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RESEARCH ARTICLE

EFFECTS OF SINGLE OR MIXED DIETARY SUPPLEMENTATION OF INULIN OR MANNAN-OLIGOSACCHARIDE (MOS) ON GROWTH, FEED UTILIZATION, FATTY ACID PROFILES, GUT MORPHOLOGY, AND HEMATOLOGY IN NILE TILAPIA FRY

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ABSTRACT: The present study was designed to investigate the effect of supplementation of inulin and mannan-oligosaccharides (MOS), single or combined, on the growth performance, feed utilization, hematology, fatty acid profiles, and intestinal morphology of Chamo strain Nile tilapia *Oreochromis niloticus* (L.) fry. Nile tilapia fry (initial weight 1.37 ± 0.116 g) were fed four diets supplemented with prebiotics at 0 g of prebiotic/kg of fish feed (Diet-T_1), 5 g kg^{-1}inulin-supplemented diet (Diet-T_2), 6 g kg^{-1} MOS-supplemented diet (Diet-T_3), or a combination of 2.5 g kg^{-1} inulin and 3 g kg^{-1} MOS (Diet-T_4). Each diet was randomly assigned to three aquaria and hand-fed 6% of their body weight divided into three portions daily. Fish fed 6 g kg^{-1} of MOS had higher weight gain, Daily Growth Rate (DGR), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) although the effect was not significant ($p>0.05$). Fish fed Diet-T_3 and Diet-T_4 had the highest content of polyunsaturated fatty acids (PUFAs). Fish fed Diet-T_3 and Diet-T_4 had significantly higher villi length in the proximal and middle portion of the intestine compared to other feeding groups. There was also significant difference ($p<0.05$) recorded in villus width in fish fed Diet-T_3 and other feeding groups. Fish fed Diet-T_3 also showed significantly higher goblet number at proximal and middle portion of the intestine. RBC, HCT, HGB neutrophil and monocyte levels were higher in the 6 g MOS kg^{-1} fed fish. MCHC, platelets, and eosinophil levels were higher in fish fed 5 g kg^{-1} inulin supplemented diet. In conclusion, dietary supplementation of MOS could confer benefits on growth performance, fatty acid profiles, haematology, and intestinal morphology of Chamo strain Nile tilapia.

Key words/phrases: Chamo strain Nile tilapia, Fatty acid profiles, Hematology, Intestinal morphology, Inulin, MOS.

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INTRODUCTION

According to Subasinghe *et al*. (2009), production from capture fisheries has levelled off and most of the main fishing areas have reached their maximum potential. Sustaining fish supplies from capture fisheries will, therefore, not be able to meet the growing global demand for aquatic food and aquaculture is considered to be an opportunity to bridge the supply and demand gap of aquatic food in most regions of the world (Bharathi *et al*., 2019). Nile tilapia is one of the most farmed fish globally, with a significant contribution improving local livelihoods, especially in developing countries (Munguti *et al*., 2022). Feed represents around 50–80% production cost in aquaculture and successful aquaculture practice depends on a nutritionally balanced diet and low cost of production (Bharathi *et al*., 2019). Feed additives can be used in fish feed to extend beyond the satisfying basic nutritional requirements of the target species to improve growth and feed utilization, but also to support the health, induce physiological benefits and stress resistance of the fish (Encarnação, 2016; Tewodros Abate *et al*., 2018). According to Ringø *et al*. (2010); Kiron (2012); Ganguly *et al*. (2013); Pohlenz and Gatlin III (2014), administration of non-nutritive compounds in the diet has become a viable means of enhancing health of various aquatic species, mainly by the provision of cellular fractions such as β-glucans, mannan-oligosaccharides, fructo-oligosaccharides or sulfated polysaccharides or yeast and algae extracts.

Prebiotics are short chain carbohydrates that are non-digestible or minimally digested by digestive enzymes in stomach or small intestine of host but selectively enhance the activity of some groups of beneficial bacteria in colon (Gibson *et al*., 2004; Ringø *et al*., 2010; Leong and Duque, 2019). There are a number of prebiotics such as inulin, fructo-oligosaccharides (FOS), short-chain fructo-oligosaccharides (scFOS), mannan-oligosaccharides (MOS), galacto-oligosaccharides (GOS), xylooligo-saccharides (XOS), and arabinoxylo-oligosaccharides (AXOS) are tested in different fish species (Ringø *et al*., 2010). Several reports showed that adding single or mixed prebiotics in fish diet had positive effect on fish performance. For instance, fructo-oligosaccharides (FOS) in common carp (*Cyprinus carpio*) fry (Hoseinifar *et al*., 2014); xylo-oligosaccharide in juvenile grass carp (*Ctenopharyngodon idellus*) (Zhang *et al*., 2020); xylo-oligosaccharide in tilapia (Poolsawat *et al*., 2021). Growth and feed utilization of the crabs (*Eriocheir sinensis*) fed on 3 g kg^{-1} MOS + 1.5 g kg^{-1} β-glucan supplemented had improved growth compared with the other feeding groups (Lu *et al*., 2019). Jami *et al*. (2019) found that Caspian trout

(*Salmo trutta*) fed mixed prebiotics, 3 and 4 g of β-glucan and MOS, respectively, per kg basal diet significantly decreased FCR and increased WG, protein efficiency ratio (PER), and final weight.

