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EVALUATION OF GROWTH PERFORMANCE AND BLOOD BIOCHEMICAL CHANGES IN FISHES (*LABEO ROHITA*) SUPPLEMENTED WITH FRUCTOOLIGOSACCHARIDES UNDER HEAT STRESS

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ABSTRACT
This study was conducted to examine the effects of heat stress (HS) on growth performance and selected blood biochemical attributes of fishes supplemented with fructoloigosaccharide (FOS). The main objectives revolved around evaluating the negative impacts of HS and amelioration of those harmful effects by using FOS along with exploring the potential benefits of FOS in improving growth and biochemical parameters of (*Labeo rohita*) fishes reared under cyclic heat stress. Moreover, this study offers insightful information related to fish health, management and aquaculture practices. One hundred-eight (n=108) rohu fishes (*Labeo rohita*) were selected and were equally divided into five groups with each group having 3 replicates (n=18) on 21st day. The details of the grouping are T_0, T_1, T_2, T_3, T_4, T_5, in which T_0 was considered as control group which was fed with basal diet. T_1 and T_2 groups were supplemented with 1% and 2% FOS respectively, without HS. The group T_3 was positive control whereas, T_4 and T_5 were exposed to HS at 40 ºC supplemented with 1% and 2% FOS respectively. The results revealed that supplementation of fructooligosaccharides significantly improved growth performance and selected biochemical attributes of fishes during 63 days of rearing. The T_0 group exhibited significant decrease in growth and selected biochemical parameters compared to the treatment groups however, after treating with FOS the negative effects of HS were significantly reduced. Moreover, T_1 showed positive improvements in all the selected parameters of this experiment. Growth parameters were found higher in T_2 as in final weight (10.85%), weight gain (51.06%), survival rate (2.09%) at ($P \leq 0.05$) supplemented with 2% FOS as compared to T_0 and T_1 but the aforementioned parameters were found lower in T_3 under influence of 40ºC of HS as compared to T_0. Although biochemical parameters were found higher in T_2 as in albumin (119.38%), globulin (132.20%), ALP (7.81%) and total protein (34.28%) As compared to T_0 and T_1 but afforested parameters such as globulin and total protein were significantly decreased under HS as compared to rest of the treatment. Furthermore, several serum biochemical markers, including urea (40.86%),

cholesterol (28.58%), and triglycerides (31.24%) were significantly decreased in T_2 as compared to T_0 and T_1. In conclusion, the results revealed that supplementation of FOS alleviate the negative effects of HS in fishes (*Labeo rohita)*.

Key words: Cyclic heat stress, Fructooligosaccharides, growth performance, serum biochemical markers, urea, cholesterol, triglycerides.

INTRODUCTION

Aquaculture production has experienced the profligate growth in the animal food production in the last decade (Stankus, 2021). It is estimated to rise to 53% by the year 2030 (FAO, 2020). Almost 1 billion people make their living through aquaculture industry which is rapidly expanding (Obi *et al.*, 2016). In 2018, 46% of the world's total fish production was attributed to aquaculture (ACTION, 2020) . It has been reported that 50% of animal protein consumed by humans comes from aquaculture which is one of the food industry's fastest-growing segments globally. There are some problems such as heat stress (HS) that stop aquaculture from flourishing. (McKenzie *et al.*, 2021). In many parts of the world HS is one of the major environmental factor compromising production performance of animals (El-Tarabany *et al.*, 2017). The HS negatively effects growth of fish. Amur sturgeon *(Acipenser schrenckii*) and Atlantic salmo *(Salmo salar)* (Handeland *et al.*, 2008) are two fishes in which negative effects of HS have been reported which include reduced food intake whereas, in common carp *(Cyprinus carpio communis)* abnormal swimming patterns and respiratory disorders were observed. The HS is a serious issue that demands serious concern due to global warming (Hansen *et al.*, 2010). Ruminants, poultry and pigs are susceptible to HS due to their high metabolic rate, higher basal metabolic heat production, rapid growth and high level of production (Tajima *et al.*, 2007). The HS in fishes negatively impacts their feeding, growth and disease resistance by altering intestinal microbiota, disrupting intestinal barrier function and causing metabolic disorders.(Zhou et al., 2022). Changes in the climate is making HS gradually harmful to fish aquaculture. Cold-water fish like rainbow trout are particularly sensitive to water temperature fluctuations, leading to stress and negative impacts on food consumption, behavior, growth and survival above 18°C. (Zhu *et al.***,** 2019**)**. Fructo-oligosaccharides (FOS) are prebiotics derived from sucrose through the action of certain enzyme. They are a low-calorie and non-carcinogen sweetener that promotes ion absorption in the stomach, reduces fat, cholesterol levels and supports the growth of beneficial gut bacteria **(**Bali *et al.* (2015)

