Indian Journal of Ecology (2023) 50(4): 1163-1169
DOI: https://doi.org/10.55362/IJE/2023/4029

Manuscript Number: 4029
NAAS Rating: 5.79

Synergistic Effect of Prebiotic with Gut Isolated Probiotic Bacteria on Survival, Growth and Carcass Composition of *Labeo rohita*

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***Abstract*:** Feeding trial (120 days) was conducted to evaluate the synergistic effects of dietary supplementation of prebiotic and probiotic bacteria (*Lactobacillus plantarum* FLB1), isolated from the intestine of Indian Major Carp (*Labeo rohita*) on the survival, growth and carcass composition of *L. rohita* fingerlings. Six experimental diets were formulated containing basal diet as control (T1) and prebiotics, i.e. β-glucan (BG) and Mannan oligosaccharide (MOS) were added in basal diet (T1) at 2 different levels (T2 @ 0.2% BG + 10^8 *L. plantarum*, T3 @ 0.5% BG + 10^8*L. plantarum*, T4 @ 0.2% MOS+10^8*L. plantarum*, T5 @ 0.5% MOS + 10^8*L. plantarum*, and T6 @ 10^8*L. plantarum)* and fed to the fish for a period of 120 days @ 5% body weight in two parts, daily. Hematological parameters were observed at 30, 60 and 120 days intervals while growth parameters at 30, 60, 90 and 120 days intervals. Total body length (cm) and fish body weight (g) were significantly higher in T3. Average hemoglobin content, total erythrocyte count, total leukocyte count, hematocrit and erythrocyte sedimentation rate of fishes fed with T3 diet were significantly improved. Mean corpuscular volume, corpuscular hemoglobin and corpuscular hemoglobin content revealed positive effect in pre- and pro-biotic fed fishes. There was significant improvement in the carcass composition in T3 in terms of highest flesh protein as compared to other treatments and control.

***Keywords*:** Prebiotics, Probiotics, β-glucan (BG), Mannan oligosaccharide (MOS), Synergistic effect

In India, inland fish production has crossed 16.24 million tons during the year 2021 contributing over 76% to the total fish production (pib.gov.in 2023). However, disease outbreak is a major hurdle in the intensification and harnessing of full potential in carp farming. Overuse of antibiotics and therapeutics is leading to negative impacts like development of antimicrobial and drug resistance, accumulation of chemical residues in tissue and reduced consumer preferences. As a therapeutic measure in aquaculture (tanks, ponds or cages), antibiotics (mixed with specially formulated medicated feed) are orally administered to fishes for shorter duration. However, fishes do not effectively metabolize those antibiotics and therefore, a large portion (75%) is released back into the environment in the form of feces (Burridge et al 2010). Further, the quality and quantity of antibiotics and other related compounds used in aquaculture differed significantly between countries. Hence, the rise in bacterial antibiotic resistance and residues have become a global concern which calls for the need to develop alternative therapies for controlling bacterial pathogens in animal production, especially in aquaculture. Although vaccines are being developed and marketed to address this problem, however, these cannot be a universal disease control measure in aquaculture (Lara-Flores 2011).

Among alternative therapies, a variety of useful feed additives like medicinal herbs, immunostimulants, probiotics and prebiotics having beneficial effects on the host are being used in aquaculture. Certain non-digestible carbohydrates *viz.* polydextrose, lactosucrose, resistant inulin and oligofructose, transgalacto oligosaccharides (TOS), isomalto-oligosaccharides (IMO), palatinos, xylooligosaccharides (XOS), mannan oligosaccharides (MOS), lactose, hemicellulose, soybean oligosaccharides, glucooligosaccharides (GOS), gluconic acid and β-glucan display authentic prebiotic properties (Verkhnyatskaya et al 2019). The oligosaccharides prebiotics are reported to reduce β-glucuronidase and nitroreductase activities resulting in the enhancement of immunity, modulation of mucin production and expression of immune regulatory genes (Arturo et al 2010). This study was carried out to investigate the effect of different doses of prebiotic compounds combined with fish gut probiotic on rohu, *Labeo rohita*.

