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Impact of Dietary Yeast Supplementation on the Antioxidant Enzymes, Digestive Enzymes and Muscle Proximate Composition of *Labeo rohita*

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ABSTRACT

The production of aquaculture has expanded rapidly to satisfy the rising demands of an expanding human population and by 2050 additional intensification is anticipated. Active dry yeasts are often used in probiotic products for their immune-stimulatory benefits, provided by vitamins, B-glucans, and nucleotides. The goal of the current study was to determine how dietary yeast used as a feed supplement, affected the muscle composition, digestive enzyme activity and antioxidant enzyme activity of Labeo rohita, taking into account the significance of this readily accessible and affordable source of protein and energy. The current experiment was carried out in indoor tank hatcheries. The current study indicated that adding dietary yeast to *Labeo rohita*'s diet had a positive impact. All treatment groups and control group showed similar results of whole body proximate without any significant difference among treatments. Digestive enzymes analysis like protease with the highest activity observed in T4 (3.53U/mg), followed by T3 (3.31 U/mg), T2 (3.06 U/mg), T1 (2.65 U/mg) and T0 (2.34 U/mg) and amylase activity reaching its peak in T4 (4.84 U/mg), followed by T3 (4.66 U/mg), T2 (4.47 U/mg), T1 (4.32 U/mg) and T0 (4.19 U/mg). While lipase enzyme activity showed a non-significant trend ($P > 0.05$), indicating no differences between groups, protease and amylase showed significant results that rose significantly ($P < 0.05$) with larger yeast inclusion levels. Superoxide dismutase (SOD) and catalase enzyme activity were found to differ significantly by antioxidant enzyme analysis ($P < 0.05$), with T4 having the highest values (18.41 U/mg for SOD and 62.70 U/mg for catalase), and T0 having the lowest (10.87 U/mg for SOD and 55.67 U/mg for catalase). Peroxidase enzyme activity, on the other hand, showed a non-significant trend ($P > 0.05$) with slight variations between groups.

INTRODUCTION

The production of aquatic creatures in fresh, brackish and seawater is known as aquaculture (Stickney 2000). It encompasses the cultivation of these organisms in controlled or semi-controlled environments for various purposes including commercial, recreational or resource management (NOAA 2011). Fish, which include whitefish, oily fish, and shellfish, are an essential source of high-quality protein, vitamins, and minerals. Oily fish, like sardines, have a fat content ranging from 10% to 25%, while whitefish, like haddock and seer, have very little fat, usually less than 1%. According to Fellows and Hampton (1992), oily fish is especially high in important fatty acids and fat-soluble vitamins (A, D, E, and K) that are necessary for normal body activities. Approximately 50–60% of an adult's daily protein needs can be met with 150 g of fish (FAO 2018).

Consuming oily fish that is high in long-chain omega-3 fatty acids can help lessen the risk of cardiovascular disease and reduce systemic inflammation (Khan 2021). According to Helen et al. (2018), it may not have a substantial impact on the total death rate from cardiovascular diseases, but it can lower the chance of deadly heart attacks. High-quality fishmeal contains between 60% and 72% crude protein by weight. The amino acid content and digestibility of protein are intimately linked to the nutritional value of protein in diets. 32% to 45% of the total protein by weight can be found in typical fish diets (FAO 2023). In terms of specific nutrient content fish provides 22g of protein, 12g of total fat and essential vitamins and minerals per 100 grams (USDA 2022).

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With a total production of 87.7 million tons and a farm gate value of $281.5 billion in 2020, aquaculture is one of the industries with the fastest rate of growth in the world (FAO 2022). In Pakistan, however, per capita fish consumption is low at only 1.9 kg, despite the country's vast fishing potential (FAO 2019). With a coastline of 1,120 km and a significant exclusive economic zone, Pakistan has seen an increase in aquaculture production from 159,083 metric tons in 2018 to 164,527 metric tons in 2021 (World Bank 2023). The country has approximately 60,470 hectares dedicated to fish ponds with the majority located in Sindh, and around 16,000 fish farms operating across various provinces (FAO 2023).

