameters of Tench (*Tinca tinca* L. 1758) after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead. Turkish journal of veterinary & animal sciences, 29(5): 1163-1168.

Shamna, N., P. Sardar, N. P. Sahu, V. Phulia, M. Rajesh, F. J. Fawole & G. Angel, 2017. Heamato-immunological and physiological responses of *Labeo rohita* fingerlings to dietary fermented Jatropha curcas protein concentrate. Animal Feed Science and Technology, 232: 198-206.

Swain, H. S., B. K. Das, A. Upadhyay, M. H. Ramteke, V. Kumar, D. K. Meena & K. D. Rawat, 2022. Stocking density mediated stress modulates growth attributes in cage reared *Labeo rohita* (Hamilton) using multifarious biomarker approach. Scientific Reports, 12(1): 9869.

Tok, N. C., K. K. Jain, D. L. Prabu, N. P. Sahu, S. Munilkumar, A. K. Pal & P. Kumar, 2017. Metabolic and digestive enzyme activity of *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings in response to alternate feeding of different protein levels in the diet. Aquaculture Research, 48(6): 2895-2911.

Tongsiri, S., K. Mang-Amphan & Y. Peerapornpisal, 2010. Characterization of amylase, cellulase and proteinase enzyme in stomach and intestine of the mekong giant catfish fed with various diets consisting of Spirulina. Current Research Journal of Biological Sciences, 2(4): 268-274.

Trosvik, K. A., S. D. Rawles, K. R. Thompson, L. A. Metts, A. Gannam, R. Twibell & C. D. Webster, 2012. Growth and body composition of Nile tilapia, *Oreochromis niloticus*, fry fed organic diets containing yeast extract and soybean meal as replacements for fish meal, with and without supplemental lysine and methionine. Journal of the World Aquaculture Society, 43(5): 635-647.

Van Heugten, E., D. W. Funderburke & K. L. Dorton, 2003. Growth performance, nutrient digestibility, and fecal microflora in weanling pigs fed live yeast. Journal of animal science, 81(4): 1004-1012.

Venmarath, A., S. S. Karkal, P. V. Suresh & T. G. Kudre, 2024. Extraction optimization, partial purification, and characterization of sialoglycoproteins from *Labeo rohita* roes. International Journal of Biological Macromolecules, 133462.

Walter, H. E., 1984. Proteases and their inhibitors. 2. 15. 2 Method with haemoglobin, casein and azocoll as substrate. Methods of enzymatic analysis., 270-277.

Weiwei, D., M. Kangsen, X. Wei Z. Yanjiao, X. Dandan & A. Qinghu, 2016. Effects of replacing fish meal with plant- based protein on growth, physiological and biological indices, and intestinal histology in tongue sole, *Cynoglossus semilaevis* Güntuer. Journal of Fishery Sciences of China, 23(01): 125-137.

Wintrobe, M.M. 1967. Clinical hematology. Lea and Febiger, Philadelphia.

Zhang, P. Y., F. Yang, J. P. Hu, D. Han, H. K. Liu, J. Y. Jin, Y. X. Yang, J. H. Yi, X. M. Zhu & S. Q. Xie, 2020. Optimal form of yeast cell wall promotes growth, immunity and disease resistance in gibel carp (*Carassius auratus gibelio*). Aquacult Rep., 18: 100465.