MATERIAL AND METHODS

Preparation and maintenance of experimental tanks: The study was conducted in outdoor cemented tanks (20m^2) where 3-5cm soil bottom layering was done to stimulate natural conditions and liming @ 300kg/ha for disinfection and as per requirement (pH balance) throughout the experimental period. Water exchange (1/4th of water) was performed with freshwater once a week.

Procurement, conditioning and stocking of experimental fish: Fingerlings of rohu (720 nos.) distributed randomly in all experimental tanks (40/tank) in triplicates. Proximate analysis of the feed ingredients, formulated feeds and fish flesh, water quality parameters, microbiological, hematological, and biochemical parameters studies were carried out. Prebiotics β-glucan (BG) and Mannan oligosaccharide (MOS) were added in basal diet (Table 1). The bacterial count of *L. plantarum* mixed in feed adjusted through spectrophotometer was @ 10^8 cfu/g. To achieve accurate final concentrations in the diet, the bacterial suspension was gradually added to feed pellets with a hand sprayer for uniform distribution in the laminar airflow chamber under sterilized conditions. The fishes were fed with experimental diet for a period of 120 days @ 5% body weight daily in two parts.

Water quality parameters: Water samples were collected fortnightly in the morning hours for analyzing the physico-chemical parameters (APHA 2013) *viz*. temperature, pH, dissolve oxygen (DO), total alkalinity (TA), total hardness (TH), orthophosphate (PO_4^{-2}) and ammoniacal nitrogen (NH_3-N).

Blood collection: Prior to collection of blood, fishes were anesthetized with clove oil @ 30-50 mg/l(1-part clove oil and 9-parts 94% ethanol) (Hajek et al 2006) and blood were collected via caudal vein puncture and then pooled from a random sample consisting of five fish from each replicate. Hematological parameters were observed at 30, 60 and 120 days intervals while growth parameters at 30, 60, 90 and 120 days. Blood (heparinized 150 IU/ml) collected from each group were analyzed for the hematological parameters *viz.* hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), hematocrit (Ht) and erythrocyte sedimentation rate (ESR). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin content (MCHC) were calculated by the method of Mukherjee (1988). Hemoglobin (Hb) concentration was estimated by acid haematin method (Sahli 1962).

Growth of fish: The growth of fish was assessed in terms of total body length and weight at monthly intervals during 120 days of experimental period. At the end of the experiment, growth (length and weight) parameters in terms of total length gain (TLG), percent total length gain (%TLG), net weight gain (NWG), percent net weight gain (%NWG), specific growth rate (SGR), growth index (GI), condition factor (K) and feeding efficiency in terms of protein efficiency ratio (PER), feed conversion ratio (FCR) and feed conversion efficiency (FCE) for each treatment was calculated.

Flesh composition: Carcass composition was assessed at the end of experiment. Flesh quality (% wet weight basis) in terms of total proteins, total lipids, total carbohydrates, ash content and moisture were estimated for each treatment and control.

Statistical analysis: The differences between parameters were analyzed by using SPSS software 16 version.

RESULTS AND DISCUSSION

Physico-chemical parameters of water: Physico-chemical parameters were under optimal range (Boyd 1988) with insignificant differences among the treatments (Table 2).

Growth of fish: Survival rate was 100% in all the treatments along with control. The total body length gain (cm) was significantly higher in T3 (9.95) followed by T5 with non-significant differences (Table 3). The body length gain was significantly low in T1 (7.43). The total body net weight gain (g) was maximum in T3 (59.73) with % net weight gain of 581.86 followed by T5 and minimum in T1 (33.34). The specific growth rate was also significantly higher in T3 (1.60) followed by T5 (1.50) and minimum in T1 (1.19) (Table 4). The growth Index depicted the same pattern with highest value in T3 followed by T5.