Rohu (*Labeo rohita*) is a key species in South Asian aquaculture, requiring induced spawning as it does not breed in standing water (FAO 2018). Rich in omega-3 fatty acids, vitamins A, B, C, and D, and able to stave off ailments like osteoporosis, it is widely consumed in Bangladesh, Nepal, and several Indian states (Paul 2015). The nutritional values of Rohu include 16 g of protein and essential minerals per 100 grams (ICAR 2022). Historically, artificial feeding in Pakistan was minimal but with advancements farmers now utilize artificial feeds significantly increasing yields. For example, all-male tilapia culture technology has led to stocking densities of 3,000 fish per acre yielding around 2,500 kg per acre (NACA 2019).

Yeast (*Saccharomyces cerevisiae*) has emerged as an effective feed supplement in aquaculture providing a protein-rich source that can enhance fish health and reduce reliance on fishmeal (Tawwab 2020). Yeasts are easy to cultivate and can improve intestinal health and immune function in fish (Rigo et al. 2019). Studies indicate that dietary yeast can boost growth and digestive enzyme activity in cultured species including Rohu (Reda and Selim 2015; Talpur et al. 2012). The nutritional profile of baker's yeast shows high protein content (40.44 g per 100 g) and essential vitamins (USDA 2021). This study aims to explore the impact of dietary yeast supplementation on muscle composition, digestive enzyme activity and antioxidant enzyme levels in Rohu (*Labeo rohita*), thereby contributing to sustainable aquaculture methods in Pakistan.

MATERIALS AND METHODS

The Rohu fish (*Labeo rohita*) was collected from Lasani fish farm, Lahore. Fifteen aquaria were set up, filled with water, aerated and covered with nets. Each aquarium housed 20 fish with an average weight of 3.69g and length of 7.3cm. For 70 days, fish were fed at a rate of 3% of their body weight twice a day. Five diets were formulated with varying yeast concentrations (0% to 20%) and other consistent ingredients like fish meal, soybean meal and wheat flour. The proximate composition of the feeds showed minor variations with crude protein between 34.23% to 34.81% and energy content ranging from 2813.3 to 3129 Kcal/Kg.

Table 1

Stocking density of Labeo rohita per replica

Treatments	T_0 (Control)			T_1			T_2			T_3			T_4		
Replicates	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Fish	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Table 2

Proximate composition of experimental feed

Proximate Composition	Diet 1 (T0)	Diet 2 (T1)	Diet 3 (T2)	Diet 4 (T3)	Diet 5 (T4)
Dry Matter %	90.65	91.05	94.33	94.26	94.28
Crude Protein %	34.23	34.61	34.81	34.55	34.68
ME Kcal/kg	2813.2	2892.4	2971.2	3051	3128
Crude Fat %	5.83	5.71	5.83	5.58	5.42
Ash %	9.51	10.67	9.91	10.27	10.18

Table 3

Ingredients of the experimental feeds

Ingredients	Diet 1 (T0)	Diet 2 (T1)	Diet 3 (T2)	Diet 4 (T3)	Diet 5 (T4)
Fish meal (g)	20	20	20	20	20
Soybean meal (g)	17	17	17	17	17
Wheat flour(g)	13	13	13	13	13
Sunflower meal(g)	5	5	5	5	5
Canola meal(g)	15	12	9	6	3
Corn gluten 30%(g)	15	13	11	9	7
Vegetable oil(g)	3	3	3	3	3
Rice polish(g)	10	10	10	10	10
Yeast(g)	0	5	10	15	20
Vitamin premix(g)	1	1	1	1	1
Mineral mixture(g)	1	1	1	1	1
Total	**100**	**100**	**100**	**100**	**100**

Fish Proximate Analysis

Fish total body and muscle proximate composition analysis was carried out using the AOAC 2006 technique. Fish from every treatment were ground into a fine powder, dried in a hot air oven for a full day, and then placed in storage until additional examination. 1g samples were dried at 105°C for 12 hours, weighed,

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chilled in a desiccator, and then dried again until a constant weight was reached in order to estimate moisture or dry matter. The following formula was used to get the moisture content:

Moisture % = WI- W2/ Weight of samples * 100

Where W1 represents the sample weight prior to drying and W2 represents the weight following drying. The percentage of dry matter was found to be 100% moisture content.

