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Incorporation of inulin in the diets of Amur common carp (*Cyprinus carpio haematopterus*) feed to enhance growth, haematological parameters and immunological parameters

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Abstract
Aquaculture now viewed as strong option for increasing fish production as it plays a vital role in providing food security to the world. There is a need for enhanced disease resistance, feed efficiency and growth performance of cultured fish species. Dietary supplementation of different feed additives. Immunostimulants such as probiotics and prebiotics usually in small quantities for the purpose of fortifying it with certain nutrients have been found to be beneficial for improving immune status, feed efficiency and growth performance of finfishes. Inulin dietary supplements have been demonstrated to improve the development and health of fish. This study investigated the impact of varying doses of Inulin, a naturally occurring prebiotic, on the growth, nutritional utilization, liver histology, immune responses and immunological parameters of Amur common carp. The present study of prebiotic inulin performance on fish health and growth in Amur common carp was investigated for 90 days. The basal diet in all trials (30% protein) was incorporated with different levels of prebiotic 5 g/kg (T_1), 10 g/kg (T_2), and 15 g/kg (T_3) inulin and control diet (T_0) with no prebiotics in triplicates. A stocking density of 15 fish (4±0.5 g) per tank was stocked in an FRP tank and fed at 5% of body weight for 90 days to evaluate growth performance, hematology, and immune response. The T_2 group outperformed the T_1, T_3 and control groups in terms of growth measures such the Protein Efficiency Ratio (PER), weight increase, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR). The fishes fed with treatment T_2 showed significantly positive elevation in immune parameters such as NBT assay, lysozyme activity, and phagocytic activity, followed by T_1, T_3 and control groups. The Treatment of T_2-fed fish showed higher serum parameters such as serum albumin serum globulin. The highest serum protein and albumin and globulin ratio was observed in T_0 (control). Hematological markers such as RBC, WBC, hemoglobin, hematocrit, MCV, MCH and MCHC revealed no significant differences but increased levels in the T_2 group compared to T_1, T_3 and control groups. This study concluded that the utilization of Inulin at different concentrations exhibits advantageous prebiotic properties in fish, leading to favorable outcomes in growth and overall well-being. Therefore, inulin compounds have significant promise as prebiotics in fish diets.

Keywords: Haematology, inulin, immunostimulants, immune response, prebiotic

1. Introduction

The major portion of the population, particularly the fishing community, relies on the fishing industry as a source of income, helping to ensure the nation's nutritional security. India is the world's fourth-largest exporter of fish products, contributing around 7.7% of global fish production. In the year 2019-20, the total fish production amounted to 141.64 lakh tones, with the marine sector accounting for 37.27 lakh tones and the inland sector providing 104.37 lakh tones. Aquatic animals raised in culture frequently contract viral and bacterial illnesses because their immune systems are often weakened by stressful environments. The aquaculture business is unfamiliar with the idea of useful feed. It symbolizes a developing new model for creating meals that go beyond meeting the essential nutritional needs of the cultured organism. It would be worthwhile to explore this research even further because it could be very beneficial to the business (Li and Gatlin, 2004) [23]. In culturable fishes such African catfish, carp, catfish, tilapia and others, several growth promoters are currently used to raise feed-to-muscle conversion rates and improve fat and protein distribution. (Nowosad *et al*., 2023) [29]. Research has shown that using growth promoters in farmed aquatic species may enhance feed efficiency, production levels, immunological function and overall fish health. Prebiotics are carbohydrate sources that act as food sources for beneficial live microbes and their metabolic products are frequently added to fodder when raising agricultural animals.

Prebiotics have been shown to have a good impact on the host's healthy gut bacteria, including lactic acid bacteria (LAB), which are engaged in gut metabolism (Carbone and Faggio, 2016) [8]. Prebiotics are non-digestible carbohydrates (NDCs) that specifically promote the development and metabolism of gut-resident, health-promoting microbes. Bifidobacteria and other lactic acid-producing bacteria, as well as other commensal microorganisms, are encouraged to colonize and grow in abundance by Inulin. The fishing industry required novel, inexpensive sources of inulin-type fructans due to the dietary prebiotic Inulin's positive effects on several fish species productivity. The majority of the inulin that is easily accessible for use in animal feed, according to study by Hoseinifar *et al.* (2016), is found in chicory, which has also been shown to enhance fish growth performance, immunological parameters, and intestinal flora. In the digestive tracts of monogastric animals, this compound of non-digestible soluble fiber is used as a substrate for fermentation (Dawood and Koshio, 2016) [14].

Amur carp is a popular fish species worldwide, known for its exquisite taste and high nutritional value. As the demand for Amur carp increases globally, there has been an increased focus on improving their growth rate and overall health through innovative feeding strategies. In recent years, the use of inulin-based diets has gained popularity as a promising approach to promoting the growth and health of Amur carp (Wang *et al*., 2022) [11]. Inulin is a natural prebiotic fiber that has been found to enhance the growth and health of Amur carp. Studies have shown that inulin-based diets can improve the quality of gut microflora, leading to better nutrient absorption and immune function in Amur carp (Radhakrishnan *et al*., 2020) [33]. In addition to its prebiotic properties, Inulin also has low caloric value and can serve as a substitute for other high-calorie feed ingredients (White and Hekmat, 2018) [42], thereby potentially reducing the cost of feed and promoting sustainable aquaculture practices. Overall, the inulin diet can be a promising strategy for promoting the growth and health of Amur carp while also contributing to sustainable aquaculture practices (Verdegem *et al*., 2021) [15].

