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Effects of Inulin and Mannan-oligosaccharide on growth, feed utilization, fatty acid profiles, and intestinal morphology of Chamo strain Nile tilapia (*Oreochromis niloticus*) fry reared under suboptimal temperature

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ABSTRACT: The effects of inulin and mannan-oligosaccharide (MOS) on growth, feed utilization, fatty acid profiles, and intestinal morphology of Chamo strain Nile tilapia (*Oreochromis niloticus*) fry reared at suboptimal temperature were investigated in this study. Chamo strain Nile tilapia is a local strain in Ethiopia that has been selected for fast growth and disease resistance under tropical conditions. However, its performance under cold stress or sub-optimal temperature is unknown. Four experimental diets were formulated to contain 0% (control), 5 g kg^{-1} inulin, 6 g kg^{-1}MOS and a combination of 2.5 g kg^{-1} inulin and 3 g kg^{-1} MOS. Each diet was fed to triplicate groups of Nile tilapia fry (initial weight 1.6 ± 0.074 g) for nine weeks at a suboptimal temperature of 22.88 ± 0.48 ^{o}C. The results showed that prebiotic supplementation did not significantly affect the survival, final weight, weight gain, SGR, FCR and ash content of the fish except for the slight improvements observed with MOS alone and in combination with inulin. However, MOS alone or a combination with inulin improved the villi length and width, and some PUFA levels of the fish compared to the control group. The fish fed with the combination of inulin and MOS had the highest crude lipid content and villi length in the proximal and middle intestine among all groups. The fish fed with MOS alone had the highest villi width and some PUFA levels among all groups. These results suggest additions of these prebiotics have improved the villi length and width, and some PUFA levels of Chamo strain Nile tilapia and could be used as sustainable additive in tilapia nutrition under suboptimal temperature conditions Therefore; further studies are needed to optimize the prebiotic supplementation for Nile tilapia under different conditions.

Keywords/ phrases: Chamo strain Nile tilapia; Fatty acid profile; Intestinal morphology; Inulin; MOS; Suboptimal temperature

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most important aquaculture species in the world, due to its high adaptability, fast growth, and good flesh quality. Although the tolerance of Nile tilapia to wide range of environmental conditions including temperature is obvious, the optimum growth rate can be obtained when the water temperature is between 27 and 30 °C (Azaza *et al*., 2008). In Ethiopia, one of the major constraints in the development of Nile tilapia aquaculture is the low water temperature that stays below the optimal level in high altitude areas. Under low water temperature conditions, Nile tilapia suffers from poor growth performance, feed utilization, immune (Gewaily *et al*., 2021) response, and intestinal health (Nivelle *et al*., 2019). Low water temperature can induce stress and reduce the activity of digestive enzymes, leading to impaired nutrient absorption and increased susceptibility to diseases (Magouz *et al*., 2020). Moreover, low water temperature can alter the composition and function of the intestinal microbiota, which plays a crucial role in maintaining the intestinal homeostasis and host health (Negash Kabtimer *et al*., 2022). Therefore, finding effective dietary strategies that maintain the regular activity and health of fish especially under stressful

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conditions for Nile tilapia is of great interest for the aquaculture industry. One of the potential strategies is the use of functional feed additives that can modulate the intestinal microbiota and metabolism of Nile tilapia (Yassine *et al*., 2021; Magouz *et al*., 2020).

Nowadays, different functional feed additives such as prebiotics are being used in tilapia nutrition to ensure the dietary nutrients are ingested, digested, absorbed, and transported to the cells (Tewodros Abate *et al*., 2018). Prebiotics are non-digestible carbohydrates that can selectively stimulate the growth and activity of beneficial bacteria in the gut, thereby improving the intestinal barrier function and enhancing the host resistance to pathogens. Inulin and mannan-oligosaccharide (MOS) are two types of prebiotics that have been shown to have beneficial effects on the gut health and immune system of various animals, including fish (Genc *et al*., 2007; Reza *et al*., 2009; Dimitroglou *et al*., 2010; Razeghi Mansour *et al*., 2012; Eshaghzadeh *et al*., 2015; Tiengtam *et al*., 2015). Inulin is a fructan that can stimulate the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* in the gut (Wang *et al*., 2021b; Hutchinson *et al*., 2023), while MOS is yeast cell wall extract that can bind to pathogenic bacteria and prevent their adhesion to the intestinal mucosa (Levy-Pereira *et al*., 2018; Asbury *et al*., 2022). Moreover, both inulin and MOS can influence the production of short-chain fatty acids (SCFAs) in the gut, which are important energy sources and signaling molecules for the host. SCFAs can regulate the intestinal pH, modulate the gene expression and metabolism of intestinal cells, and affect the systemic immune response and inflammation.

However, the effects of inulin and MOS on Nile tilapia reared at suboptimal temperature are still unclear. Previous studies have reported inconsistent results on the effects of prebiotics on fish growth and health under cold stress. Some studies have found positive effects of prebiotics on improving the growth performance, feed efficiency, immune response, and intestinal morphology of fish under low temperature conditions (Wang *et al*., 2021a; 2021b; Wang *et al*., 2022), while others have found no significant effects or even negative effects (Mugwanya *et al*., 2022). These discrepancies may be due to different types and doses of prebiotics, different fish species and strains, different experimental temperatures and durations, and different methods of measuring the outcomes. Therefore, more studies are needed to elucidate the mechanisms and optimal conditions for using prebiotics to enhance the cold tolerance of fish.

Therefore, the aim of this study was to investigate the single or combined effects of inulin or MOS on growth, feed utilization, fatty acid profiles, and intestinal morphology of Chamo strain Nile tilapia fry reared at suboptimal temperature. Chamo strain Nile tilapia is a local strain in Ethiopia that has been selected for fast growth and disease resistance under tropical conditions (Adamneh Dagne and Abelneh Yimer, 2018). However, its performance under cold stress is unknown. We hypothesized that inulin and MOS would have synergistic effects on improving the cold tolerance of Nile tilapia by modulating its intestinal microbiota and metabolism.