In all the treatments, 100 % survival rate at the end of experiment indicated no harmful effect of prebiotics (β-glucan and MOS) and probiotic (*L. Plantarum*) supplemented diets. The length and growth parameters revealed improved fish growth performances in synbiotic and probiotic incorporated diets (T2-T6), with significant improvement in T3 (0.5% β-glucan + 10^8 of *L. plantarum/* g feed*)* and T5 (0.5% MOS + 10^8 LAB) as compared to that of control. No information is available on synergistic effect of β-glucan and *L. plantarum* or MOS and *L. plantarum* on rohu, however, Giri

Table 1. Details of treatment for experiment

Treatments					
Control diet	Basal diet supplemented with gut probiotic bacterial (GPB) culture				
T_1	T_2	T_3	T_4	T_5	T_6
Basal diet*	Basal diet + 0.2% BG + 10^8 *L. plantarum*	Basal diet + 0.5% BG + 10^8 *L. plantarum*	Basal diet + 0.2% MOS+10^8*L. plantarum*	Basal diet + 0.5% MOS+10^8*L. plantarum*	Basal diet + 10^8 *L. plantarum*

*Rice bran[1] (49%) + Mustard meal[1] (49%) + Vit-Min. mixture (1.5 %) + Salt (0.5%)
[1]De-oiled rice bran, β-glucan (BG) and MOS (Mannan Oligosaccharide)

et al (2013) reported higher specific growth rate and feed utilization efficiency in the juveniles of *L. rohita* when fed with *L. plantarum* only. The positive interactive effects of heat-killed *L. plantarum* (HK-LP) and β-glucan (BG) on body weight gain, specific growth rate, feed intake and protein efficiency ratio was reported by Dawood et al (2015) in juvenile red sea bream, *Pagrus major*. Probably, β-glucan was degraded in the digestive gland by glucanases to produce energy, hence, permitting the use of more proteins for growth. Mishra et al (2006 a,b) reported increased SGR and reduced FCR in *L. rohita* when β-glucan was supplemented in diets as immunostimulant or through direct injection.

Feeding efficiency: Feeding efficiency of prebiotic and probiotic supplemented feed was observed in terms of FCR, FCE and PER (Table 5). FCR was significantly lower in T3

Table 2. Mean physico-chemical parameters of water in different treatments

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Temperature (^{o}C)	30.64±0.31	30.78±0.32	30.79±0.32	30.81±0.33	30.73±0.31	30.70±0.32
pH	7.73±0.04	7.74±0.03	7.80±0.03	7.76±0.03	7.78 ±0.03	7.71 ±0.03
DO (mg/l)	6.47 ±0.04	6.50 ±0.03	6.46 ±0.06	6.44 ±0.06	6.55 ±0.04	6.40 ±0.05
TA ($CaCO_3$ mg/l)	154.52 ±1.73	161.63 ±2.05	156.96 ±2.10	160.26 ±2.34	160.07 ±1.86	164.33 ±1.87
TH ($CaCO_3$ mg/l)	245.04 ±2.75	241.96 ±3.03	243.15 ±2.59	242.30 ±2.71	239.19 ±3.04	243.48 ±2.36
NH_3-N (mg/l)	0.04±0.002	0.04±0.002	0.04±0.002	0.04±0.003	0.04±0.002	0.04±0.002
Orthophosphate (mg/l)	0.07±0.002	0.07±0.001	0.08±0.002	0.07±0.002	0.08±0.001	0.07±0.001
NO_3^-N (mg/l)	0.30±0.01	0.32±0.01	0.31±0.01	0.32±0.01	0.30±0.01	0.29±0.01

See Table 1 for treatment details. Values are Mean ± S.E. ($p \leq 0.05$)
DO – Dissolved oxygen, TA – Total Alkalinity, TH – Total hardness

Table 3. Length parameters of rohu, *Labeo rohita* (Ham.) during the experimental period

