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Effect of Inulin Fortified Diet on the Growth, Carcass Composition, Haematological Responses, Intestinal Microbiota and Histological Alterations of GIFT

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ABSTRACT

The experiment was conducted in the year 2021 at wet laboratory, Advanced Research Farm Facility, Dr. M.G.R. Fisheries College and Research Institute, TNJFU, Madhavaram, Tamil Nadu, India to investigate the effect of inulin on the growth performance, haematology, carcass composition, intestinal microbiota and histology alterations of Genetically Improved Farmed Tilapia (GIFT). Four experimental diets were designed to incorporated inulin at 0 (basal diet), 0.5 g kg^{-1}, 1.0 g kg^{-1} and 1.5 g kg^{-1}. Fish with an initial mean weight of 0.85±0.02 g were allotted 12 tanks of 50 L capacity at a density of 20 fish per tank and fed with 5% of their body weight, two times a day. At the end of the experiment, the growth performance, feed utilization and haematology parameters were improved in fish fed with inulin at 1.5 g kg^{-1} of diet compared to control and other dietary treatments. A diet with 1.5 g kg^{-1} of inulin improved the total bacteria and lactic acid bacteria in the intestine of GIFT. The addition of inulin altered the intestine and liver histology. In the intestine, villi are short and dilated in fish fed with inulin on the 60th day, whereas hepatocytes and sinusoids showed vacuolation and congestion in the liver in fish fed with inulin on the 30th and 60th day. No histological change was observed in the gill. Substantially dietary inulin at 1.5 g kg^{-1} had beneficial effects on the growth performance, haematology and intestinal microbiota of GIFT.

KEYWORDS: GIFT, growth, haematology, histology, inulin, carcass composition

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1. INTRODUCTION

Fisheries and aquaculture is one of the most swiftly-widening industries in the World (Tacon, 2020) and has been playing an important role in the economic development front on account of its contribution to food and nutritional security, national income, employment opportunities as well as generating livelihood options (Kumar and Shivani, 2014). It is the primary source of animal protein for billions of people Worldwide, where capture fishery and aquaculture serve the livelihoods of more than 10% of the global population.

Tilapia is a hardy herbivorous fish that feeds on algae or small aquatic plant cells and is primarily raised in freshwater systems using cages, ponds, raceways or open waters. The water conditions in the farming operations have an important impact on product quality and taste (Joshna et al., 2024). Tilapia has been called the aqua-chicken because of the breeding improvements and mass production. Nile tilapia, *Oreochromis niloticus* (L.) is an important species for freshwater aquaculture. Improving fish performance and disease resistance of cultured organisms are major challenges facing fish culturists. Tilapia are second only to carps as the most widely farmed freshwater fish in the world. Advantages of GIFT are faster growth rates than other farmed strains. Improved survival in polluted waters and those they can be raised in extensive systems without the need for commercial feeds (Anusha et al., 2024). The benefit of three fish crops per year raises the yield potential and income generation from the smallest of ponds. GIFT consumes rice bran to weeds and even sewage but it is mainly plant-eating.

Different chemotherapeutic agents such as antibiotics and disinfectants are routinely used in the treatment and prevention of a large number of diseases in farmed fish. The antibiotic should be safe to the host, permitting their use as therapeutic agents for the treatment of infectious bacterial disease. Mostly for treating diseases, antibiotics are used but sometimes antibiotics are used to prevent the disease by treating the water and fish and also acknowledged that preventing disease in prophylactic method is profitable because it prevents loss and allows fish to grow quickly. However, unacceptable and uninterrupted use of antibiotics cannot be recommended as it may lead to a potential increase of antibiotic resistant bacteria, environmental pollution and residue increase in fish (Ringo et al., 2006).

Prebiotics are non-digestible carbohydrates that selectively stimulate the growth and metabolism of health promoting bacteria present in the host gut. This leads to an increased growth rate and better health of the host (Ahmdifer, 2011). The fiber can't be digested by the digestive enzymes in the gastrointestinal system but has a distinctive action in the gut by enhancing the growth of gastro intestinal flora. Prebiotic has an important role in maintaining good fish health by influencing both the immune system and the health of fish. The frothing of inulin in the colon- enhances the short chain fatty acid production which stimulates the villi to grow and improves the absorption of nutrients. Later have a positive influence on zoo-technical performances of fish through a high specific growth rate and lower feed conversion ratio (FCR) and also increases the general disease resistance of fish. Prebiotics are easy to incorporate in the ration, non-carcinogenic, low calorific value, stimulate beneficial gut microbes and have no residual effect. Therefore, the objective of this study is to evaluate the effect of inulin, on the growth performance of GIFT.