MATERIALS AND METHODS

Experimental Setup for Growth Trial

The experiment was conducted for nine weeks at the Center for Aquaponics and Recirculating Aquaculture System (CARAS), Department of Zoological Sciences, Addis Ababa University (AAU). Fingerlings of Chamo strain Nile tilapia with an initial mean weight of 1.6 ± 0.074 g were randomly distributed into 12 aquaria (60 liters each) in a closed recirculation system, with 25 fish per aquarium. The fish were assigned to four treatments with three replicates each. They were acclimatized to the experimental conditions for two weeks by being fed with the control diet. During the trial, the fish were hand-fed to apparent satiation three times daily (08:00, 12:00, and 16:00) for nine weeks. The feed amount was adjusted weekly based on the new mean fish weight in each treatment.

The recirculation system was supplied with continuous flow (2.5 lit. min^{-1}) of filtered aerated water from a sump tank heated at 22.88 ± 0.48 ^{o}C and subjected to natural 12-h light: dark cycle. Water quality parameters measured during the experiment were within acceptable ranges for tilapia and averaged (± SD): pH, 7.7 ± 0.34; ammonia, 0.06 ± 0.01 mg l^{-1}; nitrite, 0.20 ± 0.25 mg l^{-1}; nitrate, 20 ± 0.84 mg $l^{-1}$and dissolved oxygen, 6.54 ± 0.44 mg l^{-1}.

Experimental Design and Diet Preparation

The experiment followed a completely randomized design with four treatment diets in triplicates. A basal diet was formulated (as fed basis) to contain 39.5% protein and 15.0% lipid using fish meal and full fat soybean as the main protein and lipid sources. The basal diet was supplemented with different levels and types of prebiotics (Table 1) to prepare the experimental diets. Prebiotic inulin (>90% pure) extracted from tuber of *Helianthus tuberosus* and MOS (>90% pure) (Yangling Ciyuan Boiotech Co., Ltd., China) were used as the prebiotic sources. The four treatment diets were: basal diet/control diet (Diet-T_1) (0 g of prebiotics/kg fish feed), inulin-supplemented diet (Diet-T_2) (5 g kg^{-1}), MOS-supplemented diet (Diet-T_3) (6 g kg^{-1}) or mixed prebiotic-supplemented diet (Diet-T_4) (2.5 g kg^{-1} inulin and 3 g kg^{-1} MOS).

Growth Performance

All fish were weighed every week and growth performance parameters were calculated according to the following formulae:

Weight Gain (%) = $\frac{(W2)-(W1)}{(W1)}$ x 100, where, W1-initial weight, W2-final weight

Specific growth rate (SGR, % /day) = $\frac{(In(W2)-In\,(W1))}{No.\ of\ cultured\ days}$ x 100, where, W1-initial weight, W2-final weight

Daily Growth Rate (DGR, g / day) = $\frac{Finalweight-Initialweight}{Cultureddays}$

Food Conversion Ratio (FCR) = $\frac{Totalweightofdryfeedgi}{Totalweightgainbyfis}$

Survival rate (SR, %) = $\frac{N2}{N1}$x 100, where N_2 = No. of fish harvested and N_1= No. of fish stocked

Diet and Carcass Composition

At the end of the experiment, ten fish were randomly sampled from each aquarium for the final carcass composition analyses. Fish were euthanized by immersing into an overdose of clove oil solution according to the Guidelines of the European Union (Directive 2010/63/UE) and dried in an oven at 60^0C until a constant weight was achieved. Dried fish carcasses were ground for proximate composition analysis.

The experimental diets and fish carcasses were analyzed for crude protein, lipid, moisture and ash using standard methods of the AOAC (2002) procedures: dry matter (dried at 100 ^{0}C to constant weight), ash by the gravimetric method (combusted at 550 ^{0}C to constant weight), crude protein (N × 6.25) by the Kjeldahl method, and crude lipid by the Soxhelet extraction method. All analyses were performed in triplicate. The ingredients and proximate composition of the experimental diet are presented in Table 1.

Table 1. Proximate chemical composition (g kg^{-1}) of ingredients and experimental diets.

	Experimental diets			
	Diet-T_1	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**
Ingredients (g kg^{-1} dry weight)				
Fish meal	350.6	350.6	350.6	350.6
Soybean meal	325.4	325.4	325.4	325.4
Corn	120	120	120	120
Wheat grain	180	180	180	180
CMC	20	20	20	20
vit/ min premix	4	4	4	4
Inulin	0	5	0	2.5
MOS	0	0	6	3
Proximate composition (% as fed basis)				
Dry matter	91.20	91.34	91.61	92.20
Crude protein	39.51	39.21	39.37	39.13
Crude fat	15.01	15.83	15.22	15.31
Crude fiber	2.50	2.54	2.55	2.61
Ash	3.54	3.57	3.47	3.43
NFE	30.64	30.19	31.00	31.72
Gross energy (kJ g^{-1})	20.45	20.63	20.57	20.67

CMC-Carboxy Methy Cellulose
NFE-Nitrogen Free Extract

Fatty Acid Profile Determination

The fatty acid (FA) profiles of the frozen fish carcasses were determined by extracting and methylating the fat samples following the method of Trbovic *et al*. (2018) and analyzing them using gas chromatography (GC-Agilent 7890B) equipped with a flame ionization detector and an HP88 capillary column (30m x 0.25mm x 0.20µm). Helium was used as a carrier gas. The percentage area of the fatty acids was estimated using NIST17 library and expressed as percentages of the total content.