The study of hematologic factors can be used to evaluate the state of aquatic animals in reaction to stress, pollutants, nutrients, and biological and metabolic conditions. The assessment of blood cells, blood biochemistry and hormones could be helpful for illness detection and monitoring the physiological state of fish (Stoskopf, 1993) [40]. The use of inulin-based diets has been found to enhance the growth and health of Amur carp, making it popular as a promising approach for promoting aquaculture. Studies have demonstrated that Inulin is a natural prebiotic fiber that enhances gut microflora's quality and leads to better nutrient absorption (Naor *et al*., 2012) [28]. This study comprised a quantitative analysis of growth, immune response, and blood parameters in amur common carp fed diets supplemented with prebiotic Inulin.

2. Methodology

2.1 Collection of Fish

A total of 180 healthy Amur carp fingerlings were procured from the Fisheries Research and Information Centre, Hesaraghatta, Bangalore (mean individual weight 4.0±0.5 g). They were reared in FRP Tanks and fed with a balanced diet at 5% body weight. Twice a week, the water was partially renewed and it was routinely checked. 25 ºC was maintained as the temperature. For a few weeks, fishes were kept in the aquariums for adoption.

2.2 Preparation of Experimental Diet

For protein optimization, three test diets were formulated with a protein content of 30% by using the square technique (Hardy, 1980). Three experiment diets were prepared by incorporating inulin 5 g, 10 g and 15 g per kg of feed and after that, it is referred to as T_1, T_2 and T_3. The control diet was prepared without incorporation of Inulin and is referred to as T_0 (Tables 1).

2.3 Experimental Design

The experiment was conducted in 250-liter FRP tanks at the Fisheries Research and Information Centre, Hesaraghatta, Bengaluru. Uniform in size Amur common carp fingerlings, with an average length and weight of 6.0±0.5 cm and 4.0±0.5 g respectively, were introduced into tanks at a stocking density of 15 individuals per tank. The experiment was conducted over a period of 90 days. The water exchange was done twice every week to remove the left-over feed and fecal matter. The fish were sampled once every 15 days to record their growth and survival.

Table 1. Proportion of various ingredients in different experimental diets.

Ingredients (%)	Treatment			
	T_0	T_1	T_2	T_3
Fish-meal	36.3	36.3	36.3	36.25
Groundnut oil cake	36.3	36.3	36.3	36.25
Rice bran	12.8	11.8	10.8	9.25
Tapioca	13.8	13.8	13.8	13.75
Vitamin-mineral mixture	1	1	1	1
Inulin	0	0.5	1	1.5
Total (100 g)	100	100	100	100

2.4 Growth and Survival performance

Fishes of all replicates were weighed after 90 days of feeding trials, and body gain was measured. Total weight gain (WG), Specific growth rate (SGR), Protein efficiency ratio (PER), Feed conversion ratio (FCR) and mortality were observed throughout the experimental period.

$$\text{SGR (\%)} = \frac{[\text{Ln(Final weight)} - \text{Ln(Initial weight)}]}{\text{No. of days}} \times 100$$

$$\text{Weight gain (\%)} = \frac{[\text{ Final weight(g)} - \text{ Initial weight(g)}]}{\text{Initial weight(g)}} \times 100$$

$$\text{FCR} = \frac{\text{Dry weight of the feed given}}{\text{Gain in wet weight of fish}}$$

$$\text{PER} = \frac{\text{Gain in body}}{\text{Protein intake}}$$

2.5 Blood sampling
The fish from each replication were subjected to anaesthesia and blood samples were obtained by breaking the caudal vein using a 2 ml syringe that had been washed with an anticoagulant (Heparin). The blood was immediately transferred to heparin-coated vials. Blood was collected in 2ml centrifuge tubes without any anticoagulant and centrifuged for serum collection after centrifugation supernatant is pooled and stored at-20 °C.

2.6 Hematological parameters:
Hematology parameters such as hematocrit, hemoglobin count, mean corpuscular hemoglobin (MCH), mean Leucocyte count, cell volume, erythrocyte count and mean corpuscular haemoglobin concentration and values were analyzed. An automatic hematology analyzer was used for the analysis of blood parameters. The MCV, MCH and MCHC values acquired from the hematology analyzer were further verified using standard formulae.

$$\text{MCHC} = \frac{\text{Hemoglobin (g/dl)}}{\text{Hematocit (\%)}} \times 100$$

$$\text{MCH} = \frac{\text{Hemoglobin (g/dl)}}{\text{RBC count (million mm}^{-3})} \times 10$$

$$\text{MCV} = \frac{\text{Hematocrit (\%)}}{\text{RBC count (million mm}^{-3})} \times 10$$

2.7 Immunological parameters
The immunological parameters, including serum albumin, serum globulin and total serum protein, were assessed using a serum analyzer (Clinical Chemical Analyzer, AGDTM 2020) using kits provided by AGDTM Biomedicals Pvt. Ltd., located in Mumbai, India. The Nitroblue tetrazolium (NBT) assay was performed using Anderson and Siwicki (1995) [5] method (Anderson and Siwicki, 1995) [5]. The turbidimetric technique proposed by Parry *et al*. (1965) [32] was used to quantify lysozyme activity (Parry *et al.*, 1965) [32]. *In vitro*, phagocyte functional assays were carried out using the Cunningham method, with minor changes to the incubation times (Smith and Romme, 1997) [38].