For crude fat estimation, 2g of dried sample was placed in a Soxhlet apparatus at 650°C. After 2 hours, samples were cooled and dried and the fat content was calculated as:

% Either Extract = (W1 + W2) – W3/Sample Weight * 100

Where W2 is the weight of the sample, W1 is the weight of the filter paper, and W3 is the weight following extraction.

Crude protein was estimated using Kjeldahl's apparatus where samples were digested in a mixture ($CuSO_4$, $FeSO_4$, and K_2SO_4), diluted and further processed with NaOH, boric acid and methyl red indicator. Digested samples were distilled and titrated against sulfuric acid until a light pink color appeared. Protein was calculated using:

% N = D x 0.00014 x V/W x Volume of 40 % NaOH used x 100

Where W is the sample weight, V is the volume of H2SO4 used, and D is the dilution factor (250 ml).

For ash content, a dried crucible was weighed (W1), 2g of sample added (W2) then burned on a hot plate and ignited in a muffle furnace at 650°C. The ash percentage was calculated as:

Ash % = W3 - W1/W2 × 100

Determination of Antioxidant Enzymes

The enzyme extract was ready after the dissected liver muscle was rinsed in 0.2 M phosphate buffer (pH 6.5), homogenized, and centrifuged at 10,000 rpm for 15 minutes at 4°C. For peroxidase assay, its activity was measured by reducing H_2O_2 at 470 nm using reagents such as guaiacol and phosphate buffer with calculations performed using the formula:

$$\text{Activity } (Units/ml) = \frac{\Delta A/3}{26.6 \times 60/3000}$$

Catalase activity was measured by reducing H_2O_2 at 240 nm using 60 mM phosphate buffer and 10 mM H_2O_2, with the formula:

$$Activity\ (\frac{Units}{ml}) = \frac{\Delta A/min \times dilution \times 2ml}{0.04mM^{-1}cm^{-1} \times 0.05ml}$$

Superoxide dismutase (SOD) activity was determined by inhibiting the photoreduction of NBT using 0.067 mM phosphate buffer, EDTA/NaCN, riboflavin, and NBT solutions. SOD activity was calculated as % inhibition:

$$\%age\ inhibition = \frac{Blank\ (Abs) - Sample\ (Abs)}{Blank\ (Abs)} \times 100$$

Determination of Digestive Enzymes

To prepare enzyme extracts from fish, intestines, pancreas and liver were collected and the tissues were homogenized in a 5% sucrose solution (1 g sample in 19 mL sucrose). After centrifuging this homogenate for 15 minutes at 5 °C at 5000 ×g, the supernatant was frozen at -20 °C in preparation for further enzyme testing. 85.5 g of sugar were dissolved in 1000 mL of distilled water to create a 0.25 M sucrose solution. For enzyme assays, amylase activity was assessed using a 2% starch solution with absorbance measured at 540 nm after incubation with DNS. Protease activity was determined via casein digestion, measuring absorbance at 280 nm. Lipase activity was measured using p-nitrophenylpalmitate (pNPP) as a substrate, with the reaction monitored at 410 nm. Each assay followed specific procedures for reagent preparation including phosphate buffers and TCA solution ensuring pH and concentrations were accurately maintained.

RESULTS AND DISCUSSION

Muscle Proximate Composition

There were minor variances observed, but there were no significant differences ($P > 0.05$) in the muscle's moisture content among the groups. T0 had the highest moisture content at 77.55%, while T1 had the lowest at 77.11%. The overall trend was T0 (77.55%) > T2 (77.29%) > T3 (77.20%) > T1 (77.11%). A graphical illustration is provided in Figure 1.

A non-significant trend ($P > 0.05$) was also seen in the crude protein content, with T4 (22.90%) and T1 (22.89%) showing the highest values. The overall trend was T4 (22.90%) > T1 (22.89%) > T3 (22.79%) > T2 (22.70%) > T0 (22.44%). This is illustrated in Figure 2.