Intestinal morphology Study

At the end of the feeding trial, two fish from each triplicate aquarium were anesthetized in a solution of 1-3 ml of eugenol/clove oil mixed in 10 ml of ethanol diluted in 1 liter of water. The whole gastrointestinal tract was removed and the proximal, middle and distal portions of the intestine were collected. Each tissue was placed in a 10 ml sample vial half filled with 10% formalin fixative. Slide preparation and scanning were performed at the Pathology Department of St. Paul's Hospital Millennium Medical College, Addis Ababa. Each sample was embedded in paraffin wax, sliced (5µm thick), mounted on glass slides and stained with hematoxylin and eosin. Two longitudinal slices were prepared from each sample. The sample slides were photographed with a digital camera connected to a microscope (MoticEasyScan Pro 6 (USA) (Resolution: 40X: 0.26µm/pixel-20X: 0.52µm/pixel; Scanning Camera: 5.0 MP (2/3″ high speed Sensor); Scanning Mode: three Dimensional stacking). The number of goblet cells/villus (GC), the villus height (VH), and the villus width (VW) were measured using a Dell OptiPlex 7450 computer connected to the scanner. For each sample, six villi measurements were performed.

Data Analysis

All results are presented as mean ± standard deviation. Data were analyzed by One-Way Analysis of Variances (ANOVA) using Minitab version 17. Differences between the means were tested at the 5% probability level using Tukey's test as a post-hoc test.

RESULTS

Growth Performance

Table 2 shows how the prebiotics affected the growth performance of Nile tilapia. The survival and all growth parameters of the fish were not significantly ($p > 0.05$) different among the groups fed with different prebiotic-supplemented feeds and the control group. However, the fish fed with Diet-T_4 had better final weight, weight gain (WG), specific growth rate (SGR) and food conversion ratio (FCR) than the other groups.

Table 2.The growth performance (mean ± SD) of Nile tilapia fed diets containing different types and levels of prebiotics.

	Diets				
Parameters	**Diet-T_1**	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**	*P-value*
Initial weight (g)	1.51 ± 0.310	1.63 ± 0.283	1.67 ± 0.288	1.68 ± 0.486	0.865
Final weight (g)	3.57 ± 0.132	3.97 ± 0.356	4.05 ± 0.483	4.16 ± 0.333	0.253
Weight gain (%)	136.20 ± 8.74	143.8 ± 21.9	142.3 ± 28.9	147.8 ± 19.8	0.923
DGR (g/day)	0.069 ± 0.020	0.0417 ± 0.029	0.0423 ± 0.018	0.0443 ± 0.021	0.925
SGR (% day^{-1})	1.537 ± 1.098	1.590 ± 1.619	1.582 ± 0.902	1.619 ± 1006	0.999
FCR	4.296 ± 2.012	5.437 ± 2.498	4.650 ± 3.610	3.996 ± 2.140	0.763
SR (%)	100	100	100	100	

There were no significant differences between treatment groups

Fish Carcass Composition

Table 3 shows the proximate composition of the fish carcass after the 9-week feeding trial. Prebiotic supplementation did not significantly affect ($p > 0.05$) the whole body moisture, crude protein, crude lipid, and ash contents of the fish in this study.

Table 3. Proximate chemical composition (%) of Nile tilapia carcass at the end of 9-week feeding trial (Mean ±SD).

Parameters	Diet-T_1	Diet-T_2	Diet-T_3	Diet-T_4	*p*-value
Moisture	7.16 ± 3.16	7.77 ± 4.46	7.81 ± 4.96	7.62 ± 5.11	0.999
Crude protein	47.16 ± 5.56	44.98 ± 5.69	44.89 ± 6.30	45.97 ± 5.76	0.975
Crude lipid	37.53 ± 3.63	39.31 ± 2.03	36.43 ± 6.84	40.02 ± 6.64	0.306
Ash	9.27 ± 1.05	8.49 ± 2.27	7.52 ± 3.39	8.43 ± 2.50	0.911

There were no significant differences between any groups

Fatty Acid Profile

Table 4 shows the fatty acid profiles of Nile tilapia carcasses after the 9-week feeding trial. The main saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in all groups were palmitic acid (16:0) and oleic acid (18:1 n-9), while the most abundant polyunsaturated fatty acids (PUFA) were linoleic acid (18:2n-6), alpha linolenic acid (18:3n-3) and docosahexaenoic acid (22:6n-3). The fatty acid profiles of the fish were affected by the prebiotic supplementation. The fish fed with Diet-T_3 and Diet-T_4 had higher ΣPUFAs and PUFAs/SFAs ratio than the other groups. They also had higher levels of some PUFAs, such as 18:3n-3, 20:3n-6, 20:4n-6, 22:4n-6, 20:5n-3, 22:5n-6 and 22:6n-3, compared to the fish fed with Diet-T_2 and the control group (Diet-T_1).

Table 4. Fatty acid profiles of Nile tilapia carcasses fed experimental diets for 9 weeks.

	Final % of area			
Fatty acid	**Diet-T_1**	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**
12:0	0.10	0	0.09	0.09
14:0	1.92	1.86	1.82	2.00
15:0	0.86	0.79	0.61	0.82
16:0	18.55	18.37	16.58	17.94
17:0	0.87	0.81	0.82	0.78
18:0	6.99	6.74	6.26	6.44
ΣSFAs	**29.29**	**28.57**	**26.38**	**28.02**
16:1n-9	4.85	4.73	4.90	4.91
18:1*n-9*	23.38	23.87	22.10	22.68
18:1*n-13*	3.19	3.22	3.01	2.73
20:1*n-9*	0.84	0.86	0.79	0.76
ΣMUFAs	**32.26**	**32.68**	**30.8**	**31.08**
18:2*n-6*	25.17	24.71	25.04	25.16
18:3*n-3*	3.55	3.65	4.11	4.03
20:2*n-6*	1.02	0.94	1.07	0.98
20:3*n-6*	1.13	1.11	1.26	1.17
20:4 *n-6*	1.63	1.71	2.00	1.81
20:4*n-6*	0.33	0.33	0.43	0.41
20:5 *n-3*	0.28	0.29	0.40	0.35
22:4*n-6*	0.52	0.59	0.66	0.61
22:5*n-6*	0.64	0.56	0.76	0.69
22:5*n-3*	1.64	1.21	1.42	1.36
22:6*n-3*	2.98	3.57	5.59	4.27
DHA/EPA	10.64	12.31	13.98	12.2
Σn-6and n-3 PUFAs	**38.89**	**38.67**	**42.74**	**40.80**
Percent (%) PUFA	**38.72**	**38.50**	**42.55**	**40.62**
Percent (%) MUFA	**32.12**	**32.54**	**30.67**	**30.94**
Percent (%) SFA	**29.16**	**28.44**	**26.26**	**27.90**
PUFA/SFA ratio	**1.33**	**1.35**	**1.62**	**1.46**