2.8 Statistical analysis
The experiment was conducted in triplicate, with each tank containing 15 fish as the experimental unit. The statistical methodology used in this research was the application of one-way analysis of variance (ANOVA) to ascertain the existence of a statistically significant disparity in the average growth, survival, and immunological response in relation to the treatment variable. The researchers proceeded to perform the Duncan Multiple Range Test using the SPSS software (version 20.0) in order to further examine these observed variations.

3. Results
The Growth performances and survival rate in Amur carp fingerlings were determined (Table 2). During the experimental period, the dietary supplemented Inulin showed improved feed conversion ratio, specific growth rate, weight gain, protein conversion ratio, and survival, with significant differences in these parameters within the diets. Survivability in the experimental period is almost similar in all the treatments. For the Amur fingerlings, after a 90-day feeding trial, the diets fed with 5 g, 10 g and 15 g showed better growth performances, including SGR, weight gain, and PER with high significance (p<0.05) and with that, the lowest FCR were seen in the treatments fed with inulin diets compared to control diets (p<0.05). The survival rates in Amur carp fingerlings have shown significant improvements in Inulin incorporated diets compared to control diets (p<0.05).

Table 2. Under various treatments and in the control group, FCR, SGR, PER, WG and fish survival were measured.

Parameters	Treatments			
	T_0	T_1	T_2	T_3
WG (%)	128.43±2.588^a	170.97±6.722^b	254.61±5.501^c	140.30±6.204^a
SGR (% per day)	0.92±0.012 ^{a}	1.11±0.029 ^{b}	1.41±0.018 ^{c}	0.97±0.029 ^{a}
FCR	1.66±0.034 ^{c}	1.33±0.026 ^{b}	1.11±0.025 ^{a}	1.63±0.030 ^{c}
PER	2.54±0.033 ^{a}	3.51±0.044 ^{c}	4.99±0.107 ^{d}	2.87±0.047 ^{b}
Survival (%)	95.8	97.8	100	97.8

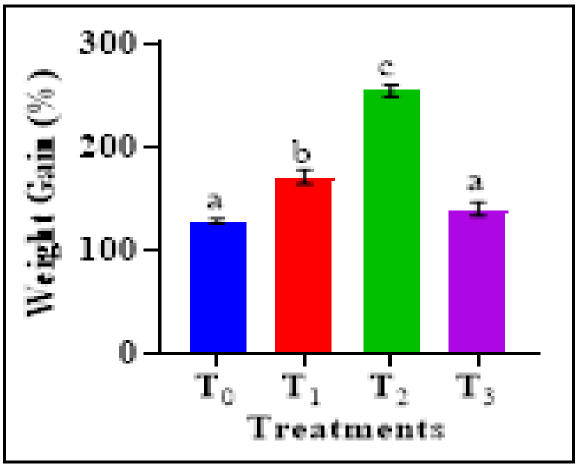

Fig 1: Influence of Inulin on carp weight increase

Figure 1 depicts the influence of Inulin on carp weight increase. T_0 has the least amount of inulin influence on fish weight. Compared to the control group T_0, T_2 had the largest effect of Inulin on fish weight gain. T_3 showed the inulin effect on fish weight to be the least significant.

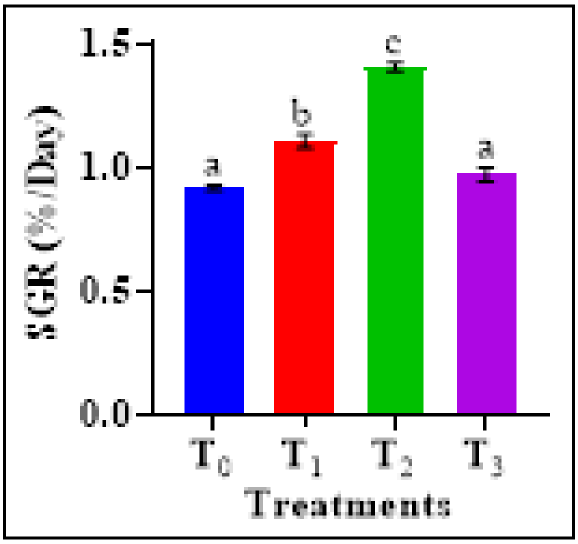

Fig 2: Influence of Inulin on carp specific growth rate

Figure 2 shows the impact of Inulin on fish (carp) specific growth rate. T_2 had the highest influence of Inulin on fish-specific growth rate compared to control T_0, followed by T_1 and T_3.

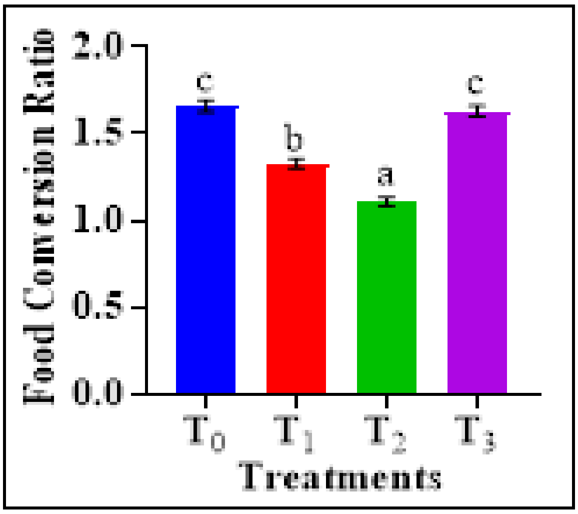

Fig 3: Food conversion ratio in fish carp after administering inulin

Figure 3 illustrates the food conversion ratio in fish carp after administering the dietary supplement inulin. The influence of the food supplement on T_3 is equal to that of the control group T_0. The dietary supplement was shown to have minimal impact on T_2.