2. MATERIALS AND METHODS

2.1. Ethics statement

The use of animals and experimental protocol followed in the study was approved Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Ministry of Environment and Forests (Animal Welfare Division), Government of India). The ethics committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India. The study was conducted at wet laboratory, Advanced Research Farm Facility, Madhavaram, Tamil Nadu, India.

2.2. Experimental fish

A total of 500 apparently healthy GIFTs were procured from the State Fisheries Department, Krishnagiri, Tamil Nadu, India (initial mean weight of 0.055±0.024 g and average length of 2.03±0.21 cm) and transferred to the wet laboratory, Advanced Research Farm Facility, Madhavaram, Tamil Nadu, India. The fishes were acclimatized for 30 days and fed with a commercial feed containing crude protein at 25% of the diet. GIFT with an initial mean body weight of 0.85±0.02 g was randomly distributed in 12 troughs, with 20 fish per trough, with three replicates per diet. During the 60-day feeding trial, the fish were fed with the amount of feed equal to 5% body weight of their body weight, 2 times per day at 09:00 and 17:00. Uneaten food and faeces were siphoned out before the next feeding. Aeration was continuously provided throughout the experimental period using an air blower (Hailea hi-flow diaphragm air pump). During the feeding trial, water quality parameters were monitored daily and the mean values were as follows: water temperature at 27±1°C, pH at 8.5±0.6, dissolved oxygen at 5±0.4 mg l^{-1} and ammonia–N at 0.03±0.01 ppm.

2.3. Experimental diets

Dietary ingredients were finely ground, thoroughly mixed using a vertical ingredient mixer (Jinan Sunpring Machinery Co Ltd, China) and then extruded at 60 to 70°C to prepare 1.5 mm floating pellets using a twin screw extruder. A total

of four experimental diets were formulated in this present study including one control diet without the inclusion of inulin and three treatment diets with graded levels of inulin at 0.5, 1 and 1.5 g kg^{-1} of diet which were named INU 0.5, INU 1 and INU 1.5, respectively (Table 1). A commercially available inulin was procured from Hi-media. All the experimental diets were stored in air tight plastic containers and kept at room temperature.

Table 1: Formulation and chemical composition of the experimental diets (% of diet)

Ingredients	Control	INU 0.5	INU 1	INU 1.5
Fish meal	8	8	8	8
Soybean meal	10	10	10	10
Corn gluten meal	15	15	15	15
Corn flour	18	18	18	18
Cassava flour	0.3	0.3	0.3	0.3
Wheat flour	10	10	10	10
Rice bran (Defat)	38.5	38	37.5	37
Vitamin premixa	0.1	0.1	0.1	0.1
Mineral premixb	0.1	0.1	0.1	0.1
Inulin	-	0.5	1	1.5
Chemical composition (% dry matter)				
Moisture	8.6	8.28	8.64	8.68
Crude protein	25.79	25.56	25.53	25.78
Crude lipid	3	2.19	2.14	2.5
Crude fibre	5.75	5.25	5.30	5.75
Ash	8.78	8.2	8.47	8.49

[a]Composition of vitamin premix (quantity per kg): Vit. A: 1,00,00,000 IU, Vit. B1: 5,000 mg, Vit. B2: 5,000 mg, Vit. B3: 6,000 mg, Vit. B5: 6,000 mg, Vit. B6: 6,000 mg, Vit. C: 60,000 mg, Vit. D3: 20,00,000 IU, Vit. E: 10,000 EU, Vit. H : 200 mg; [b]Composition of mineral premix (quantity per kg): Magnesium: 2,800 mg, Iodine: 7.4 mg, Iron: 7,400 mg, Copper: 1,200 mg, Manganese: 11,600 mg, Zinc: 9,800 mg, Chlorides Cobalt: 4 mg, Potassium: 100 mg, Selenium: 4 mg, Calcium Carbonate: 27.25%, Phosphorous: 7.45 mg, Sulphur: 0.7 mg, Sodium: 6 mg, Calpan: 200 mg, Aluminium: 1,500 mg, Choline Chloride: 10,000 mg

2.3. Growth parameter and nutrient utilization

At the end of the trial, all the fishes were individually counted and weighed to estimate their weight gain, feed conversion ratio (FCR), feed efficiency ratio (FER), average daily gain, specific growth rate, protein efficiency ratio (PER) and survival rate (Priyatharshni et al., 2024).