DHA- Docosahexaenoicacid; EPA- Eicosapentaenoicacid

Intestinal Morphology

Figure 1 shows the intestinal morphology parameters (villi length, villi width and goblet cell number) of Nile tilapia fed with different prebiotic-supplemented diets. The prebiotics significantly increased the villi length compared to the control diet. The villi length in the proximal and middle intestine was significantly ($p<0.05$) affected by the diet (Fig. 1A and 1B). The fish fed with Diet-T4 had the longest villi in the proximal intestine among all groups. The fish fed with Diet-T4 also had significantly longer villi than the control group in the middle intestine. Moreover, the prebiotics increased the villi width compared to the control diet. The fish fed with MOS (Diet-T3) had the widest villi among all groups.

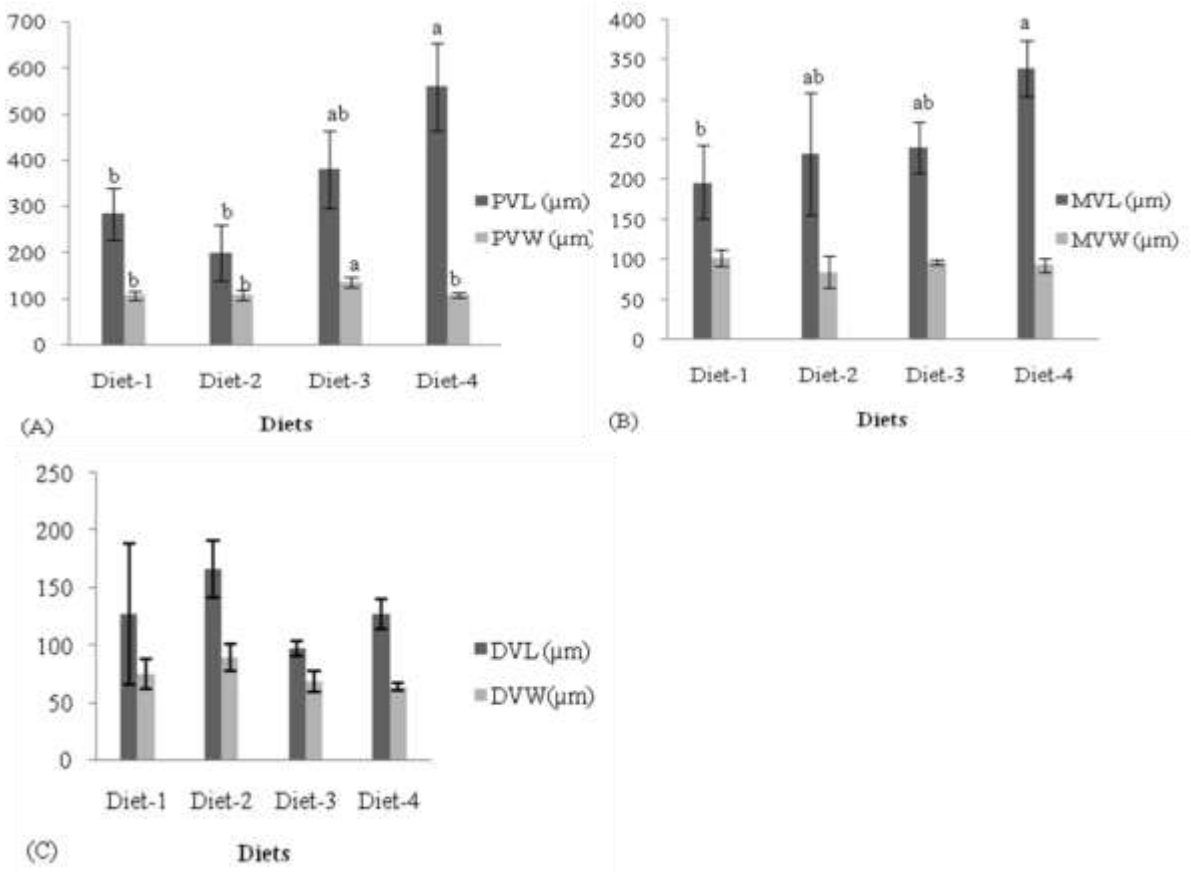

Figure 1.Graph showing proximal villi length (PVL) and villi width (PVW) (A), middle villi length (MVL) and villi width (MVW) (B), and distal villi length (DVL) and villi width (DVW) (C) of intestine of Nile tilapia in µm.

Mean scores of goblet cell number per villus at proximal, middle and distal portion of intestine are presented in Table 5. There was no significant ($p > 0.05$) difference observed in the number of goblet cells among different feeding groups or within different intestinal portions. However, the highest mean goblet cell numbers, 19.33±8.50 and 15.00 ± 5.20 were recorded in middle and proximal portion of intestine in fish fed MOS, respectively. However, the least mean goblet cell number (6.67 ± 3.06) was recorded in those fish fed control diet. This results of the gut morphology also showed that the intestines of Nile tilapia had normal levels and order of absorption, and no abnormality was detected.

Table 5.Goblet cell number per villus at proximal, middle and distal parts of intestine of Nile tilapia fed with diets containing different types and levels of prebiotics.