Month	Days	Treatments					
		T1	T2	T3	T4	T5	T6
May	0	8.70±0.15	9.00±0.06	8.78±0.10	8.80±0.06	8.67±0.11	8.79±0.14
	30	9.87±0.16	10.73±0.38	10.63 ±0.45	10.76±0.49	9.61 ±0.22	10.91±0.15
June	60	11.37±0.07	12.35±0.27	11.94±0.22	12.25±0.33	11.39±0.32	12.64±0.43
July	90	13.23±0.15	14.12±0.19	14.96±0.34	13.91±0.11	14.29±0.06	14.45±0.20
August	120	16.13±0.15	17.25±0.41	18.73±0.12	17.04±0.15	18.21±0.12	17.01±0.15
Total length gain		7.43±0.20	8.25±0.43	9.95±0.06	8.24±0.12	9.54±0.04	8.22±0.18
Length gain %		85.55±3.49	91.67±5.12	113.44±3.42	93.64±1.38	110.02±1.39	93.64±3.23

See Table 1 for treatment details

Table 4. Weight parameters of rohu, *Labeo rohita* (Ham.)in different treatments during the experimental period

Month	Days	Treatments					
		T1	T2	T3	T4	T5	T6
May	0	10.50±0.15	10.47±0.18	10.27±0.07	10.38±0.22	10.47±0.18	10.33±0.18
	30	16.23±0.29	19.63±0.75	26.53±0.64	19.51±0.54	21.73±0.48	19.10±0.53
June	60	24.30±0.23	28.53±0.65	35.55±1.86	28.63±0.77	31.53±0.58	28.37±0.74
July	90	35.43±0.61	41.00±0.40	46.03±1.23	40.94±0.64	42.27±0.24	39.98±0.23
August	120	43.84±1.97	56.60±4.20	70.00±0.51	52.16±0.52	63.05±1.32	50.51±0.11
Net weight gain		33.34±2.05	46.14±4.16	59.73±0.53	41.78±0.54	52.58±1.27	40.18±0.16
% NWG		317.96±22.38	440.74±39.31	581.86±7.35	402.96±11.48	502.60±13.29	389.11±8.01
Specific growth rate		1.19±0.05	1.40±0.06	1.60±0.01	1.35±0.02	1.50±0.02	1.32±0.01
Growth index		2.18±0.22	3.41±0.39	4.82±0.07	3.03±0.11	4.03±0.13	2.89±0.08

See Table 1 for treatment details

(2.97) followed by T5 whereas, FCE and PER was significantly higher in T3 (0.34, 1.32) and T5 (0.33.1.29) as compared to other treatments and control. Similar result was observed in previous works in FCR in all pre- and pro-biotic supplemented feeds. For instance, the extracellular enzymes might be activated by pre- and pro-biotic supplementation resulting in high feed utilization and improved growth performance. Cultured species are primarily affected by beneficial bacteria through the enhancement of host nutrition due to the stimulation of digestive enzymes resulting in a higher growth and feed efficiency ratio (Suzer et al 2008). Furthermore, the presence of beneficial bacterial cells in the intestine improves microbial balance, which in turn improves nutrient absorption and utilization Lara-Flores 2003). Moreover, the prebiotics addition in fish feed along with probiotics improve feed digestion and availability of nutrients (Gibson et al 2004) leading to improvement in overall fish nutrition and feed efficiency. Khattab et al (2004) revealed positive effect of biogen (synbiotic) @ 0.1% in terms of best growth performance and feed efficiency (reduced FCR) in *O. niloticus*.