There was a discernible upward trend in the crude fat content ($P < 0.05$). T4 had the highest crude lipid value, at 1.74%. T4 (1.74%) > T3 (1.69%) > T2 (1.22%) > T1 (1.16%) > T0 (0.90%) was the general pattern. A graphical representation is shown in Figure 3.

The percentage of ash content showed a significant upward trend ($P < 0.05$), with T1 having the highest value (3.53%). Overall trend was T1 (3.53%) > T2 (3.50%) > T0 (3.44%) > T3 (3.24%) > T4 (3.22%). The ash content is illustrated in Figure 4.

Table 4

Effect of dietary yeast on muscle proximate composition of *Labeo rohita*

Parameters	T_0 (0 %)	T_1 (5 %)	T_2 (10 %)	T_3 (15 %)	T_4 (20 %)	P < 0.05
Moisture	77.55±0.47^a	77.11±0.02^a	77.29±0.40^a	77.20±0.04^a	77.11±0.08^a	0.54

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Protein	22.44±0.47[a]	22.89±0.02[a]	22.70±0.40[a]	22.79±0.49[a]	22.90±0.08[a]	0.54
Ash	3.44±0.09[a]	3.53±0.00[a]	3.50±0.07[b]	3.24±0.01[b]	3.22±0.04[b]	0.00
Fat	0.90±0.15[a]	1.16±0.04[b]	1.22±0.03[b]	1.69±0.11[c]	1.74±0.02[c]	0.01

Figure 1

A graphical depiction of Labeo rohita's moisture percentage

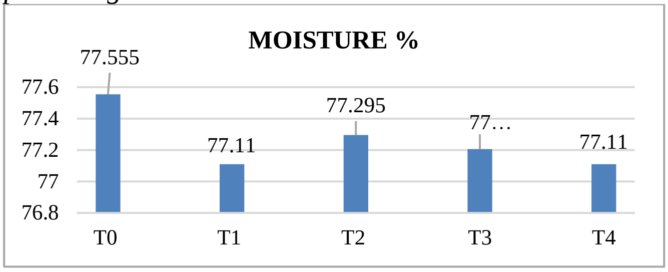


Figure 2

A graphical depiction of Labeo rohita's crude protein percentage

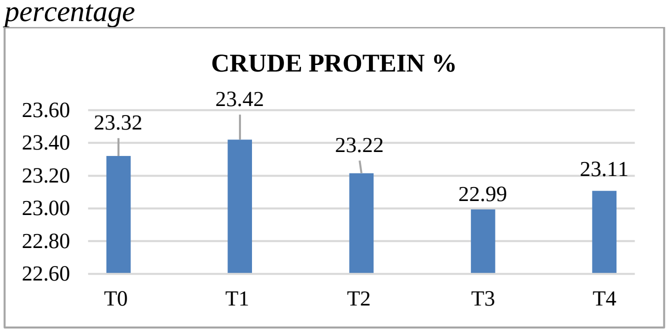


Figure 3

A graphical depiction of Labeo rohita's crude lipid/fat percentage

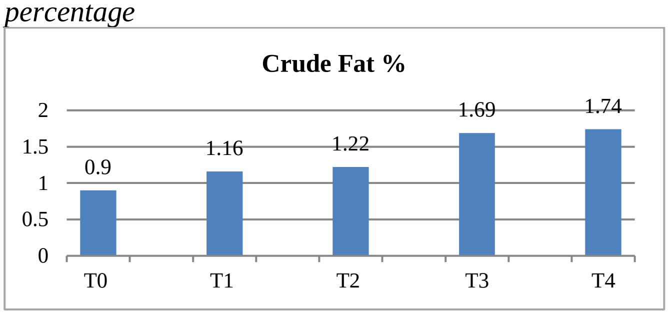
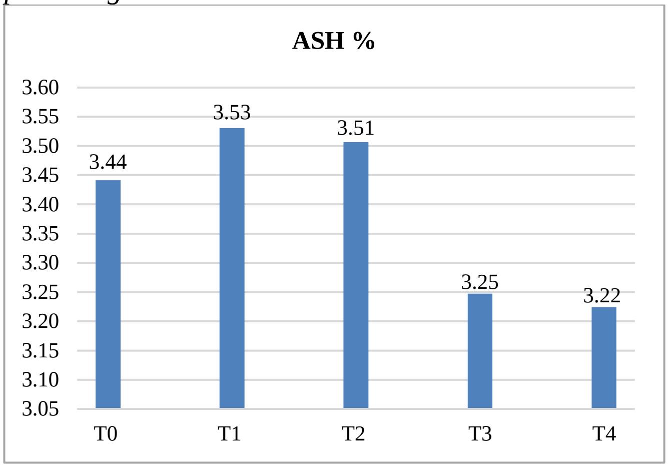