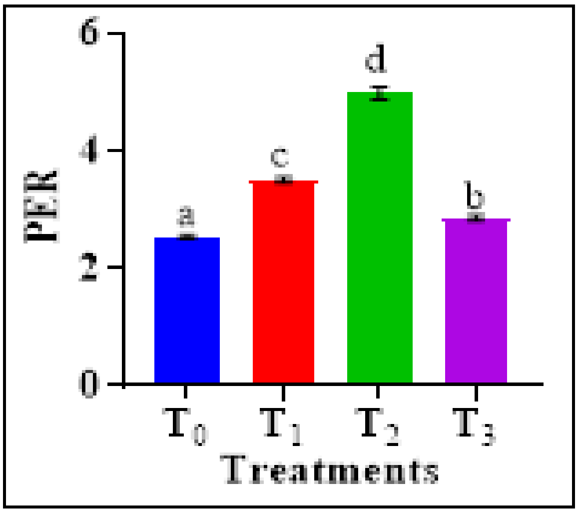

Fig 4: Protein conversion ratio in fish after administration of inulin

Figure 4. shows the protein conversion ratio in fish after administration of the dietary supplement inulin. The effect of the dietary supplement inulin was observed maximum in T_2 compared to control T_0. The minimal effect of the dietary supplement inulin was observed in T_3.

The hematological parameters were analyzed (Table 3). The dietary supplementation of Inulin has shown improved hemoglobin levels, RBCs, WBCs, Haematocrit, MCV, MCH and MCHC concentrations with high significance compared to the control diet (p<0.05).

Table 3: Hematological parameters of Amur common carp (Cyprinus carpio haematopterus) were recorded in different treatments and control groups.

Parameters	Treatments			
	T_O	T_1	T_2	T_3
Hemoglobin (g/dl)	10±0.58 ^{a}	11±0.58 ^{a}	11.8±0.72 ^{a}	10.4±0.31^{a}
RBC (10^6/mm^3)	4.60±0.12 ^{a}	4.90±0.03 ^{b}	5.10±0.12 ^{b}	4.87±0.04 ^{ab}
WBC (10^3/mm^3)	6.90±0.57 ^{a}	8.80±0.10 ^{c}	9.40±0.21 ^{d}	7.50±0.15^{b}
Hematocrit (%)	30±1.73 ^{a}	33±1.73 ^{a}	35.4±2.16 ^{a}	31.2±0.92 ^{a}
MCV (fl)	65.22±1.47^{a}	67.35±0.82^{a}	70.66±1.62^{a}	64.10±1.86^{a}
MCH (pg)	21.0±1.15^{a}	22.44±0.66^{a}	23.55±1.10^{a}	21.35±1.22^{a}
MCHC (%)	30.0±1.73^{a}	33.0±2.65^{a}	32.0±1.15^{a}	31.8±1.08^{a}

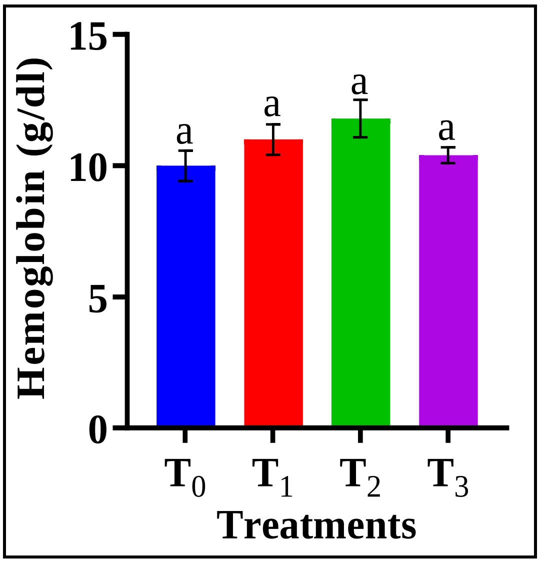

Fig 5: Impact of Inulin on hemoglobin

The impact of Inulin on hemoglobin is seen in Figure 5. T_1 has an equivalent impact on hemoglobin in the presence of Inulin as compared to control T_0. T_2 has the highest degree of influence of Inulin on hemoglobin.