	Diets				
Parameters	**Diet-T_1**	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**	***P*-Value**
Proximal	6.67 ± 3.06	10.33 ± 5.03	15.00 ± 5.20	11.00 ± 1.73	0.170
Middle	12.00 ± 3.00	13.00 ± 1.73	19.33 ±8.50	9.00 ± 2.65	0.135
Distal	9.33 ± 2.31	11.00 ± 3.00	8.67 ±2.31	8.333 ± 1.528	0.539
Pooled StDev	2.80872	3.52767	5.90668	2.02759	

There were no significant differences between any groups

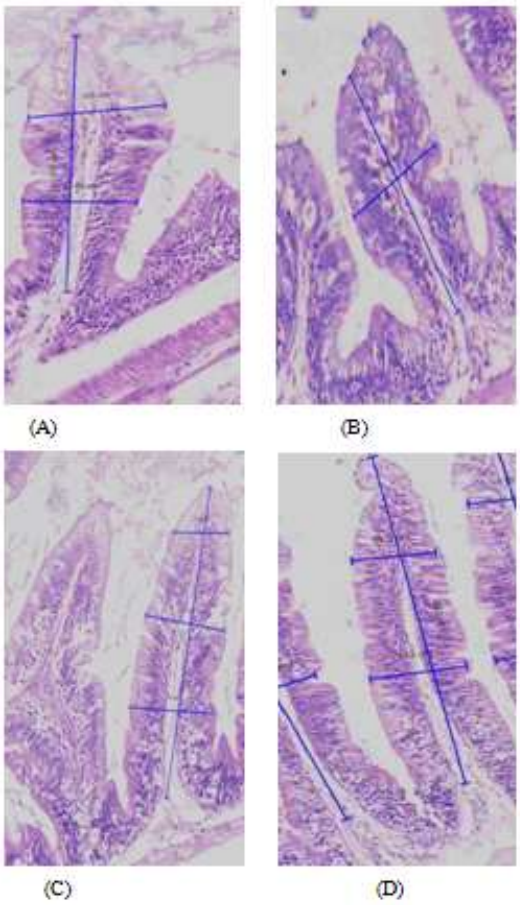

Figure 2. Proximal villi length and width scanned by Motic Easy Scan Pro 6 (USA): (A) Diet-T_1; (B) Diet-T_2; (C) Diet-T_3 and (D) Diet-T_4.

DISCUSSION

The present study showed that supplementing fish feed with inulin, MOS or combination of both has no significant effects on final weight, food conversion ratio or specific growth rate of the Nile tilapia. However, inclusion of these prebiotics has improved the villi length and width, and some PUFA levels fish compared to the control group. The lack of significant effects of prebiotics on the growth performance and feed efficiency of Nile tilapia in this study is in contrast with some previous studies that reported positive effects of prebiotics on these parameters in other fish species (Ganguly *et al*., 2013; Tewodros Abate *et al*., 2018; Xu *et al*., 2022). Azevedo *et al*. (2015) for example, reported 0.02% MOS (from *Saccharomyces cerevisiae*) in the diet of juvenile Nile tilapia (*O. niloticus*) for 6 weeks enhanced the final biomass, relative biomass gain, and feed conversion, but did not affect the feed intake and survival rate. Cechim *et al*. (2015) on the other hand, observed no improvement in growth performance of Nile tilapia in net-cage systems due to MOS supplementation. However, 0.4% MOS in the diet for 60 days increased the intestinal villi height of fish. Yuji-Sado *et al*., (2017) also found no effect of MOS (0.2, 0.4, and 0.6%) in the diet of Nile tilapia fed for 60 days on the growth and immune system of fish. However, 0.4% MOS inclusion diet increased the intestinal fold height, and the 0.4 and 0.6% MOS diets increased the intestinal muscular layer thickness. Tiengtam *et al*., (2015) reported that prebiotic supplementation of 5 g kg^{-1} inulin or 10 g kg^{-1} Jerusalem artichoke (JA) in the diet of juvenile Nile tilapia for 8 weeks improved the growth performance (FW, SGR, FI, FCR, Survival) and the intestinal villi height and goblet cell number of fish. The slight improvement in the WG, SGR and FCR in fish fed with the diet containing combination of inulin and MOS (Diet-T_4) could be a positive signal and conforms with

the suggestion that prebiotics be used in combination than in single forms (Sutriana *et al*., 2021).

However, conflicting results obtained by various studies can be explained by variation in the types, levels and sources of prebiotics used, as well as the fish species, age, size, diet composition, duration of prebiotic supplementation and environmental conditions involved in the experiments (Mugwanya *et al*., 2022). A possible reason for the absence of significant enhancements in growth and feed efficiency of Nile tilapia could be the sub-optimal temperature of the rearing environment used in the present study. Rearing temperature is an important factor that is connected with the physiology as well as the normal micro flora colonization in the gastrointestinal tracts of fishes (Liu *et al*., 2021). Prebiotics used in this study were not elicit significant responses compared with others report. This might be due to variation of microbiota diversity in the fish gut in different environmental and culturing conditions. Therefore, further studies are needed to optimize the prebiotic supplementation for Nile tilapia under different conditions.

Body protein content was not affected by MOS or inulin supplementation in the present study. This is in contrast to the negative effect of prebiotics in body protein composition reported by Genc *et al*. (2007) on hybrid tilapia fed 3 or 4.5 % MOS which showed significant improvement in the body protein concentration than fish fed the control diet. The present results showed that dietary supplementation with inulin or combination of inulin and MOS slightly increased the body fat content in Chamo strain Nile tilapia. Eshaghzadeh *et al*. (2015) reported that inclusion of 5 g kg^{-1} or 10 g kg^{-1}inulin in the diet of common carp fry significantly improved the carcass lipid content. Changes in lipid content in carcass of fish could be attributed to the enhanced lipid absorption and metabolism by the prebiotics (Ringø *et al*., 2010; Ganguly *et al*., 2013). Prebiotics may stimulate the growth and activity of beneficial bacteria in the gut, which can produce short-chain fatty acids (SCFAs) that can be used as energy sources by the host or modulate lipid metabolism (Ringø *et al*., 2010). Prebiotics may also increase the expression of genes involved in lipid synthesis and transport (Lokesh *et al*., 2022; Porter *et al*., 2022).