Hemato-immunological parameters: The hemato-immunological parameters of fish including Hb, TEC, TLC, Ht or Packed Cell Volume (PCV), MCV, MCH, MCHC and ESR were analyzed at 30, 60 and 120 day interval. Significant difference was observed in hemoglobin content of all the treatments between 30 days and 60days interval except T1 where no significant difference in Hb was observed up to 120days. Elevated hemoglobin content at 60 days in T2, T3, T4, T5 and T6 reduced after 120 days but remained higher in comparison to 30 days. T3 had the significantly higher hemoglobin content at 60 days interval within itself and in comparison, to other treatments (Table 6). At 60 days interval, RBC count was also observed significantly higher in T3 (2.17) followed by T5 and lowest in T1 (1.82). The WBC were significantly higher in all the pre and probiotics supplemented treatments (T2-T6) as compared to control T1. The WBC count was observed highest in T3 (6.01) followed by T5 (5.32), T2, T4, T6 and T1 after 30 days supplemented feeding. Post 60 and 120 days of feeding, WBC reduced significantly in T3 (5.22) and T5 (5.01). Nayak et al (2007) reported higher leucocyte count in rohu, treated with probiotic bacterium *B. subtilis*. Misra et al (2006) revealed significantly increased leucocyte count after 28 day and highest on 42nd day with reduction on 56th day, when BG was added @ 100 to 500 mg/kg. Hassan et al (2014) also recorded highest levels of Hb, Ht, TEC and TLC in *T. niloticus* when fed with *B. licheniformis* (4.8x10^{5}cfu/g) in combination with 5g/kg yeast extract.

The results with respect to hematology in the present study clearly indicated the improvement of health status of fish in T3 (*L. plantarum* @ 10^8 cfu/g + 0.5% β glucan) followed by T5 (*L. plantarum* @ 10^8 cfu/g + 0.5% β glucan) and T6 (*L. plantarum* @ 10^8 cfu/g) in terms of Hb, TEC, PCV and ESR at day 60 whereas TLC also followed the same pattern, but maximum value was at day 30. MCV, MCH and MCHC followed the same pattern and showed variations in accordance to Hb, Ht and TEC. In teleost, probiotic can positively stimulate several immune-hematological parameters, including mononuclear phagocytic cells (monocytes and macrophages), polymorphonuclear leukocytes (neutrophils) and natural killer cells (Balcazar 2003). The results of the present study depicted positive effect of supplementation of probiotic and prebiotic on fish health in terms of higher hemoglobulin along with improved TEC, TLC and Ht. The improvement in hematological parameters is due to the ability of the probiotics to stimulate blood formation (Renuka et al 2014). Further, because of the complex structure of ß glucan, have superior ability to activate the immune response and act as biological response modifiers (Miura *et al* 1996). Larger molecular weight glucans

Table 5. Changes in total feed consumption (g) of *Labeo rohita* (Ham.) in different treatments during the experimental period

Month	Days	Treatments					
		T1	T2	T3	T4	T5	T6
May	30	15.75±0.23	15.70±0.26	15.40±0.10	15.57±0.33	15.70±0.26	15.50±0.26
June	60	24.35±0.44	29.45±1.13	39.80±0.96	29.27±0.81	32.60±0.66	28.65±0.79
July	90	36.45±0.35	42.80±0.97	53.33±2.79	42.95±1.15	47.30±0.87	42.55±1.11
August	120	53.15±0.92	61.50±0.60	69.05±1.85	61.41±0.96	63.40±0.36	59.97±0.35
Total feed		129.70±0.64	149.45±0.63	177.58±2.17	149.20±2.68	159.00±1.96	146.67±1.25
Feed conversion ratio		3.92±0.28	3.29±0.30	2.97±0.06	3.57±0.02	3.03±0.09	3.65±0.04
Feed conversion efficiency		0.26±0.02	0.31±0.03	0.34±0.01	0.28±00	0.33±0.01	0.27±0.003
Protein efficiency ratio		1.01±0.06	1.21±0.11	1.32±0.05	1.10±0.01	1.29±0.03	1.07±0.01

See Table 1 for treatment details

(yeast ß glucan) activate leukocyte, stimulating phagocytic, cytotoxic, antimicrobial activities and production of reactive oxygen species. Studies have shown that insoluble ß glucan of larger molecular weight have greater biological activity than that of its soluble and low molecular counterparts (Ooi and Liu 2000).