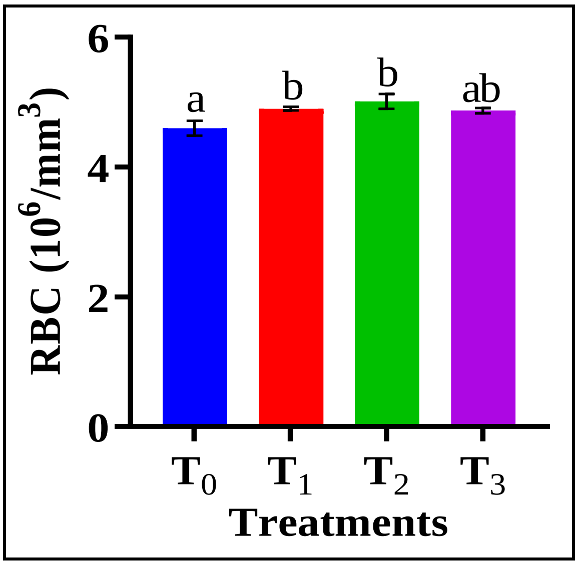

Fig 6: Influence of dietary supplementation of Inulin on the fish's RBC

Figure 6 presents the influence of dietary supplementation of Inulin on the fish's RBC (106/mm3) levels. Treatment T_2 has the most significant observable influence of Inulin when compared to the control treatment T_0, whereas treatment T_3 displays a comparatively weaker effect. The effect of Inulin in the treatment group labelled as T_1 was determined to be minimal

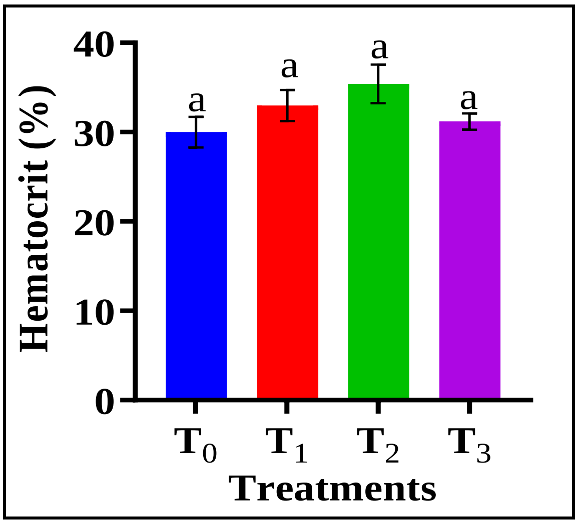

Fig 7: Impact of dietary supplementation of Inulin on the hematocrit levels of fish

The influence of dietary supplementation of Inulin on the hematocrit levels of fish is shown in Figure 7. Treatment T_2 has the highest observed impact of Inulin compared to the control treatment T_0, with treatment T_1 exhibiting a lesser effect. The observed impact of Inulin was found to be minimal in treatment group T_3.

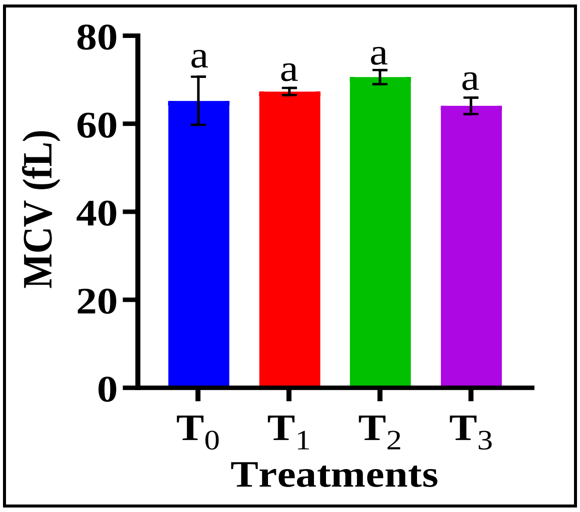

Fig 8: Influence of dietary supplementation of Inulin on fish's MCV (ft) levels

Figure 8 presents the influence of dietary supplementation of Inulin on fish's MCV (ft) levels. Treatment T_2 has the most significant observable influence of Inulin when compared to the control treatment T_0, whereas treatment T_1 displays a comparatively weaker effect. The effect of Inulin in the treatment group labelled as T_3 was determined to be minimal.

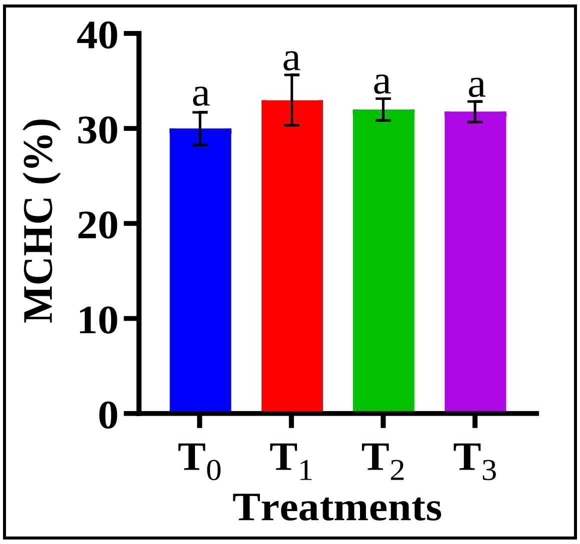

Fig 9: Impact of dietary supplementation of Inulin on fish's MCH concentration levels

The impact of dietary supplementation of Inulin on fish's mean corpuscular hemoglobin concentration levels is shown in Figure 9. Treatment T_1 has the most pronounced and statistically significant impact on Inulin compared to the control treatment T_0, followed by T_2 and T_3.

The outcome of the dietary inulin supplementation on immune parameters is shown in Table 4. The Inulin-supplemented fishes have significantly improved values of NBT, Lysozyme activity and Phagocytic activity compared to control-fed fishes ($p<0.05$). The study revealed that fishes fed control diets exhibited higher blood protein, globulin, and albumin globulin ratio levels than those fed Inulin. This difference was statistically significant across the various treatments ($p<0.05$). Following the administration of various doses of an inulin-based dietary supplement, the observed Lysozyme activity (10^3/l) in fish is shown in Table 4. When compared to the control treatment T_0, the T_2 therapy shows greater levels of Lysozyme activity (10^3/l) in fish. Following the administration of dietary inulin supplementation, the lowest measured NBT activity in fish was seen in T_1.