The (FOS) are a beneficial carbohydrate found in a variety of functional food products which include dairy items, bakery items, beverages, jams, candies, chocolates, breakfast cereals, meat, ice cream and confectionery (Kherade *et al.* (2021). The FOS is a type of oligosaccharide that is recognized as safe and have recently drawn particular attention as food ingredients because of their unique aspects and advantages in health (Tódero *et al.*, 2019). Introducing fish to new environments boosts aquaculture yields and water resource management. Fish contribute to 15% of animal protein in human diets (Casal, 2006). Aquaculture is a swiftly growing food industry which involves raising fish, shellfish and aquatic plants. During the last decade aquaculture has supplied one-third of the world's seafood (Reverter *et al.*, 2014). Fish from both inland and marine capture fisheries are highly consumed and traded globally playing a crucial role in supporting livelihoods, food security and improving economic growth (Loring *et al.*, 2019). According to FAO (2020) about 120 million people in developing nations depend on fisheries for their livelihoods with over 90 percent of them engaged in small-scale fisheries (Loring *et al.*, 2019). This study aims to explore the potential of FOS as a dietary supplement to mitigate the negative effects of HS on fish growth. Specifically, this study will examine whether FOS can enhance growth performance and alleviate hazardous effects of HS on fishes *(Labeo Rohita)*. By altering dietary strategies the research aims to improve fish performance and resilience under HS. This investigation aims to evaluate the growth performance and selected serum biochemical changes in fish supplemented with FOS during cyclic HS contributing to the development of sustainable aquaculture techniques for countering climate change challenges.

Material and Methods:

2.1. Animal Ethics:

All the experimental procedures were approved by Animal Care and Use Committee Bahauddin Zakariya University, Multan, Pakistan.

2.2. Experimental setup:

The experiment was conducted in fisheries research lab IPAB and the examination of the parameters was executed in the laboratory of Department of Environmental Science, Bahauddin Zakariya University (BZU) Multan. In this experiment one hundred-eight rohu fish (*Labeo Rohita*) were purchased from local fish farm having an average body weight of 15gm which were kept under environmentally controlled conditions.

2.3. Experimental Design:

One hundred-eight rohu fishes (*Labeo Rohita*) with an average weight of 15±2 gm were selected and divided into six groups T0, T1, T2, T3, T4, T5. All groups were fed with basal diet on daily basis (twice daily) and grown at normal temperature (26°C to 28°C) for 3 weeks. Three replicates were selected for each treatment. The experimental continued for 6 weeks and selected fish groups were subjected to HS during the last three weeks of experiment. After 21 days, the weight of the fish increased from 15±2 gm to 23.5 gm. Twenty- one days old fish with average weight of 23.5gm was exposed to FOS and HS. The treatment design for each group was set as:

Group I: Basal diet +No heat stress + No FOS named as T_0
Group II: Basal diet +No heat stress + 1% FOS named as T_1
Group III: Basal diet+ No heat stress + 2% FOS kept as T_2
Group1V: Basal diet+ only heat stress (40ºc) + NO FOS kept as T_3
Group V: Basal diet +1% FOS + heat stress Kept as T_4
Group VI: Basal diet +2% FOS + heat stress kept as T_5

The appropriate sizes of the basal diet were utilized as the basal feed in the experiment and its composition is shown in Table 1. The percentage composition of the ingredients for basal diet was formulated in line as in previously used by (Guerreiro *et al.*, 2016; Genç *et al.*, 2020).

Table 1. Percentage composition of ingredients for basil diet

Ingredients	**%**
Crude protein	48
Crude lipid	18
Crude Ash	12
Crude cellulose	2
Phosphorous	1.5
Moisture	11
Fat	13
Fiber	2.5
Starch	5

2.4. Assessment of selected parameters:

The growth parameters such as weight gain, final weight, feed intake and survival rate were estimated using digital electronic balance. The biochemical parameters such as aspartate aminotransferase (AST) (IU/L), alanine aminotransferase, glucose(mg/dL), triglycerides, total cholesterol and total protein (TP) was measured in the automatic biochemical analyzer (Mindray BS-400 bio- chemistry analyzer) using kits assay specially designed for fish detection. The alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes were measured calorimetrically from plasma with a kit (Abcam Cambridge, UK) in 96-well microplates. Amylase activity was determined by the starch hydrolysis method, according to the Somogy–Nelson colorimetric method. According to Hidalgo et al. (1999) the casein hydrolysis method was used to measure total protease activity.