Figure 4

A graphical depiction of Labeo rohita's crude ash percentage



Digestive Enzymes Analysis

As seen in Figure 5, the protease enzyme activity rose considerably ($P < 0.05$) with increasing yeast inclusion levels. T4 had the highest activity at 3.53, followed by T3 (3.31), T2 (3.06), T1 (2.65), and T0 (2.34). Similarly, amylase enzyme activity also increased significantly ($P < 0.05$) with yeast inclusion, reaching its peak in T4 (4.84), followed by T3 (4.66), T2 (4.47), T1 (4.32), and T0 (4.19), illustrated in Figure 6. In contrast, lipase enzyme activity displayed a non-significant trend ($P > 0.05$), showing no differences between groups, as depicted in Figure 7.

Table 5

Impact of dietary yeast on Labeo rohita's lipase, amylase and protease activities

Parameters	T_0 (0%)	T_1 (5%)	T_2 (10%)	T_3 (15%)	T_4 (20%)	P < 0.05
Protease (U/mg)	2.34±0.03	2.65±0.16	3.06±0.10	3.31±0.09	3.53±0.06	0.00
Amylase (U/mg)	4.19±0.04	4.32±0.06	4.47±0.05	4.66±0.05	4.84±0.05	0.00
Lipase (U/mg)	1.36±0.05	1.46±0.08	1.50±0.16	1.52±0.15	1.50±0.17	0.64

Figure 5

A graphical depiction of effect of dietary yeast on protease of Labeo rohita

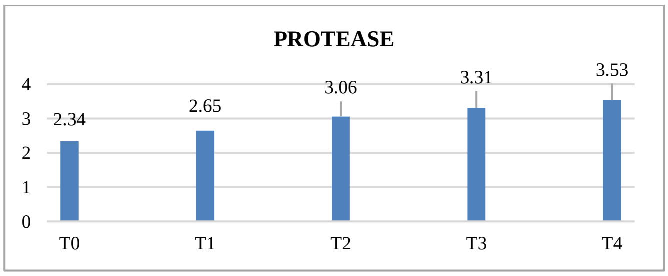


Figure 6

A graphical depiction of effect of dietary yeast on amylase of Labeo rohita

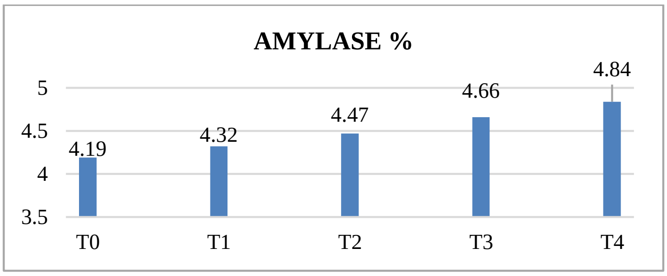


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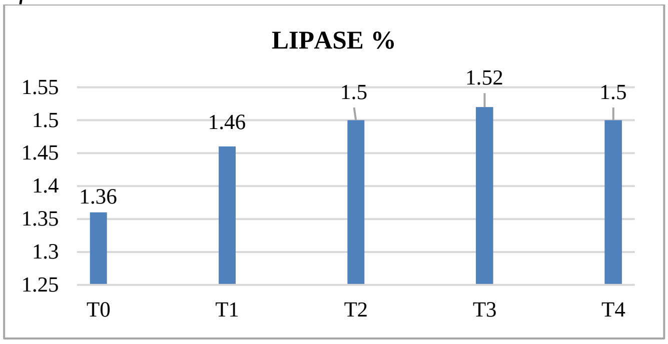
Figure 7