The growth performances were calculated as follows:

Weight gain=Final weight-Initial weight

Feed conversion ratio (FCR)=Dry feed fed (g)/weight gain (g)

Feed efficiency ratio (FER)=1/FCR

Average daily gain (ADG)=Final weight (g)-Initial weight (g)/Experimental duration

Specific growth rate (SGR)=Ln final weight (g)-Ln initial weight (g)/Experimental duration

Protein efficiency ratio (PER)=Body weight gain (g)/protein intake (g)

Survival (%)=Total number of fishes survived/ Total number of fishes stocked ×100

2.4. Carcass composition

Proximate analysis of the fish carcasses from each treatment was determined according to the standard method (Ruby et al., 2022, Joshna et al., 2023) during the 30th day and 60th day of the experiment. Crude protein content was determined by Kjeldahl method (Kjeltron Tulin equipment), crude lipid content was estimated by the soxhlet method (SoxTRON SOX-2 Tulin Equipments) and crude fibre was also analyzed by analytical method (FibroTRON FRB-2 Tulin Equipments).

2.5. Blood collection

About 1ml of blood sample was drawn from the caudal vein of four fishes from each treatment after they were starved for 24h during the 30th day and 60th day of the experiement. In order to study the haematological parameters, the blood samples were suspended in heparinized tube and then values of red blood cells (RBC) (Neubauer hematocytometer), haemoglobin (Hb) (Cyanmethaemoglobin method (Drabkin, 1946)), and haematocrit (Hct) (the microhematocrit method (Nelson and Morris, 1979) were measured. According to Wintrobe (1934), the erythrocyte indices such as MCV, MCH and MCHC were also calculated as follows:

MCV (per µl)=(Ht×10)/erythrocytes

MCH (fl)=(Hb×10)/ erythrocytes

MCHC (g/dl)=(Hb×100)/Ht

2.6. Intestinal microbiota

The analysis of intestinal microbiota was conducted in the middle (30th day) and end of the feeding trial (60th day). Two fishes were sampled in each treatment after the cessation of feeding for 48h. The fish were killed by physical destruction of the brain and opened the ventral surface with sterile scissors. The intestinal tract of fish was removed, weighted and suspended in sterile saline (0.85% (w/v) NaCl). The suspension was serially diluted to 10^{-2} and 0.1ml of the solution was spread in triplicates on Tryptic Soya Agar (TSA) and DeMan, Rogosa, and Sharpe (MRS) were also used to detect lactic acid bacteria (LAB). All of the plates

were incubated and examined for 5 days (Rengpipat et al., 1998; Mahious and Ollevier, 2005), and then the numbers of colonies were counted. Phenotypic characterization of the isolates from MRS was carried out by biochemical method (Manan et al., 2017).

2.7. Histology

Experimental fish from the control and other experimental treatment groups had their intestines, gills and liver preserved in neutral buffered formalin for 48 hours before being histologically examined using conventional procedures (Roberts, 2001). To summarize, the samples were dehydrated in ethanol. They were then cleaned with xylene before being saturated with liquid paraffin wax and implanted in paraffin blocks at 58°C. Using a Rotary microtome (Leica RM2255), samples were sectioned at 6 m and stained with Hematoxylin and Eosin with Microm HMS7. A light microscope (Olympus CX21) was used to view and photograph the stained sections.

2.8. Statistical analysis

All the data were presented as the mean values±standard deviation (SD) of three replicates. All the percentage values were subjected to arcsine transformation prior to statistical analysis. One-way ANOVA, followed by Duncan's test at the significant level of 0.05 was used to compare the differences among the four dietary groups. The data were statistically analyzed by SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Growth performance and feed utilization

At the end of the study, fish fed with 1.5 g kg^{-1} of inulin supplemented diet displayed improved growth performance and feed utilization including weight gain, feed conversion ratio, feed efficiency ratio, average daily gain, specific growth rate and protein efficiency ratio (p<0.05). There was no significant difference in survival rate (p<0.05) among treatments and control (Table 2).