2.5 Statistical Analysis

The collected data was analyzed statistically by using excel sheets and statistix 8.1 version. For the calculation of mean and standard error, MS office-2010 was used while one way Analysis of Variance (ANOVA) was done through statistical software statistix 8.1. Significant difference between the treatments was also observed through the Duncan Multiple Range Test (DMRT). The differences were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSIONS

RESULTS

The FOS are type of prebiotic carbohydrate that selectively stimulates the formation of beneficial bacteria in the gut and mitigate the impact of heat stress on fish (*Labeo Rohita*).

3.1. Effect of heat stress and fructooligosaccharides supplementation on growth Parameters:

There was a significant effect of FOS on growth parameter except feed intake. As illustrated in (fig.1a) final weight was significantly increased 4.91% in T_1 supplemented with 1% FOS while T_2 showed significant increase in final weight which was found to be 10.85% compared to T_0 and T_1. The final weight of T_3 fish *(Labeo Rohita*) under the effect of 40 ºC heat showed prominent difference as compared to control as well as rest of the treatments which was found to be less 9.84% than T_0 at $P \leq 0.05$. The final weight of T_4 was significantly increased that was found to be 2.81% compared to control while T_5 exhibited an increase in final weight that was 7.14% compared to T_4. As shown in (**fig.1b&d)** weight gain and survival rate were significantly increased in T_1 that was found to be 37.95%, 1.27% respectively supplemented with 1% FOS As compared to T_0. While T_2 showed significant increase in weight gain and survival rate that was found to be 51.06%, 2.09% respectively as compared to T_0 and T_1. The T_3 showed significant decrease in weight gain 12.75% and survival rate 11.76% As compared to T_0. As demonstrated in (**fig.1b**) that the weight gain of T_4 was significantly increased that was found to be 5.63% As compared toT_0 and T_3 while T_5 showed some extent of increase in weight gain that was 42.98% as compared to T_4 and T_3.