The total serum protein (g/dl) in fish after the administration of different doses of an inulin-based dietary supplement is shown in Table 4. The T_0 (control) treatment demonstrates elevated levels of NBT activity in fish compared to the treatment group (T_1, T_2 and T_3). The albumin (g/dl) level in fish after the administration of different doses of an inulin-based dietary supplement is shown in Table 4. The T_0 (control) treatment demonstrates elevated levels of albumin (g/dl) level in fish in comparison to the treatment group (T_1, T_2 and T_3).

Similarly, table 4 presents the observed globulin (g/dl) level in fish after the administration of varying dosages of an insulin-based dietary supplement. The T_2 treatment exhibits higher levels of globulin (g/dl) in fish compared to the control treatment, T_0. The lowest measured globulin (g/dl) level in fish was seen in T_3 following the administration of dietary inulin supplementation.

Following the administration of various doses of an insulin-based dietary supplement, the observed phagocytic activity (%) in fish is shown in Table 4. Compared to the control treatment T_0, the T_2 therapy shows greater levels of phagocytic activity (%) in fish followed by T_3. Following dietary inulin supplementation, the lowest measured phagocytic activity (%) in fish was seen in T_1.

The albumin-globulin ratio in fish after administering different doses of an inulin-based dietary supplement is shown in Table 5. The T_0 (control) treatment demonstrates higher levels of albumin-globulin ratio in fish compared to the treatment group (T_1, T_2 and T_3).

Table 4: Serum immunological parameters of Amur common carp (*Cyprinus carpio haematopterus*) recorded in different treatments and control group.

Parameters	Treatments			
	T_O	T_1	T_2	T_3
Total serum protein (g/dl)	7.5±0.153 ^{b}	6.8±0.265 ^{a}	7.2±0.10 ^{ab}	7.0±0.10 ^{ab}
Albumin (g/dl)	4.5±0.115 ^{b}	3.5±0.153 ^{a}	3.8±0.153 ^{a}	3.5±0.115 ^{a}
Globulin (g/dl)	3.0±0.577 ^{a}	3.3±0.115 ^{b}	4.4±0.115 ^{c}	2.8±0.577 ^{a}
Albumin-globulin ratio	1.5±0.058 ^{b}	1±0.029 ^{a}	0.86±0.051^a	1.25±0.076^a
NBT activity (OD at 620 nm)	0.48±0.028 ^{a}	1.37±0.029 ^{b}	1.48±0.046 ^{b}	1.39±0.069^b
Lysozyme activity (10^3/l)	52±4.041 ^{a}	170±23.094 ^{a}	990±63.509^c	700±46.188^b
Phagocytic activity (%)	24.56±0.468 ^{a}	32.14±0.364 ^{b}	39.57±0.202^d	35.31±0.323^c

4. Discussion

In the food production industry, aquaculture has emerged as the sector with the quickest rate of growth and has taken on a crucial role in maintaining global food security. The significance of adequate nutrition in the context of aquatic animal production systems cannot be stressed, as it is essential to guarantee the growth of a strong and outstanding product while keeping expenses to a minimum. The significance of nutrition in fish farming cannot be understated, given that the feed cost alone accounts for a significant amount of the total production costs, ranging from 40% to 50%. Strategies apart from antibiotics and chemotherapeutics are required to maintain a sustainable development pattern and health management since these treatments depress the host's immune system and breed resistant microorganisms (Chinabut and Puttinaowarat, 2005) [12]. Although alternative therapies like vaccinations are being explored, there is still no solid evidence for several farmed fish species (Mahious *et al*., 2006) [24]. Feed additives, including vitamins, minerals, and prebiotics, are well recognized in aquaculture as a potential means to improve the general welfare of aquatic animals and mitigate the risk of diseases. The two main reasons for the rise in functional feed additives in recent years are the challenges associated with controlling disease outbreaks in fish used for human consumption and the increasing activation of host non-specific defence mechanisms against pathogenic microorganisms (Chinabut and Puttinaowarat, 2005) [12].

Prebiotic Inulin, a fructo oligosaccharide, is crucial for maintaining immune system health in humans, domestic animals, and fish including Atlantic salmon (Salmo salar), Hybrid Striped Bass, Arctic charr (*Salvelinus alpinus*), weaning turbot (Psetta maxima), Gilt head sea bream (*Sparus aurata*) (Cerezuela *et al*., 2008) [9] and other organisms. Despite the extensive collection and analysis of data on utilizing Inulin as a prebiotic in fish, a definitive consensus has yet to be established regarding its efficacy in promoting prebiotic or immunostimulatory effects in fish (Ibrahem *et al.*, 2010) [21]. This study determined that better feed intake at the initial feeding stage would enhance fish development, health and survival effectively throughout the growth period. Fish growth is a straightforward and convenient metric for evaluating the impact of chemical or biological agents' impact on fish populations and aquaculture productivity. The performance of fish development and its health status as a result of prebiotic inulin incorporation in diets was investigated in this study.