In this study, the fatty acid profile in Nile tilapia carcasses was different among treatments with different prebiotic supplementation (Table 4). The highest percentage, 42.55 and 40.62 % of PUFAs was recorded in fish fed Diet-T_3 and Diet-T_4, respectively. This value is higher compared to Genetically Improved Farmed Tilapia (GIFT) strain fillet samples with the total PUFAs percentage of 15% (Nguyen *et al*., 2010). However, no significant differences in the FAs contents were observed among groups ($p > 0.05$). Fish fed Diet-T_3 and Diet-T_4 had higher contents of PUFAs such as 18:3*n-3*, 20:3*n-6*, 20:4*n-6*, 22:4*n-6*, 20:5*n-3,* 22:5*n-6* and 22:6*n-3*, however there is a simultaneous linear decrease in the contents of SFAs (16:0) and MUFAs (18:1*n-9* and 20:1*n-9*). This could be due to the influence of prebiotics on the fatty acid composition of gut microbiota. Prebiotics may select for bacteria that can produce or convert PUFA from dietary or endogenous sources (Guo *et al*., 2022). Prebiotics may also affect the expression of genes involved in fatty acid desaturation and elongation, such as delta-6 desaturase (D6D) and elongase of very long-chain fatty acids 2 (ELOVL2) (Monroig and Kabeya, 2018). The increased PUFA levels may have beneficial effects for fish health and human nutrition, as they have anti-inflammatory, anti-thrombotic, and anti-atherogenic properties (Oppedisano *et al*., 2020). Therefore, fish fed MOS or combination of MOS and inulin supplemented diet may have improved lipid quality and health benefits for consumers.

The percentage of DHA content exceeded those of EPA in all treatments in the current study, with the ratio of DHA/EPA ranging from 10.64 % in Diet-1 to 13.98 % in Diet-3. This may be due to the characteristics of EPA, which is easily oxidized; hence less is retained relative to DHA (Ismail *et al*., 2016).

Intestinal villi provide a vast absorptive surface area and increase in villi length and width can improve the digestive and absorptive capacity of fish. Compared with fish fed the basal diet, a greater intestinal villi length was observed in fish fed 6 g kg^{-1} MOS and combination of inulin and MOS supplemented diets. This study showed that the intestinal morphology was affected significantly by supplementing fish feed with prebiotics, which is in line with previous reports on tilapia (Genc *et al*., 2007; Schwarz *et al*., 2011; Magouz *et al*., 2020). Enhanced gut

morphology can be explained by the stimulation of intestinal cell proliferation and differentiation by the prebiotics. Prebiotics may increase the production of growth factors, such as insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF), that can promote intestinal development and function (Xu *et al*., 2022).

Improvement on the number of goblet cells may lead on to maintaining the health of intestine as they produce mucus which is derived from the major gel-forming glycoprotein components called mucins, which forms mucus gel layer in the intestine acting as a medium for protection, lubrication, and transport between the luminal contents and the epithelial lining (Deplancke and Gaskins, 2001). However, in this study, there was no significant ($p > 0.05$) difference observed in the number of goblet cells among different feeding groups or within different intestinal portions.

CONCLUSION

In conclusion, this study showed that supplementation of fish feed with prebiotics inulin and MOS had variable effects on Nile tilapia reared under suboptimal temperature. MOS alone or a combination with inulin improved the villi length and width, and some PUFA levels of the fish compared to the control group. However, prebiotic supplementation did not significantly affect the survival, final weight, weight gain, SGR, FCR and ash content of the fish except for slight improvements observed in diets supplemented with MOS alone and in combination with inulin. Therefore, further studies are needed to optimize the prebiotic supplementation level for Nile tilapia under different conditions.

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REFERENCES

1. Adamneh Dagne and Abelneh Yimer, (2018). Growth performance of three Nile tilapia (*Oreochromis niloticus* L., 1758) populations in pond system. *International Journal of Aquaculture* **8**: 65-72.doi: 10.5376/ija.2018.08.0009
2. Asbury, R., Dipede, E. and Saville, B. (2022). Prebiotic Mannan-Oligosaccharides and Their Role in the Gut Microbiota. *Current Developments in Nutrition*. **6**: 1130. https://doi.org/10.1093/cdn/nzac072.002
3. Azaza, M.S., Dhraïef, M.N. and Kraïem, M.M. (2008). Effects of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia. *Journal of Thermal Biology* **33(2):** 98–105. https://doi.org/10.1016/j.jtherbio.2007.05.007
4. Azevedo, R.V.D., Fosse Filho, J.C., Cardoso, L.D., Mattos, D.D.C., Vidal Júnior, M. V. and Andrade, D.R.D. (2015). Economic evaluation of prebiotics, probiotics and symbiotics in juvenile Nile tilapia.*Revista Ciência Agronômica* **46(1):** 72–79. https://doi.org/10.1590/S1806-66902015000100009
5. Cechim, F.E., Sales, F.B., Signor, A.A., Mária, MICHELS-SOUZA, A. and Sado, R.Y. (2015). Dietary mannan oligosaccharide influenced feed consumption and gut morphology of Nile tilapia raised in net-cage systems. *Boletim do Instituto dePesca***41(3**):519527.[Online].Available:https://www.pesca.agricultura.sp.gov.br/boletim/index.php/bip/article/view/41_3_519-527. Date accessed: 06 Sep 2022
6. Deplancke, B. and Gaskins, H.R. (2001). Microbial modulation of innate defense: Goblet cells and the intestinal mucus layer. *The American Journal of Clinical Nutrition* **73(6)**: 1131S-1141S. https://doi.org/10.1093/ajcn/73.6.1131S
7. Dimitroglou, A., Merrifield, D.L., Spring, P., Sweetman, J., Moate, R. and Davies, S. J. (2010). Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead

sea bream (*Sparus aurata*). *Aquaculture* **300(1–4)**: 182–188. https://doi.org/10.1016/j.aquaculture.2010.01.015