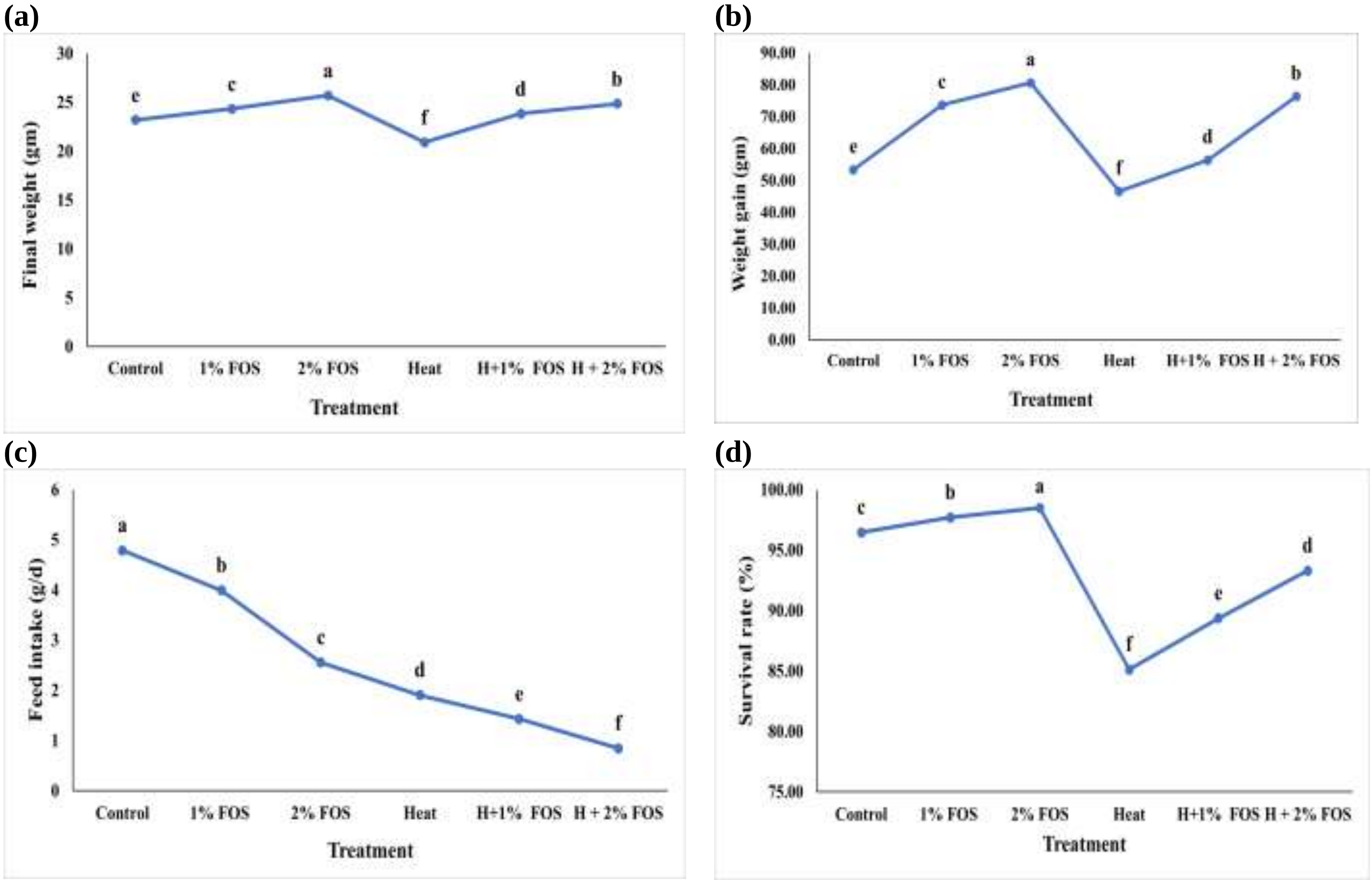

Figure 1: *Labeo rohita* fish fed with different level of fructooligosaccharides and heat on (a)final weight(gm) (b)weight gain(gm) (c)Feed intake (g/d) (d) Survival rate (%). Superscript denotes significant differences between control and treatment groups. (p<0.05)

As shown in (**fig.1d**) survival rate of T_4 that was found to be 7.35% which was significantly lower as compared to T_1 and T_0. Whereas, T_5 compared to T_4 exhibited significant decrease in survival rate which was found to be 3.28%. As demonstrated in (**fig.1c**) feed intake of T_1 was significantly reduced to 16.63% compared to T_0. Whereas, T_2 exhibited a further significant decrease in feed intake 46.62% as compared to T_0 and T_I. The results revealed that supplementation of FOS produced significant effects and countered the negative effects of HS at $P \geq 0.01$. Similarly, T_3 showed significant decrease in feed intake that was found to be 60.19% under HS compared to rest of the treatment groups as shown in (**fig.1c**) whereas, feed intake was found increase in T_4 70.14% as compared to T_3 and T_1. Feed intake of T_5 compared to T_4 was significantly decreased which was found to be 82.39% compared to T_3 and T_2.

3.2. Effect of heat stress and fructooligosaccharides supplementation on biochemical parameters:

As shown in Table 2 HS and FOS supplementation showed significant impact on albumin of fishes. The albumin of T_1 (4.61±0.16) was significantly increased 57.02% compared to T_0. The albumin content was also significantly increased in T_2 (6.45± 0.16) that was 119.38% at 2% dose of FOS compared to control at $P \leq 0.05$. Similarly, it increased significantly in T_3 (1.38± 0.12) which was significantly lower 52.94% compared to T_1 and T_2. However, compared to T_3 and the rest of the treatment groups T_4 (3.52 ±0.30) and T_5 (5.59± 0.22) exhibited increase in albumin which was 19.95% and 90.36% respectively. Globulin and total protein of T_1 (5.68± 0.12 and 6.57 ±0.29) significantly increased 80.61%, 20.25% respectively compared to T_0. Moreover, in T_2 a comparable rise in globulin (7.30 ±0.12) and total protein (**7.**33± 0.15) was found to be 132.20%, 34.28% in comparison to the T_0 and T_1. The T_3 demonstrated a considerable decrease in globulin (2.80±0.17) and total protein (2.68± 0.22) which was found to be 10.91% and 50.82% respectively, compared to the other groups whereas, globulin content of T_5 (6.79±0.04) was significantly higher and was found to be 115.78% compared to T_4. In this study (Table 2) revealed significant impact of FOS on protease which increased significantly 51.27% in T_1 (8.49±0.18).