A graphical depiction of effect of dietary yeast on lipase of Labeo rohita



Antioxidant Enzymes Analysis

Superoxide dismutase (SOD) catalase and peroxidase enzyme activity were assessed in five treatment groups (T0 to T4). Both catalase and superoxide dismutase showed markedly increasing trends ($P < 0.05$). The greatest values were found in T4 (18.41 for catalase and 62.70 for SOD), while the lowest values were found in T0 (10.87 for SOD and 55.67 for catalase). Peroxidase activity, on the other hand, varied somewhat between groups and displayed a non-significant trend ($P > 0.05$), with T0 having the highest value (306.80) and T1 having the lowest (303.53). Figures 8, 9, and 10 illustrate the antioxidant enzymes graphically.

Table 6

Effect of dietary yeast on antioxidant enzymes activity in Labeo rohita

Super oxide dismutase (U mg-1 protein)	10.87±0.79^{a}	12.89±0.89^{b}	13.72±0.31^{b}	16.28±0.55^{c}	18.41±0.55^{d}	0.00
Catalse(U mg-1 protein)	55.67±0.57^{a}	57.15±0.88^{b}	58.68±0.60^{c}	61.14±0.76^{d}	62.70±0.36^{e}	0.00
Peroxidase (U mg-1 protein)	306.80±0.917^{a}	303.53±2.47^{a}	304.37±1.13^{a}	303.99±1.71^{a}	303.40±2.72^{a}	0.262

Figure 8

A graphical depiction of effect of dietary yeast on super oxide dismutase of Labeo rohita