8. Eshaghzadeh, H., Hoseinifar, S.H., Vahabzadeh, H. and Ringø, E. (2015). The effects of dietary inulin on growth performances, survival and digestive enzyme activities of common carp (*Cyprinus carpio*) fry. *Aquaculture Nutrition* **21(2):** 242–247. https://doi.org/10.1111/anu.12155
9. Ganguly, S., Dora, K.C., Sarkar, S. and Chowdhury, S. (2013). Supplementation of prebiotics in fish feed: A review. *Reviews in Fish Biology and Fisheries* **23(2):** 195–199. https://doi.org/10.1007/s11160-012-9291-5
10. Genc, A.M., Yilmaz, E., Genc, E., Genc, E. and Aktas, M. (2007). Effects of dietary mannan oligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus x O. aureus*). *Israeli Journal of Aquaculture –Bamidgeh* **59**. https://doi.org/10.46989/001c.20509
11. Gewaily, M. S., Abdo, S. E., Moustafa, E. M., Abdel-Kader, M. F., Abd El-Razek, I. M., El-Sharnouby, M., Alkafafy, M., Raza, S. H. A., El Basuini, M. F., Van Doan, H. and Dawood, M.A. (2021). Dietary synbiotics can help relieve the impacts of deltamethrin toxicity of Nile tilapia reared at low temperatures. *Animals* **11**: 1790. https://doi.org/10.3390/ani11061790
12. Guo, P., Dong, L., Wang, F., Chen, L. and Zhang, W. (2022). Deciphering and engineering the polyunsaturated fatty acid synthase pathway from eukaryotic microorganisms. *Frontiers in Bioengineering and Biotechnology* **10**: 1052785. https://doi.org/10.3389/fbioe.2022.1052785
13. Hutchinson, N.T., Wang, S. S., Rund, L.A., Caetano-Silva, M.E., Allen, J.M., Johnson, R.W. and Woods, J.A. (2023). Effects of an inulin fiber diet on the gut microbiome, colon, and inflammatory biomarkers in aged mice. *Experimental Gerontology* **176:** 112164. https://doi.org/10.1016/j.exger.2023.112164
14. Ismail, A., Bannenberg, G., Rice, H.B., Schutt, E. and MacKay, D. (2016). Oxidation in EPA and DHA rich oils: An overview. *Lipid Technology* **28(3–4):**55–59. https://doi.org/10.1002/lite.201600013
15. Levy-Pereira, N., Yasui, G.S., Cardozo, M.V., Dias Neto, J., Farias, T.H.V., Sakabe, R., Pádua, S.B. and Pilarski, F. (2018). Immunostimulation and increase of intestinal lactic acid bacteria with dietary mannan-oligosaccharide in Nile tilapia juveniles.*Revista Brasileira de Zootecnia* **47:** e20170006. https://doi.org/10.1590/rbz4720170006
16. Liu, C., Zhao, L.-P. and Shen, Y.-Q. (2021). A systematic review of advances in intestinal microflora of fish. *Fish Physiology and Biochemistry* **47(6),** 2041–2053. https://doi.org/10.1007/s10695-021-01027-3
17. Lokesh, J., Ghislain, M., Reyrolle, M., Bechec, M.L., Pigot, T., Terrier, F., Roy, J., Panserat, S. and Ricaud, K. (2022). Prebiotics Modify Host Metabolism in Rainbow Trout (*Oncorhynchus Mykiss*) Fed with a Total Plant-Based Diet: Potential Implications for Microbiome-Mediated Diet Optimization. *Aquaculture* **561**:738699.https://doi.org/10.1016/j.aquaculture.2022.738699
18. Magouz, F.I., Dawood, M.A.O., Salem, M.F.I., El-Ghandour, M., Van Doan, H., and Mohamed, A.A.I.(2020).The role of a digestive enhancer in improving the growth performance, digestive enzymes activity, and health condition of Nile tilapia (*Oreochromis niloticus*) reared under suboptimal temperature. *Aquaculture* **526**: 735388.https://doi.org/10.1016/j.aquaculture.2020.735388
19. Monroig, Ó. and Kabeya, N. (2018). Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: A comprehensive review. *Fisheries Science* **84(6):** 911–928.https://doi.org/10.1007/s12562-018-1254-x
20. Mugwanya, M., Dawood, M.A.O., Kimera, F. and Sewilam, H.(2022). Updating the Role of Probiotics, Prebiotics, and Synbiotics for Tilapia Aquaculture as Leading Candidates for Food Sustainability: A Review. *Probiotics and Antimicrobial Proteins* ***14*(1):** 130–157.https://doi.org/10.1007/s12602-021-09852-x
21. Negash Kabtimer, Getachew Beneberu, Solomon Workneh, Curto, M., Waidbacher, H., Meimberg, H. and Domig, K. J. (2022). The gut bacterial microbiome of Nile tilapia (*Oreochromis niloticus*) from lakes across an altitudinal gradient. *BMC microbiology* **22(1**): 87. https://doi.org/10.1186/s12866-022-02496-z
22. Nguyen, N.H., Ponzoni, R.W., Yee, H.Y., Abu-Bakar, K.R., Hamzah, A. and Khaw, H.L. (2010). Quantitative genetic basis of fatty acid composition in the GIFT strain of Nile tilapia (*Oreochromis niloticus*) selected for high growth. *Aquaculture* **309(1–4):** 66–

74.https://doi.org/10.1016/j.aquaculture.2010.08.034

23. Nivelle, R., Gennotte, V., Kalala, E. J. K., Ngoc, N. B., Muller, M., Melard, C. and Rougeot, C. (2019). Temperature preference of Nile tilapia (*Oreochromis niloticus*) juveniles induces spontaneous sex reversal. *PLoS One* **14**: e0212504. https://doi.org/10.1371/journal.pone.0212504

24. Oppedisano, F., Macrì, R., Gliozzi, M., Musolino, V., Carresi, C., Maiuolo, J., Bosco, F., Nucera, S., Caterina Zito, M., Guarnieri, L. and Scarano, F. (2020). The anti-inflammatory and antioxidant properties of n-3 PUFAs: Their role in cardiovascularprotection. *Biomedicines* **8**:306.doi:10.3390/biomedicines80903