Table:2: Biochemical parameters of *Labeo rohita* fed different levels of fructooliogosaccharide

Parameters	Treatment groups					
	Control	1% FOS	2% FOS	Heat ONLY	Heat + 1% FOS	Heat + 2% FOS
Albumin (g d/l)	2.94±0.06	4.61±0.16	6.45± 0.16	1.38± 0.12	3.52± 0.30	5.59± 0.22
Cholesterol (mg d/l)	106.05±0.05	83.35±0.12	75.74±0.04	109.48±0.30	89.51± 0.38	79.11± 0.06
Globulin (g d/l)	3.14± 0.08	5.68± 0.12	7.30 ±0.12	2.80 ±0.17	4.81± 0.18	6.79± 0.04
Protease (μm/g)	5.61± 0.17	8.49± 0.18	10.78±0.16	4.35± 0.31	7.94± 0.04	9.21± 0.14
Amylase(μm/g)	2.69± 0.14	5.66± 0.16	6.93± 0.03	4.44± 0.32	7.62 ±0.43	8.79± 0.24
Glucose (mg/dl)	70.18± 0.12	72.90±0.11	85.65±0.08	77.79± 0.18	93.79± 0.18	110.16± 0.06
Total protein (g/d)	5.46 ±0.30	6.57 ±0.29	7.33± 0.15	2.68± 0.22	3.64 ±0.45	4.64± 0.20
Triglycerides(mmol/dL)	57.47± 0.21	46.49±0.20	39.51±0.39	58.26± 0.09	49.43± 0.08	42.83± 0.19
Urea (mg/dl)	19.52 ±0.19	14.89±0.08	11.54±0.17	21.43± 0.31	17.45± 0.39	16.16± 0.16
Lipase(μ/g)	2.72± 0.36	3.76± 0.20	5.69± 0.31	4.95± 0.04	6.46 ±0.22	7.65 ±0.29
ALP (U/l)	113.53±0.21	118.65±0.15	122.40±0.17	103.70±0.22	110.63 ±0.31	119.35± 0.11
ALT (U/l)	27.19±0.13	25.69± 0.03	18.59±0.14	30.65± 0.42	29.66± 0.09	22.19 ±0.13
AST (U/l)	33.21± 0.09	29.11± 0.08	25.17± 0.12	34.65± 0.45	27.55± 0.17	22.20± 0.14

values are mean±SE of three observations. Mean bearing different superscript in a row differ significantly ($p \leq 0.05$). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase. Significant level ($p \geq 0.001$)

However, T_2 (10.78±0.16**)** exhibited significant increase in protease that was found to be 91.98% as compared to T_0 and T_1 with a significance level of $P \leq 0.05$. But T_3 (4.35±0.31) exhibited significantly decrease in protease which was observed 22.43% compared to T_0. Whereas, administration of 1% FOS with heat dramatically enhanced protease in T_4 (7.94±0.04) which was 41.48% higher than T_0. Similarly, protease of T_5 (9.21± 0.14) increased that was found 64.03% as compared to T_3 and T_4 (Table 2). The ALP significantly increased in T_1 (118.65±0.15) that was observed to be 4.31% compared to T_0. Similarly, T_2 (122.40±0.17) showed further increase in ALP 7.81% than T_0 and T_1 at $P \leq 0.05$. While ALP of T_3 (103.70±0.22) significantly decreased compared to T_0. Whereas, T_5 (119.35± 0.11) showed significant decrease in ALP 2.55% compared to T_0 and T_I, at a significance level of $P \leq 0.05$. As shown in (Table 2) amylase of T_1 (5.66± 0.16) showed significantly increase that was observed 110.53% compared to T_0. Similarly, further 157.74% increase in amylase was observed in T_2 (6.93±0.03) compared to control at $P \leq 0.05$. Moreover, amylase of T_3 (4.44± 0.32) showed significant decrease which was 65.30% compared to T_0 while, amylase in T_5 (8.79± 0.24) increased 226.88% compared to T_0. As presented in Table 2 significant impacts of HS and FOS on lipase content of (*Labeo Rohita*) fish were observed. The lipase content of T_1 (3.76± 0.20) increased significantly which was 38.23% compared to T_0. Furthermore, lipase (5.69± 0.31) of T_2 increased that was 109.31% compared to T_0 at $P \leq 0.05$. Whereas, lipase content of T_3 (4.95±0.04) exhibited a significant difference compared to T_0 and other treatment groups. While, lipase of T_4 and T_5 (6.46±0.22, 7.65±0.29 respectively) significantly increased which was 137.5% compared to 181.37% of T_0 and T_1. Furthermore, glucose level was significantly increased due to FOS supplementation. The T_1 (72.90±0.11) exhibited significant increase in glucose level that was noted to be 3.88% compared to T_0. However, supplementation of FOS in T_2 (85.65±0.08) significantly increased which was 22.04% compared to the other treatment groups. While in T_3 (77.79±0.18) glucose level was significantly increased which was found to be 10.85% at HS. Although further increase was observed in T_5 (110.16±0.06) which was 56.97% higher than T_4.