Table 2: Growth performances of GIFT fed graded levels of inulin supplemented diets

	Control	INU 0.5	INU 1	INU 1.5
Initial weight (g)	0.87±0.02^{a}	0.84±0.01^{a}	0.85±0.01^{a}	0.81±0.02^{b}
Final weight (g)	3.02±0.05^{b}	3.00±0.18^{b}	2.87±0.17^{b}	4.12±0.36^{a}
Weight gain (g)	2.15±0.04^{b}	2.15±0.20^{b}	2.02±0.18^{b}	3.31±0.37^{a}
Feed conversion ratio	2.12±0.04^{a}	2.13±0.20^{a}	2.28±0.20^{a}	1.40±0.15^{b}
Feed efficiency ratio	0.47±0.01^{b}	0.47±0.04^{b}	0.44±0.04^{b}	0.72±0.08^{a}
Average daily growth	0.036 ±0.001^{b}	0.036±0.003^{b}	0.033±0.003^{b}	0.055±0.006^{a}
Specific growth rate	2.08±0.017^{b}	2.11±0.128^{b}	2.02±0.121^{b}	2.70±0.156^{a}
Protein efficiency ratio	1.83±0.031^{b}	1.84±0.168^{b}	1.73±0.156^{b}	2.81±0.312^{a}
Survival (%)	88.33±1.67^{a}	88.89±1.92^{a}	92.22±3.85^{a}	90.56±0.96^{a}

Note: Values were expressed as means ± SD of three replicate trough per treatment (n=3) and values with different superscripts indicate significant differences as determined by Duncan test (p<0.05)

Inulin is a prebiotic that helpfully affects the host by selectively stimulating the activity of health-promoting bacteria in the intestinal tract of all animals. In the present study, the highest mean body weight of GIFT was recorded in fish fed inulin at 1.5 g kg^{-1} of diet (3.31±0.37), followed by 0.5 g kg^{-1} (2.15±0.20) and 1 g kg^{-1} (2.02±0.18). Therefore, the balanced carbohydrate in the diet is an important aspect for obtaining maximum growth performance in GIFT. Maximum inulin at 1.5 g kg^{-1} of diet produced better weight gain and lower FCR than the minimum level at 1 g kg^{-1} of diet in the present study. Similar to the present study, Akrami et al., 2015 observed better growth performance of Gibel Carp Juveniles *Carassius auratus gibelio* when the dietary inulin level is supplemented at 1.5 g kg^{-1}. In contradictory Ibrahem et al., 2010 observed better growth performance of Nile tilapia *O. niloticus* when the dietary inulin level is supplemented at 5 g kg^{-1} and Yones et al., 2020 reported that the optimum requirement is 2.5 g kg^{-1} for Nile tilapia *O. niloticus*. Similarly, the optimum requirement of inulin for Rainbow trout *Onchorhynchus mykiss* is 2 g kg^{-1} as reported by Hunt et al., 2019. In contrast to the positive effects of inulin, studies by Sheikholeslami et al. (2008) on rainbow trout *Oncorhynchus mykiss*, Akrami et al., 2008 on Beluga *Huso huso* juvenile appeared diverse levels of inulin made no critical contrast on development and survival between fish nourished control and inulin supplemented diets.

3.2. Carcass composition

The whole-body chemical composition of fish fed with dietary inulin supplemented diets is presented in Table 3. The whole body proximate composition of fish fed with dietary inulin-supplemented diets was not significantly affected. However, there was significant increment from

Table 3: Carcass compositions (% of dry weight) of GIFT fed graded levels of inulin supplemented diets

	30th DAY				60th DAY			
	Control	INU 0.5	INU 1	INU 1.5	Control	INU 0.5	INU 1	INU 1.5
Protein	43.06±1.10^{b}	43.04±1.71^{b}	43.10±2.64^{b}	43.20±4.51^{b}	51.23±1.10^{a}	51.18±1.69^{a}	51.35±2.52^{a}	51.13±4.52^{a}
Fat	10.89±0.12^{b}	10.92±0.99^{b}	10.85±0.77^{b}	11.17±0.12^{b}	12.84±0.71^{a}	12.55±0.86^{a}	12.57±0.57^{a}	12.59±0.58^{a}
Fiber	0.85±0.12^{b}	0.96±0.09^{b}	1.07±0.12^{b}	1.21±0.42^{b}	1.65±0.10^{a}	1.66±0.17^{a}	1.73±0.42^{a}	1.85±0.21^{a}

Note: Values were expressed as means ± SD of three replicate cages per treatment (n=3)

the 30th day to the 60th day.