25. Porter, D., Peggs, D., McGurk, C., and Martin, S.A.M. (2022).Immune responses to prebiotics in farmed salmonid fish: How transcriptomic approaches help interpret responses. *Fish and Shellfish Immunology* **127:** 35 47.https://doi.org/10.1016/j.fsi.2022.05.055

26. Razeghi Mansour, M., Akrami, R., Ghobadi, S.H., Amani Denji, K., Ezatrahimi, N. and Gharaei, A. (2012). Effect of dietary mannan oligosaccharide (MOS) on growth performance, survival, body composition, and some hematological parameters ingiant sturgeon juvenile (*Husohuso* Linnaeus, 1754). *Fish Physiology and Biochemistry* **38(3):** 829–835.https://doi.org/10.1007/s10695-011-9570-4

27. Reza, A., Abdolmajid, H., Abbas, M. and Abdolmohammad, A.K. (2009). Effect of Dietary Prebiotic Inulin on Growth Performance, Intestinal Microflora, Body Composition and Hematological Parameters of Juvenile Beluga, *Husohuso* (Linnaeus, 1758). *Journal of the World Aquaculture Society* **40(6):** 771–779.https://doi.org/10.1111/j.1749-7345.2009.00297.x

28. Ringø, E., Olsen, R.E., Gifstad, T.Ø., Dalmo, R.A., Amlund, H., Hemre, G.-I. and Bakke, A.M. (2010).Prebiotics in aquaculture: A review. *Aquaculture Nutrition* **16(2):** 117–136.https://doi.org/10.1111/j.1365-2095.2009.00731.x

29. Schwarz, K.K., Furuya, W.M., Natali, M.R.M., Gaudezi, M.C. and Lima, P.A.G.D. (2011). Mananoligossacarídeo em dietas para larvas de tilápia. *Revista Brasileira de Zootecnia* **40(12):** 2634–2640.https://doi.org/10.1590/S1516-35982011001200003

30. Sutriana, A., Akter, M. N., Hashim, R. and Nor, S.A.M. (2021). Effectiveness of single and combined use of selected dietary probiotic and prebiotics on growth and intestinal conditions of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1978) juvenile. *Aquaculture International* **29 (6):** 2769–2791.https://doi.org/ 10.1007/s10499-021-00777-4

31. Tewodros Abate, Akewake Geremew and Abebe Getahun (2018). The Role of Functional Feed Additives in Tilapia Nutrition. *Fisheries and Aquaculture Journal* **9(02).** https://hal.science/hal-03631899

32. Tiengtam, N., Khempaka, S., Paengkoum, P. and Boonanuntanasarn, S. (2015). Effects of inulin and Jerusalem artichoke (*Helianthus tuberosus*) as prebiotic ingredients in the diet of juvenile Nile tilapia (*Oreochromis niloticus*). *Animal Feed Science and Technology* **207:** 120-129. https://doi.org/10.1016/j.anifeedsci.2015.05.008

33. Trbović, D., Polak, T., Demšar, L., Parunović, N., Dimitrijević, M., Nikolić, D. and Đorđević, V. (2018). Determination of the fatty acids in fish tissue and feed-comparison of different methods and statistical evaluation. *Acta Chromatographica* **30**: 175-179. https://doi.org/10.1556/1326.2017.00165

34. Wang, T., Wu, H.-X., Li, W., Xu, R., Qiao, F., Du, Z.-Y. and Zhang, M.-L. (2022). Effects of dietary mannan oligosaccharides (MOS) supplementation on metabolism, inflammatory response and gut microbiota of juvenile Nile tilapia (*Oreochromis niloticus*) fed with high carbohydrate diet.*Fish and Shellfish Immunology* **130:** 550–559.https://doi.org/10.1016/j.fsi.2022.09.052

35. Wang, T., Yang, J., Lin, G., Li, M., Zhu, R., Zhang, Y. and Mai, K. (2021a). Effects of Dietary Mannan Oligosaccharides on Non-Specific Immunity, Intestinal Health, and Antibiotic Resistance Genes in Pacific White Shrimp *Litopenaeus vannamei*. *Frontiers in Immunology* **12**: 772570.https://doi.org/10.3389/fimmu.2021.772570

36. Wang, T., Zhang, N., Yu, X.-B., Qiao, F., Chen, L.-Q., Du, Z.-Y. and Zhang, M.-L.(2021b). Inulin alleviates adverse metabolic syndrome and regulates intestinal microbiota composition in Nile tilapia (*Oreochromis niloticus*) fed with high-carbohydrate diet. *British Journal of Nutrition* **126(2**): 161–171.https://doi.org/10.1017/S000711452000402X

37. Xu, W., Lutz, C.G., Taylor, C.M. and Ortega, M.C. (2022). Improvement of Fish Growth and Metabolism by Oligosaccharide Prebiotic Supplement. *Aquaculture Nutrition* **2022**: 1–13.https://doi.org/10.1155/2022/5715649

38. Yassine, T., Khalafalla, M.M., Mamdouh, M., Elbialy, Z.I., Salah, A.S., Ahmedou, A., Mamoon, A., El-Shehawi, A.M., Van Doan, H. and Dawood, M.A.O. (2021). The enhancement of the growth rate, intestinal health, expression of immune-related genes, and resistance against suboptimal water temperature in common carp (*Cyprinus carpio*) by dietary paraprobiotics. *Aquaculture Reports* **20:** 100729.https://doi.org/10.1016/j.aqrep.2021.100729

39. Yuji-Sado, R., Raulino-Domanski, F., De Freitas, P.F. and Baioco-Sales, F. (2017).Growth, immune status and intestinal morphology of Nile tilapia fed dietary prebiotics (mannan oligosaccharides-MOS).*Latin American Journal of Aquatic Research* **43(5):** 944–952.https://doi.org/10.3856/vol43-issue5-fulltext-14