The current investigation found that several serum biochemical markers including urea, cholesterol, triglycerides, AST and ALT, significantly decreased in fishes fed with diets supplemented with FOS as demonstrated in Table 2. Cholesterol of T_1 (83.35±0.12) significantly decreased that was 21.40% compared to T_0. Whereas, T_2 (75.74±0.04) showed a comparable decrease that was observed to be 28.58% compared to T_0 at P≤0.05. While in T_3 (109.48±0.30) cholesterol level was significantly increased which was found to be 3.23% under influence of HS. While, cholesterol of T_4 and T_5 (89.51±0.38, 79.11±0.06 respectively) exhibited significant decrease which was observed to be 15.59%, 25.40% respectively as compared to T_0 and T_1. Results revealed that triglycerides of T_1 (46.49±0.20) significantly decreased that was seen in the form of 19.10% compared to T_0. Furthermore, a decrease was observed in T_2 (39.51±0.39) which was found to be 31.24% compared to T_1 at P<0.05 as well as control. While T_3 (58.26±0.09) showed significant increase that was 1.38% compared to T_2 and T_0. The T_5 (42.83±0.19) exhibited slight decrease in triglycerides that was observed to be 25.47% compared to T_4 and T_3.

Urea of T_1 (14.89±0.08) significantly decreased to 23.73% compared to T_0. Furthermore, a decrease in T_2 (11.54±0.17) was noted to be 40.86% compared to T_1, at significance level of P≤0.05. But in case of T_3 (21.43±0.31) exhibited significantly higher level of urea which was observed to be 9.78%. while T_4 revealed significant decrease in urea level that was observed to be 10.58% compared to T_0 and T_1. Similarly, T_5 (16.16±0.16) to some extent of revealed decreased in urea that was 17.2% as compared to T_3. In this study, liver enzymes AST was significantly decreased in T_1 (29.11±0.08) that was 12.35% compared to the control group. The AST level was significantly decreased in T_2 (25.17±0.12) that was 24.20% compared to T_0 at a significance level of P≤0.05. While T_3 (34.65±0.45) exhibited significant increase that was 4.31% compared to T_2 and T_0. Urea of T_5 (22.20±0.14) significantly decreased that was found to be 33.16% compared to T_4 and T_3. Results revealed a considerable impact of FOS on the ALT of the fish (*Labeo rohita*), as shown in Table 2. The ALT was significantly decreased in T_1 (25.69±0.03) that was 5.51% as compared to T_0. Similarly, further decrease was observed in T_2 (18.59±0.14) that was noted to be 31.64% compared to control at P≤0.05. Whereas, ALT of T_3 (30.65±0.42) significantly increased which was 12.72% compared to T_0. Moreover, T_5 (22.19±0.13) significantly decreased ALT which was 18.41% compared to T_4 and T_3.