In the present study, the whole body proximate composition was not significantly (p>0.05) affected by dietary inulin supplementation among treatments. Similarly, the whole body proximate composition was not affected in Nile tilapia fingerlings fed diets containing inulin (Tiengtam et al., 2017). In contrast to present research, crude fat was significantly increased in tilapia fed diets containing inulin (Yones et al., 2020) and crude protein was significantly increased in rainbow trout fed diets containing inulin (Ghafarifarsari et al., 2021).

3.3. Haematology parameters

The effects of dietary supplementation of inulin on haematological parameters are presented in Table 4. Statistical analysis of data showed that there was significant difference in haematological parameters such as RBC, haemoglobin, haematocrit, MCV, MCH, MCHC between treatment groups (p>0.05). The highest values were observed in fish fed with 1.5 g kg^{-1} of inulin compared to other treatments at both the 30th and 60th days of the experiment. The haematological parameter values were increased on 60th day of the experiment compared to the 30th day.

Table 4: Hematological responses of GIFT fed graded levels of inulin supplemented diets

	30th DAY				60th DAY			
	Control	INU 0.5	INU 1	INU 1.5	Control	INU 0.5	INU 1	INU 1.5
RBC (million/ cu mm)	1.29± 0.15^{c}	0.30± 0.17^{d}	1.18± 0.21^{c}	1.46± 0.45^{bc}	1.97± 0.23^{ab}	1.27± 0.36^{c}	2.11± 0.44^{a}	2.36± 0.35^{a}
Haemoglobin (g dl^{-1})	3.48± 0.12^{d}	1.10± 0.52^{e}	3.75± 0.83^{cd}	5.27± 0.60^{ab}	4.61± 0.24^{bc}	3.04± 0.89^{d}	5.31± 0.41^{ab}	5.76± 0.38^{a}
Haematocrit (%)	16.54± 0.41^{bcd}	12.47± 1.60^{d}	13.63± 1.30^{cd}	17.43± 5.12^{bc}	18.21± 0.97^{abc}	12.27± 2.10^{d}	19.77± 2.37^{ab}	22.07± 2.66^{a}
MCV (fl)	143.2± 7.53^{d}	115.97± 6.60^{e}	120.20± 2.57^{e}	156.40± 6.05^{c}	169.57± 10.31^{b}	115.77± 5.71^{e}	180.03± 2.77^{b}	193.47± 5.86^{a}
MCH (picograms)	28.56± 2.43^{d}	32.30± 3.14^{cd}	34.73± 2.15^{bc}	37.13± 2.50^{abc}	38.23± 5.22^{ab}	32.73± 1.46^{bcd}	41.60± 3.35^{a}	42.59± 2.43^{a}
MCHC (g dl^{-1})	21.39± 0.34^{d}	25.20± 3.62^{cd}	30.10± 2.25^{b}	29.53± 3.37^{bc}	26.96± 0.48^{bc}	25.33± 3.81^{cd}	27.77± 1.40^{bc}	37.23± 1.85^{a}

Values were expressed as means ±SD of three replicate troughs per treatment (n=3), and values with different superscripts indicate significant differences as determined by Duncan test (p<0.05)

The analysis of blood can disclose internal biochemical changes, physiological conditions and living situation of the fish. In the present study, the haematological values were also affected by dietary supplementation of inulin and the RBC, haemoglobin, haematocrit, MCV, MCH and MCHC were found significantly improved (p<0.05) in fish fed with inulin at 1.5 g kg^{-1} on 30th and 60th day of the experiment. The values obtained in this study were slightly higher compared to 2.13 million/cu mm (2.36 million/cu mm) of RBC and slightly lower compared to 7.14 g dl^{-1} (5.76 g dl^{-1}) of haemoglobin respectively reported by Tiengtam et al., 2017 for Nile tilapia *O. niloticus* fingerlings. The values obtained in this study were slightly higher compared to 25.96 g % (37.23 g %) of MCHC and 188.16 fL (193.47 fL) of MCV and slightly lower compared to 49.47 pg (42.59 pg) of MCH respectively reported by Ghafarifarsari et al., 2021 for Rainbow trout and Asian sea bass (Syed et al., 2018). In contrast, the dietary supplementation of inulin had no effect on haematological responses of Gold fish (Akrami et al., 2015) and Nile tilapia (Yones et al., 2020).