Prebiotic supplements have been proven to significantly reduce the spread of potentially harmful species in aquatic animal diets while simultaneously increasing the numbers of beneficial bacteria including bifidobacteria and lactic acid bacteria (Gibson and Roberfroid, 1995) [17]. Bifidobacteria and lactic acid bacteria are important because they have various positive impacts on the host, particularly in terms of digestion, mineral absorption, and immune system improvement (Rengpipat *et al.*, 2000; Seifert and Watzl, 2007) [34, 36]. The results derived from the present investigation established that Inulin was fed to juvenile Amur common carp as a dietary additive at doses of 0.5%, 1% and 1.5%. The Inulin fed fishes had the largest weight gain (WG) and mean length (p<0.05) than the control group. The Inulin supplemented had a higher specific growth rate (SGR) of (p<0.05), suggesting a larger proportion of total growth. The FCR in the Inulin fishes was substantially lower value, indicating greater feed utilization by the treated fish (p<0.05). The protein efficiency ratio (PER) in the insulin-supplemented treatments was higher than the control group and was shown to be substantially improved (p<0.05). Similar results were observed. Inulin and FOS dietary supplements have been shown to improve growth performance in several species. For instance, adding Inulin (5 and 10 gram per kilogram) as a supplement increased FCR and weight growth in rainbow trout (Ortiz *et al*., 2013) [31]. Bakke-McKellep *et al.* (2007) [6] stated that an inulin-supplemented diet did not affect body weight or length. At 1 and 2 months of the trial, the percentage of those who survived was much higher in the inulin-fed group (Bakke-McKellep *et al.*, 2007) [6]. Akrami *et al*. (2009) [2] reported that various performance measures, such as WG, SGR, PER and survival, have a negative association. According to a study conducted by Akrami *et al.* (2009) [2], fish that were given meals with 1% and 3% inulin showed a decrease in feed intake when compared to diets that were supplemented with baseline and 2% inulin.

However, no significant alterations in feed consumption were observed. This suggests that the reduced growth performance of belugas given high-inulin meals is not due to a lack of feed palatability. Mahious *et al.* (2006) [24] found that feeding Siberian sturgeon at the rate of 20 g kg^{-1} inulin for 30 days resulted in a considerable increase in growth (Mahious *et al.*, 2006) [24]. The results obtained in our experiment on Inulin are different from those published by Burr *et al*. (2010) [7], who looked at the effects of several prebiotics, including Inulin (FOS), on the digestive system, microbiome and hybrid stripe bass performance. They claimed that adding the studied prebiotics, including Inulin, did not affect the hybrid striped bass growth or performance indicators (Burr *et al*., 2010) [7]. The present experiment may encourage maximum growth and fish performance owing to the kind of fish, feeding rate, starting fish weight, and trial duration. According to Anne *et al.* (2007), the administration of dietary supplements did not provide statistically significant changes in body weight or length.

The survival of the Amur common carp was not much affected by dietary administration with prebiotic Inulin. During the trial, some death was seen mainly due to the fish escaping through the shade net. The survival rate was high in inulin-fed groups compared to the control group. However, a few findings show that prebiotics negatively impact some species at increased doses of assimilation. The current experiment had no obvious anomalies in the fish's development patterns or behavior. Safari *et al.* (2014) [35] reported that the usage of FOS in the feed of crayfish has higher health-promoting benefits. The survival rate of crayfish on a diet consisting of 1.5 g kg^{-1} FOS (Fructo oligo saccharide) was high in their study, Olsen *et al.* (2001) [30] documented adverse effects on Arctic charr from including a 15% inulin supplement in their diet. Inulin or FOS dietary supplements increased the survival rate of common carp fry, although the improvement in survival rates that was most noticeable was not noticed in the latter growing phase of common carp (Olsen *et al.*, 2001) [30].

Hematological analysis is another significant indicator for judging fish health and evaluating fish feed additives. The earliest detectable and quantitative reaction to any climate change is generally a change in the hematological profile (Hawkins *et al.*, 2006) [16]. This study determined that Inulin supplemented diets positively affected hematological parameters such as hemoglobin, mean corpuscular hemoglobin, hematocrit, mean corpuscular volume, red blood cell count, white blood cell count, and mean corpuscular hemoglobin concentration. Ahmadifar *et al.* (2010) [1] found that supplementing beluga juvenile meals with 1% inulin significantly improved WBC and lymphocyte levels. The mean values of MCH and MCHC rose as inulin supplementation increased, whereas the mean values of PCV, WBC, Hb, RBC and MCV dropped. As Ibrahem *et al*. (2010) [21] reported, supplementing feeds with 5 g kg^{-1} prebiotic Inulin resulted in no significant changes in PCV levels. The considerable modification in hematocrit in Nile tilapia, hybrid surubim (*Pseudoplatystoma* sp.) and hemoglobin in leopard grouper was not impacted by dietary Inulin. RBC, WBC, Hb, PCV, MCV and lymphocyte levels rose in the fish fed with 1% FOS (Akrami *et al.*, 2013). Hoseinifar *et al.* (2011) [20], on the other hand, found no statistically significant differences in hematological parameters for WBC, Hb and lymphocyte in beluga fed diet FOS. Previous research on the Channel catfish, Nile tilapia, Grass carp and Asian seabass hematological parameters were not significantly affected by the dietary mannan oligosaccharide. There is a clear link between the number of white blood cells in an animal's blood and the severity of the infection (Douglass *et al.*, 2010) [16].