DISCUSSIONS

The FOS are prebiotic carbohydrates that selectively stimulates the formation of beneficial bacteria in the gut and mitigate the impact of HS on fish *(Labeo rohita)*. Results of the present study conclusively demonstrated that dietary supplementation of FOS at 1% and 2% considerably boosted the digestive enzyme activities in (*Labeo rohita)* fishes which are comparable to the findings of Soleimani *et al.* (2012) who reported similar effects in Caspian roach (*Rutilus Rutilus*) fry and snout bream (*Megalobrama Amblycephala*) fingerlings (Wu *et al.*, 2013). Protease, amylase and lipase activities were reported to increase with increasing doses of dietary FOS. The causes of the higher levels are results of endogenous and exogenous microbial activities regulated by FOS. The findings of the present experiment demonstrated that fishes fed with diets supplemented with FOS exhibited significantly higher levels of selected serum biochemical markers which included glucose, albumin, globulin, ALP and total protein as shown in above table which are also comparable with the findings of Ali *et al.* (2020) but the decreased albumin and total protein in the treatment groups suggest an abnormality in kidney and liver function. In contrast to our findings Reza *et al.* (2009) in juvenile beluga reported that in an 8-week feeding trial various biochemical parameters were not significantly affected by dietary prebiotic inulin. The enzyme activities ALP, ALT and AST levels were not affected by supplementation of mannooligosaccharides at 0, 0.5, 1, 1.5, and 2% in Asian Seabass fingerlings.

The current investigation found that several serum biochemical markers including urea, cholesterol, triglycerides, AST and ALT significantly decreased in fish fed with diets supplemented with FOS which are in line to the findings of Feng and Xia (2019) who fed FOS to yellow broiler hens to lower the levels of total cholesterol and serum triglyceride which are two of the key elements of blood lipids. The FOS possesses the properties of dietary fiber that is water-soluble and may absorb lipids from the body while enhancing lipid metabolism to lower blood lipid levels. Serum urea nitrogen levels in the FOS group are lower in the experiment. The findings of the abovesaid study is in line to our results because in this experiment FOS is used as prebiotic supplement in yellow broiler which promotes development and activity of good bacteria in the gut. The digestion and absorption of nutrients are enhanced by these bacteria especially those of the *Bifidobacterium* and *Lactobacillus* species. Fish fed FOS-supplemented diets exhibited increased nutritional utilization, resulting in lower blood levels of urea, cholesterol and triglycerides randomly. According to Taati *et al.* (2011) changes in biochemical parameters may differ from one species to another depending on numbers of variables that may vary according to the dose of prebiotics added to the diet, type of feed ingredients and the experimental settings. The high levels of AST and ALT in the serum are results of HS.

The result of the current study showed that positive impact of FOS on growth parameters which included final weight, weight gain and survival rate which significantly increased while feed intake significantly decreased. These results are according to the results reported by Hu *et al.* (2019) but differ from the finding of Hashmi *et al.* (2023) in which final weight and weight gain significantly decreased after supplementation of prebiotic. The difference from our results id due to the fact that they used pectin as prebiotic whereas in our study the prebiotic used was FOS. According to the results of the current study, it was observed that the exposure of HS significantly decreased growth parameters of (*Labeo rohita*) which included final weight, weight gain, feed intake and survival rate which is similar to the findings of Mehaisen *et al.* (2017) who reported that growth performance of quails were negatively affected by HS. Moreover, recent studies have shown that colonies of chicken exposed to HS (over 30 °C) showed low production performance along with high morbidity and mortality resulting in significant economic losses on poultry farms (Niu *et al.*, 2009; Quinteiro-Filho *et al.*, 2012).

The findings of this study shows that glucose level of (*Labeo Rohita*) under HS was significantly increased which is similar to the results of Imik *et al.* (2010) because the increasing level of plasma glucose are derived from rise in free radicals along with the release of ACTH and cortisol hormones which restrict insulin release and promotes gluconeogenesis in response to HS Ajakaiye *et al.* (2010). Likewise Rashidi *et al.* (2010) also discovered that HS raised plasma glucose levels. Albumin, globulin and total protein was significantly decreased under influence of HS which is similar to the findings of Sarıca *et al.* (2017) as HS disrupts protein synthesis and accelerates its breakdown which lowers the quantity of protein in the body resultantly decreasing albumin, globulin and total protein levels related to alterations caused by HS in protein production and metabolism.

CONCLUSIONS

Nexus to the above it is concluded that cyclic heat stress (HS) adversely effects the health of rohu fishes (*Labeo Rohita*) by decreasing growth performance and negatively altering the selected biochemical parameters. Our results reveal that the detrimental effects of HS can be minimized with fructooligosaccharides (FOS) supplementation. The results of this study are indicative that rohu fishes (*Labeo Rohita*) exposed to HS exhibited significant recovery in growth performance and selected serum biochemical parameters after FOS supplementation. Results of this study reveal that FOS alleviate negative effects of HS. Therefore, it is concluded that FOS mitigate the harmful effects of HS in rohu fishes (*Labeo Rohita*).

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