3.4. Intestinal microbiota

The intestinal microbiota of GIFT fed with graded levels

of inulin is shown in Table 5. The results showed that with increased supplementation of the level of inulin, the total bacteria and lactic acid bacteria count levels (LAB) increased (p<0.05). Total bacteria and lactic acid bacteria were increased in fish fed with inulin at 1.5 g kg^{-1} diet when compared to other experimental diets at both the 30th and 60th days of experiment. The bacterial counts of the intestinal tract values were increased on the 60th day of experiment compared to the 30th day.

The immunostimulatory nature of inulin may be attributed to the stimulation of the growth of beneficial bacteria such as lactic acid bacteria in the gut. The result of the present study showed that the fish fed with inulin at 1.5 g kg^{-1} displayed an increase in total bacteria and lactic acid bacteria compared to other diets. Inulin contributes to the growth of the animals by increasing the lactic acid bacteria population in the intestinal tract. Similar to present research, total bacteria and lactic acid bacteria were significantly increased in common carp *Cyprinus carpio* (Mousavi et al., 2016) and Gibel carp juveniles (*Carassius auratus gibelio*) (Akrami et al., 2015) fed diets containing inulin. It appears that the different basal diet, level of supplementation, adaptation period, and chemical structure, animal characteristics (species, age and stage of production) might have caused these differences.

Table 5: Bacterial counts of the intestinal tract of GIFT fed graded levels of inulin supplemented diets

	30th DAY				60th DAY			
	Control	INU 0.5	INU 1	INU 1.5	Control	INU 0.5	INU 1	INU 1.5
Total bacteria	26.56±2.43^{g}	44.67 ±4.04^{e}	37.33±4.51^{f}	49.67±3.06^{de}	53.56±3.24^{cd}	63.67±4.04^{b}	57.67±3.51^{bc}	72.00±7^{a}
LAB	12.80±2.34^{e}	21.33±2.52^{de}	22.33±3.51^{de}	24.67±3.06^{d}	32.10±2.45^{c}	36.33±4.51^{bc}	40.33±5.51^{ab}	46.33±3.51^{a}

Values were expressed as means ±SD of three replicate troughs per treatment (n=3), and values with different superscripts indicate significant differences as determined by Duncan test (p<0.05)

3.5. Histological changes of intestine, gill and liver

The histological changes of the intestine, gill and liver of GIFT exposed to different concentrations of inulin are presented in Figures 1A, 1B and 1C. Due to exposure to different concentrations of inulin, no abnormalities were detected on the 30th day (Figure 1A (i)), whereas on the 60th day, 0.5 g kg^{-1} showed some deformities such as villi are short and 1.0 g kg^{-1} and 1.5 g kg^{-1} have also shown some deformities such as villi are short and dilated in the intestine (Figure 1A (ii)). In the gill, there is no deformities on the

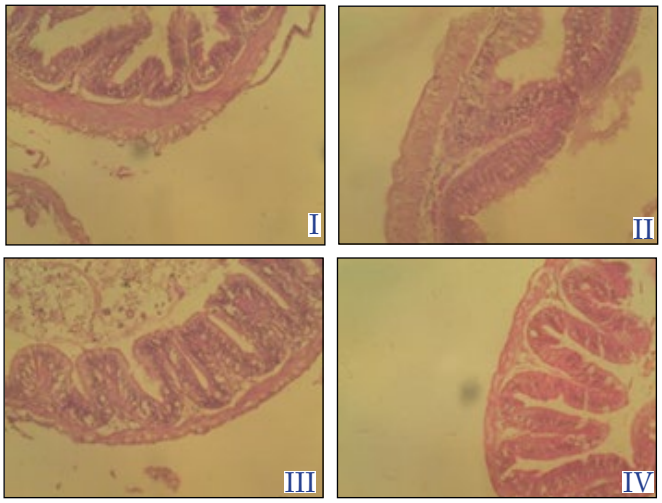

Figure 1A (i): Micrographs of the intestine of GIFT on the 30th day of the experiment; I: control, II: 0.5 g kg^{-1}, III: 1.0 g kg^{-1}; IV: 1.5 gkg^{-1}, no abnormalities detected