Fish serum immunological indicators are critical because they give information on proper metabolism and overall health. Many serum metabolites, such as total serum protein, albumin, and globulin, are represented by the amount of stress and pathogenetic factors in the environment. In the current experiment, Inulin supplemented meals had no detrimental effect on serum immunological markers; in fact, several were improved. The nitroblue tetrazolium (NBT) test indicates the generation of oxidative radicals by neutrophils and monocytes for pathogen defense (Anderson and Siwicki, 1995) [5]. In macrophages/monocytes and granulocytes, the respiratory burst, which creates superoxide radicals (ROS), is well understood in mammals. Bactericidal properties are seen in these ROS. This mechanism is thought to have a role in the death of microorganisms (Johnston, 1978) [22]. During phagocytosis or stimulation, phagocytes create a substantial amount of superoxide anion. After interaction with superoxides, the NBT reduction product produced is an excellent predictor of fish health or vaccine efficiency (Anderson *et al*., 1992) [4]. In the present study, the diet comprising 10 g/kg inulin had improved (p<0.05) NBT activity over other treatment and control groups. Similarly, Ibrahem *et al.* (2010) [21] discovered that the neutrophil oxidative radical production test yielded insignificant results for Inulin after 1 month but exhibited an increase after 2 months compared to the control group. In contrast, the administration of inulin supplementation yielded a statistically insignificant increase in the quantity of NBT-positive stained cells (Wang and Wang, 1997) [11]. The fish that were treated with a diet supplemented with FOS displayed a considerable decrease in neutrophil oxidative radicals compared to those that were given a baseline diet or diets supplemented with MOS, Previda, or GOS (Zhou *et al.*, 2010) [43]. Similarly, blood neutrophil oxidative radical production in hybrid striped bass was substantially related with dietary yeast inclusion (Li and Gatlin, 2004) [23].

Increased amounts of lysozyme, an essential enzyme in the blood that actively lyses germs, have been considered a natural protective barrier in fish. In this study, all three treatments indicated significant differences (p<0.05) in all groups, with the Inulin groups having higher lysozyme activity than the control groups. Aquatic animals are consistently subjected to a diverse range of opportunistic infections, hence necessitating the ongoing engagement of their innate immune system. This crucial facet of the immune response functions as the first defense barrier for the host organism (Soleimani *et al.*, 2012) [39]. Cerezuela *et al.* (2008) [9] observed a substantial rise in lysozyme activity in gilthead sea bream in the Inulin-supplemented group, similar to our findings. The gilthead seabream fed 10 g kg-1 Inulin had a considerably stronger favorable effect on several innate immunological markers than the fish fed various doses of the prebiotic (Cerezuela *et al*., 2013) [10]. Akrami *et al.* (2012) [2] found that supplementing MOS (15 or 30 g kg^{-1}) increased lysozyme activity considerably. Compared to the 1.5 g kg^{-1} MOS and control groups, fish given 4.5 g kg^{-1} MOS exhibited considerably higher serum lysozyme activity. In the present study, the diet comprising inulin diets had improved (p<0.05) Phagocytic activity over the control group. The research findings support those of (Misra *et al*., (2006) [25]; Sirimanapong W *et al.*, 2015) [37], who found that Glucan increases Phagocytic Index and Phagocytic activity percentage considerably. (Selvaraj *et al*., 2006) and the results obtained in some of the research findings of Cuesta *et al.* (2004), who found that glucan increases Total leucocytes count (TLC) significantly. The total serum protein content is a fundamental indicator of fish health (Mulcahy, 1971) [26]. The primary serum proteins, namely albumin and globulin, are important in the immune response. The results of this research demonstrate that there was no statistically significant difference (p>0.05) seen among the three treatments based on the three metrics. However, it is worth noting that the control group exhibited greater levels compared to the treated groups. Similar to our results, the experimental feeds had no effect on albumin, total serum protein, or globulin (Akrami *et al*., 2009) [2]. The plasma total protein level of fish fed prebiotic inulin-rich diets was decreased than that of the control group. Compared to the control and 3 % fed fish, fish fed with 2 % dietary oligofructose considerably reduced blood cholesterol levels. The A/G ratio was considerably lower (p<0.05) in all treatment groups than in the control group. The drop in the A/G ratio indicates that the animal has improved immunity, which might be responsible for the increase in globulin levels compared to albumin. The fact that total serum protein and globulin levels have increased shows fish have a powerful immune system (Nayak, 2010) [27]. Hence, it is plausible that the inclusion of dietary prebiotics, particularly Inulin, might potentially provide advantages to the immune system and elicit immunostimulatory responses, contingent upon the specific kind of prebiotic, species of fish, and duration of supplementation. These results imply that exploring the effects of using each prebiotic as an immunostimulant in each fish species is necessary.

5. Conclusion

The results obtained in this study showed that dietary prebiotic inclusion at various doses of feed has improved growth performance and survivability in Amur carp. In addition, the dietary inclusion of prebiotics increased the hematological and immunological parameters. Regarding growth metrics, hematological parameters, survival rate and biochemical parameters, the therapy T_2 produced the best results. All fish survived after being given to Amur carp (Cyprinus carpio haematopterus), indicating no effect on the fish's survival rate. Between treatments and the control group, all measures have significant associations (p<0.05). In every therapy, the survival rate was 100%. Hence, the incorporation of dietary prebiotic Inulin as a supplement in animal feed is a viable option that offers advantages in terms of improving growth parameters and strengthening the immune response in amur carp.

6. References

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