Flesh Composition

Total protein: The protein content was significantly higher in all the pre- and pro-biotics supplemented treatments (T2-T6) as compared to T1 (12.58). Among ß glucan + probiotics treatments (T2-T3), the total protein content was significantly higher in T3 (14.90) whereas in MOS + probiotics treatment (T4-T5), the difference for total protein content were insignificant. The results revealed that supplementation of pre and/or probiotic (T2-T6) in feed improved the flesh quality in terms of total protein content (Table 7).

Total lipid: Total mean lipid content in fish flesh ranged from 1.80 to 1.87 g/ 100 g (on wet wt. basis) in treatments T1, T2,

Table 6. Comparative hematological parameters of rohu, *L. rohita* (Ham.) at different time interval in different treatments

Parameters	Days	Treatments					
		T1	T2	T3	T4	T5	T6
Hb (g %)	30	4.23±0.00	5.06±0.04	5.41±0.0	4.91±0.02	5.20±0.00	4.94±0.03
	60	4.33±0.03	5.53± 0.03	6.44±0.07	5.36± 0.03	6.21±0.06	5.20± 0.05
	120	4.32± 0.04	5.22±0.05	5.73±0.09	5.01±0.04	5.25±0.05	4.96±0.03
RBC ($x10^6$ /mm^3/l)	30	1.71±0.02	1.75±0.01	1.75±0.03	1.82±0.01	1.86±0.02	1.87±0.03
	60	1.82±0.01	2.06±0.01	2.17±0.02	2.07±0.02	2.11±0.02	2.02±0.01
	120	1.79±0.04	1.96±0.05	2.08±0.04	1.91±0.03	2.04±0.00	1.90±0.03
WBC ($x10^3$ /mm^3/l)	30	3.87±0.02	4.84±0.02	6.01±0.08	4.55±0.05	5.32±0.07	4.48±0.05
	60	3.95±0.08	4.65±0.04	5.22±0.06	4.35±0.05	5.01±0.08	4.29±0.02
	120	3.83±0.04	4.35±0.06	4.96±0.12	4.16±0.05	4.67±0.02	4.07±0.02
PCV (%)	30	21.93±0.08	23.63±0.31	24.56±0.06	22.96±0.08	23.83±0.03	23.33±0.12
	60	23.50±0.20	26.43±0.14	27.76±0.14	26.46±0.23	26.76±0.38	25.93±0.03
	120	23.47± 0.12	25.10±0.15	26.45±0.25	25.05±0.28	25.43±0.46	24.84±0.05
MCV (μm^3)	30	126.84±1.62	134.55±2.16	139.95±2.69	126.21±1.50	127.95±1.69	124.42±2.43
	60	128.90± 1.67	127.93±1.77	127.79± 1.37	127.71± 2.57	126.70± 2.54	128.39± 0.59
	120	130.79±2.48	128.03±3.32	126.85± 1.52	131.23± 1.92	124.69± 2.62	130.36±2.35
MCH (g %)	30	24.46±0.39	28.81±0.44	30.84±0.65	26.98±0.09	27.91±0.33	26.35±0.35
	60	23.77± 0.27	26.77±0.30	29.66±0.64	25.88±0.15	29.42±0.51	25.74±0.43
	120	24.08±0.83	26.66±0.82	27.51±0.96	26.28± 0.45	25.73±0.18	26.06±0.48
MCHC (g %)	30	19.28±0.07	21.42±0.41	22.03±0.04	21.38±0.20	21.82±0.03	21.19±0.24
	60	18.44±0.30	20.93±0.30	23.21±0.38	20.28±0.29	23.22±0.12	20.05±0.24
	120	18.40±0.28	20.82±0.12	21.67±0.49	20.03± 0.35	20.66±0.52	19.99±0.16
ESR	30	2.36±0.005	2.31±0.003	2.27±0.005	2.32±0.003	2.30±0.008	2.29±0.003
	60	2.35±0.00	2.18±0.01	2.06±0.03	2.20± 0.00	2.07± 0.01	2.21± 0.01
	120	2.82± 0.01	2.27± 0.01	2.21±0.01	2.28±0.01	2.28±0.01	2.30±0.00