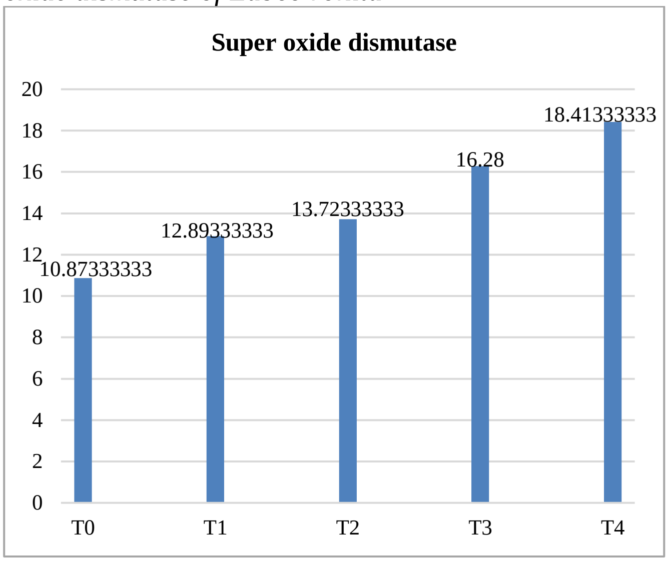


Figure 9

A graphical depiction of effect of dietary yeast on catalase of Labeo rohita

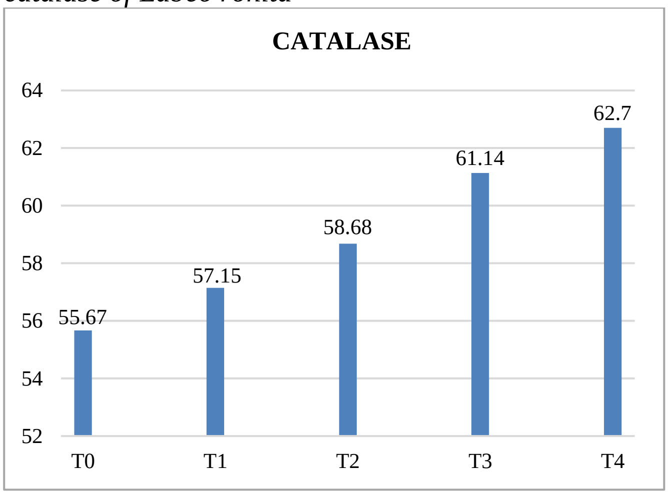
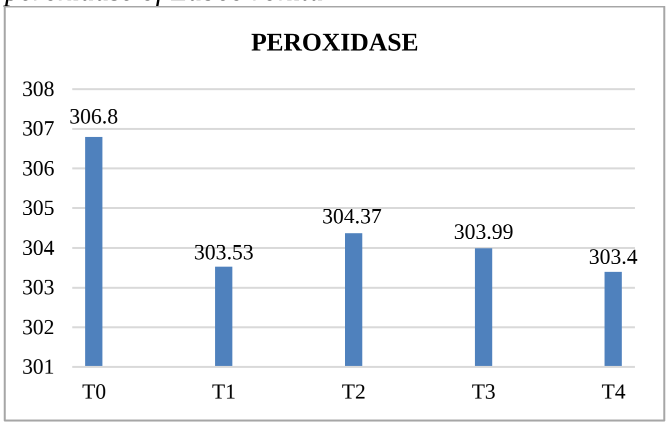


Figure 10

A graphical depiction of effect of dietary yeast on peroxidase of Labeo rohita



DISCUSSION

It has been suggested that yeast may be a viable fish meal replacement (Rumsey et al., 1990). Around the world, a wide range of yeast products and feed components containing yeast are produced, marketed, and utilized extensively in animal diets. The possible effects of adding yeast, yeast derivatives, and yeast-containing substances to animal feeds on growth performance and health benefits have been the subject of much investigation. In probiotic products, active dry yeasts are frequently utilized either alone or in conjunction with helpful bacteria (Shurson 2018). The use of alternative products, such as yeast products, to improve animal health and growth performance has become much more popular as a result of recent government restrictions and the removal of growth-promoting antibiotics from animal feed in the US and the EU (Shurson 2017).

The purpose of this study was to examine the effects of feeding *Saccharomyces cerivisiae* to *Labeo rohita* muscle body composition, activity of the digestive enzymes, and activity of the muscle antioxidant enzyme.

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Whole body proximate analysis revealed no significant differences in the protein, lipid, and moisture content, in line with studies by Kobeisy and Hussein (1995), Oliva-Teles and Goncalves (2001), and Fournier et al. (2002) that found no significant differences in the muscle composition. But fish fed a meal supplemented with yeast showed a considerable decrease in ash contents. Hassan et al. (2016) also found a trend toward a decrease in the ash contents of the entire body in tilapia as the amount of yeast added increased.

Digestive enzyme analysis in our study showed a marked rise in the activity of the enzymes amylase and protease. The trend in lipase enzyme activity, however, was not statistically significant. Significant increases in the activity of digestive enzymes were reported by Darafsh et al. (2019). Animals' ability to digest food and the development of their digestive tracts are reflected in the improvement of their digestive enzymes. As a result, it can be used to determine dietary requirements as well as to indicate nutritional status in the early stages of life (Albro et al., 1985).

Aquatic animals' antioxidant capacity is typically utilized to indicate their state of health (Yu et al., 2018). One of the main defenses against damage caused by free radicals is the presence of antioxidant enzymes. For instance, according to Reda et al. (2018), SOD and CAT lessen oxidative damage and preserve the equilibrium of free radicals. To shield cells and tissues from oxidative stress, animals control the amounts of SOD and CAT activity (Gong et al., 2019). According to our findings, SOD and CAT activity significantly increased, whereas GPx showed no discernible rise. Abdel-latif et al. 2023 found that feeding a meal enriched with yeast coupled with many probiotics significantly increased the activity of SOD, CAT, and GPx.

CONCLUSION

In conclusion, the addition of dietary yeast to *Labeo rohita's* diet did not have a deleterious effect on the species' overall proximate composition. The digestive enzymes protease and amylase displayed significant results that increased significantly with higher yeast inclusion levels while lipase enzyme activity displayed non-significant trend. The antioxidant enzymes analysis revealed beneficial effects in yeast supplemented diets with superoxide dismutase and catalase enzymes revealed significant differences while peroxidase enzyme activity showed minor variations across groups.

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