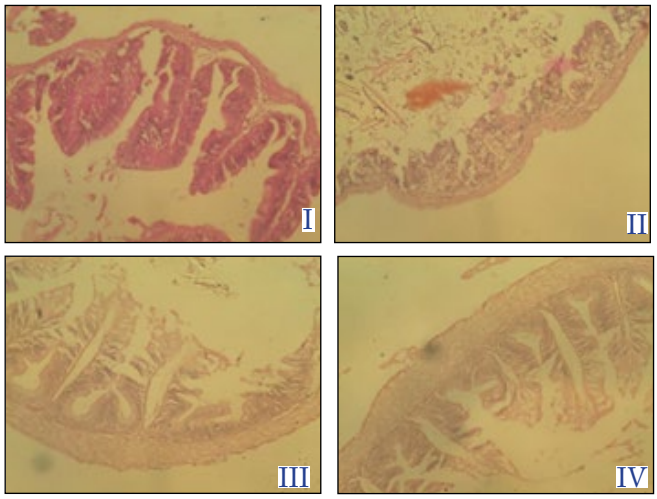

Figure 1A (ii): Micrographs of the intestine of GIFT on the 60th day of the experiment; I (control): no abnormalities detected; II (0.5 g kg^{-1}): Villi are short; III (1.0 g kg^{-1}): Villi are dilated; IV (1.5 g kg^{-1}): Villi are dilated

30th day (Figure 1B (i)) and 60th of the experiment (Figure 1B (ii)). In the liver, on the 30th day, 0.5 g kg^{-1} showed deformities such as hepatocytes show mild vacuolation, 1.0 g kg^{-1} showed moderate congestion in sinusoids and 1.5 g kg^{-1} have shown mild congestion in sinusoids (Figure 1C (i)), whereas on the 60th day, 0.5 g kg^{-1} showed deformities such as hepatocytes show moderate vacuolation and sinusoids show mild congestion, 1.0 g kg^{-1} have shown mild congestion in sinusoids and 1.5 g kg^{-1} have shown severe congestion in sinusoids (Figure 1C (ii)).

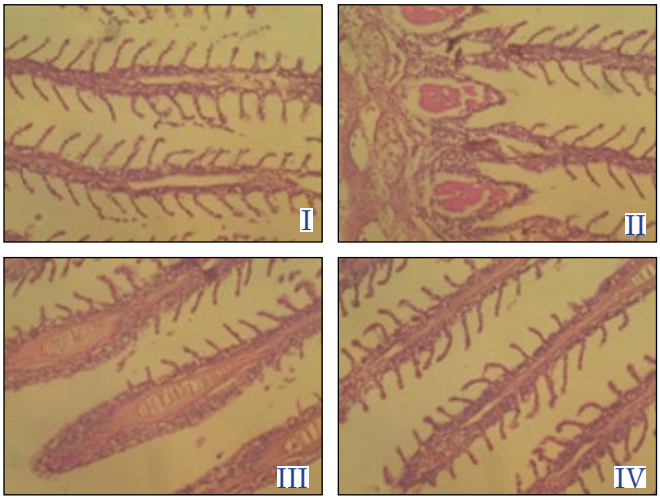

Figure 1B (i): Micrographs of the gill of GIFT on the 30th day of the experiment; I: control, II: 0.5 g kg^{-1}, III: 1.0 g kg^{-1}; and IV(1.5 g kg^{-1}): No abnormalities detected

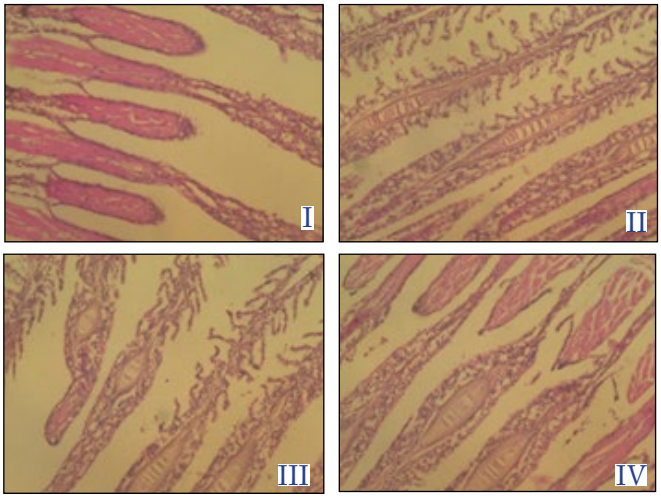

Figure 1B (ii): Micrographs of the gill of GIFT on the 60th day of the experiment; I (control), II (0.5 g kg^{-1}), III (1.0 g kg^{-1}) and IV(1.5 g kg^{-1}): No abnormalities detected