See Table 1 for treatment details

Table 7. Flesh composition (% wet weight basis) of *L. rohita* (Ham.) in different treatment at completion of experiment

Parameters (%)	Treatments					
	T1	T2	T3	T4	T5	T6
Total proteins	12.58±0.11	14.07±0.09	14.90±0.12	14.03±0.07	14.37±0.13	14.04±0.12
Total lipid	1.80±0.03	1.87±0.09	1.87±0.09	1.77±0.12	1.80±0.10	1.85±0.12
Total carbohydrate	3.76±0.03	3.37±0.13	3.50±0.15	3.43±0.13	3.43±0.12	3.03±0.05
Ash	1.84±0.03	1.79±0.01	1.77±0.02	1.71±0.04	1.70±0.02	1.69±0.01
Moisture	79.92±0.17	79.16±0.022	78.00±0.10	79.13±0.07	78.17±0.05	79.19±0.24

See Table 1 for treatment details

T3, T4, T5, and T6 (Table 7) and the differences among treatments were insignificant (T6=T5=T4=T3=T2=T1). The results indicated that probiotic supplementation did not affect the flesh total lipid content significantly.

Total carbohydrate: In different treatments, the total carbohydrate content in fish flesh was significantly higher in T1 (3.76g/100 gm) with lowest in T6 (3.03g/100 gm). The results revealed significant decrease in total carbohydrate content of flesh with pre and/or probiotic supplements.

Ash content: Maximum ash content was observed in T1 (1.84g/100gm) and lowest in T6 (1.69/100gm) with significant difference. The result indicated that pre and/or probiotic supplementation in fish feed resulted in decrease in ash content of fish flesh.

Moisture content: In different treatments, moisture in fish flesh was highest in T1 (79.92) and lowest in T3 (78.00) and the differences among treatments were significant (Table 7). Among ß Glucan + probiotics treatments (T2-T3), the total moisture content was significantly low in T3 (78.0), whereas in MOS + probiotics treatment (T4-T5), the mean total moisture content was significantly low in T5 (78.17).

Flesh quality in terms of total protein and fat revealed improved body composition of rohu in the present study. Toutou et al (2016) also reported improved biochemical composition in terms of enhanced protein and fat content of grass carp when fed with either probiotics alone or in combination with prebiotic in the form of commercial synbiotic (Microban aqua). According to Abdel Tawwab et al (2010), yeast supplementation improved the protein content of flesh with no significant differences in lipid content of *O. niloticus*. These findings are also in agreement with present study in terms of improved flesh quality with probiotic with β-glucan/MOS (extracted from yeast) supplemented diets.

CONCLUSION

The diet having only probiotic (*L. plantarum*@ 10^8) revealed higher net profit (6.91%) in comparison to control on the basis of the overall fish growth performance, health status with special reference to hematological parameters and flesh composition, *L. plantarum* @ 10^8 cfu/g + 0.5 % ß-glucan can be given as booster diet to fingerlings *L. rohia* for 60 days during the growth period along with routine feeding. Hence, dietary supplementation of prebiotic β-glucan along with *L. plantarum* @ 10^8cfu/g in diet is effective in enhancing the immunity and overall health status of *L. rohita*.

AUTHOR'S CONTRIBUTIONS

Prem Kumar-Conducted the experiment and analysis work w.r.to all parameters and manuscript writing; Vaneet Inder Kaur- Conceived the idea, planning and execution of experimental study and critical inputs in manuscript; Anuj Tyagi-Helped in isolation of probiotic bacteria and feed formulation; Sachin O. Khairnar- helped in analysis work pertaining to hemato-immunological parameters and flesh composition.

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Received 08 March, 2023; Accepted 20 July, 2023