The inclusion of inulin in the food did not adversely affect the fish's ultimate weights in our study, even if it did cause alterations in the histology of the liver and gut. In another study conducted by Olsen et al., 2001 fed with a high level of 15% dietary inulin to on-growing Artic charr (*Salvelinus alpinus*) after 4 weeks of feeding, histological analysis revealed that inulin caused destructive effects in the intestine. Cerezuela et al., 2013 reported that inulin has a negative impact on the intestinal morphology of gilthead sea bream. In contradictory, Refstie et al., 2006 reported that histology of the distal intestine revealed that inulin does not induce any morphological changes in Atlantic salmon and Tiengtam et al., 2015 reported that dietary supplementation with inulin (5.0 g kg^{-1}) resulted in greater villus height in all parts of the intestine.

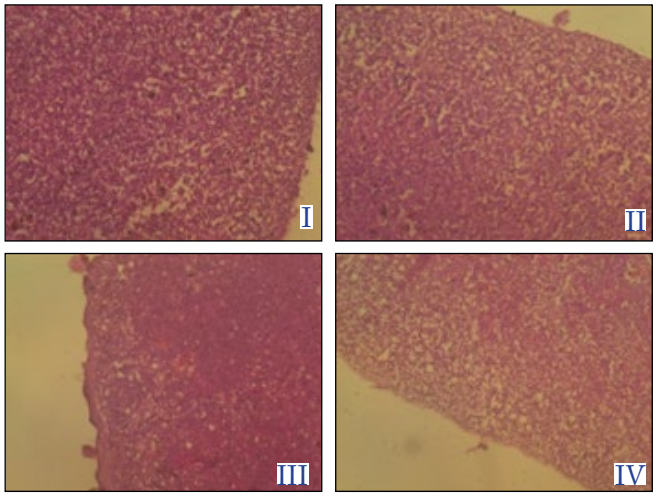

Figure 1C (i): Micrographs of the liver of GIFT on the 30th day of the experiment; I control: No abnormalities detected; II (0.5 g kg^{-1}): Hepatocytes show mild vacuolation; III (1.0 g kg^{-1}): Sinusoids show moderate congestion; IV (1.5 g kg^{-1}): Sinusoids show mild congestion

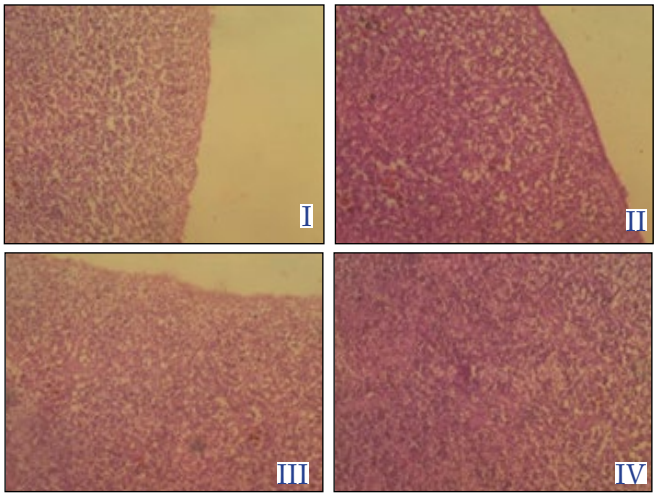

Figure 1C (ii) Micrographs of the liver of GIFT on the 60th day of the experiment; I (control): No abnormalities detected; II (0.5 g kg^{-1}): Hepatocytes show moderate vacuolation, Sinusoids show mild congestion; III (1.0 g kg^{-1}): Sinusoids show mild congestion; IV (1.5 g kg^{-1}): Sinusoids show severe congestion

4. CONCLUSION

The dietary inulin at 1.5 g kg^{-1} positively influenced the growth performance of GIFT far than other treatments and improved the health of fish through enhancing haemato-biochemical parameters and intestinal microbiota. However, the whole-body chemical composition was not significantly affected by dietary inulin supplementation.

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