Prebiotics inulin and MOS are common feed additives in different fish species. Adding MOS 5 g kg $^{-1}$ in high carbohydrate diet increased the weight gain and body length of juvenile Nile tilapia (Wang *et al*., 2022). According to Khosravi-Katuli *et al*. (2021), gilthead sea bream (*Sparus aurata*) fed 4 g kg^{-1} MOS supplemented diet had higher growth parameters compared to other feeding groups. FCR and PER were significantly influenced in milkfish (*Chanos chanos*) fed on 2 or 3 g kg^{-1} MOS supplemented diets (Harikrishnan *et al*., 2023). Supplementing feed with 4 g kg^{-1} MOS had improved growth parameters and health conditions in African catfish (*Clarias gariepinus*) (Genç *et al*., 2020). Eshaghzadeh *et al*. (2015) reported that supplementation of 5 or 10 g kg^{-1} inulin in carp (*Cyprinus carpio*) fry feed significantly increased survival rate. Li *et al*. (2018) found that combined supplementation of inulin (5 g kg^{-1}) and MOS (5 g kg^{-1}) remarkably enhanced innate immune response and pathogen resistance of shrimp (*Litopenaeus vannamei*).

However, the dietary supplementation of prebiotics was rarely studied on tilapia, especially the effects of MOS and inulin on the growth performance of tilapia. Therefore, this study aimed to investigate the potentials of dietary MOS and inulin on growth, feed utilization, body composition, fatty acid profiles, hematology, and intestinal morphology of Chamo strain Nile tilapia fry reared at optimal temperature.

MATERIALS AND METHODS

Experimental setup for growth trial

The experiment was conducted for 12 weeks at experimental facilities in the Centre for Aquaponics and Recirculating Aquaculture System (CARAS), Department of Zoological Sciences, Addis Ababa University. Fingerlings of Chamo strain Nile tilapia were produced in the hatchery unit within the center and were stocked at an average weight of 1.37 ± 0.116 g with 25 fish per aquarium (60 litres each) in a closed recirculation system. 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 12 weeks. The amount of feed was adjusted every week according to 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 28.12 ± 0.44°C and subjected to natural 12-h light: dark cycle.

Water quality parameters measured during the experiment averaged (± SD): pH, 7.3 ± 0.10; ammonia, 0.05 ± 0.08 mg l^{-1}; nitrite, 0.21 ± 0.10 mg l^{-1}; nitrate, 20 ± 0.21 mg l^{-1} and dissolved oxygen, 6.39 ± 0.38 mg l^{-1} and they were within acceptable ranges for tilapia culture.

Experimental design and diet preparation

The experimental design was completely randomized with four treatment diets in triplicates. A control/basal diet was formulated (as fed basis) to contain 39.51% protein and 15.01% lipid using fish meal and full fat soybean as main protein and lipid sources. Experimental diets were prepared by supplementing the basal formulated diet with different levels and types of prebiotics (Table 1). Prebiotic inulin (>90% pure) extracted from tuber of *Helianthus tuberosus* and MOS (>90% pure) (Yangling Ciyuan Biotech Co., Ltd., China) were used in this experiment. The four treatment diets were: basal diet/control diet (Diet-T_1) (0 g of prebiotics/kg fish feed), 5 g kg^{-1} inulin-supplemented diet (Diet-T_2), 6 g kg^{-1} MOS-supplemented diet (Diet-T_3), or combination/mixture of 2.5 g kg^{-1} inulin and 3 g kg^{-1} MOS-supplemented diet (Diet-T_4).

Growth performance

All fish were weighed every week and growth performance parameters were calculated according to the following formulae: Weight Gain (%) = (W_2-W_1/ W_1) x100; Specific Growth Rate (SGR) = 100 [lnW_2-lnW_1]/T, Daily Growth Rate (DGR) = (W^2-W^1/T) and Feed Conversion Ratio (FCR) = FO/WG. W_1 is initial weight (g), W_2 is final weight (g), T is experimental time (days), FO is feed offered (g) and WG is weight gain (g).

Diet and carcass composition

Ten fish were randomly sampled from each aquarium at harvest for the final carcass composition analyses. Fish were killed by immersing into an overdose of clove oil solution and dried in an oven at 60°C until a constant weight was achieved. Dried fish carcasses were ground in order to analyze proximate composition.

Analysis for crude protein, lipid, moisture and ash of experimental diets as well as fish carcass were carried out using standard methods of the AOAC (2002) procedures: dry matter (130°C to constant weight), ash by the gravimetric method (combusted at 550°C to constant weight), crude protein (N x 6.25) by the Kjeldahl method, and crude lipid extracted by the Soxhlet extraction method. All analyses were performed in triplicate. Ingredients and proximate composition of the experimental diet are presented in Table 1.

Table 1. Ingredients and proximate composition (g kg^{-1}) of the experimental diets.

	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 - Carboxyl methyl cellulose; NFE - Nitrogen free extract

Fatty acid profile determination

Frozen fish carcasses were used to determine the fatty acid (FA) profiles. Fat samples were extracted and methylated in accordance with Trbović *et al.* (2018) and analyzed using gas chromatography (GC-Agilent 7890B) equipped with flame ionization detector and HP88 capillary column (30 m x 0.25 mm x 0.20 µm). Helium was used as a carrier gas. The fatty acid methyl esters (FAMEs) were identified by comparison of retention times of known standards. The individual fatty acid concentrations were expressed as percentages of the total content.

Hematology

At the end of the experiment, hematological analysis was done at clinical chemistry laboratory of Ethiopian Public Health Institute (EPHI). Ten fish were randomly sampled from each treatment. Fish were anesthetized by immersion in water containing 1–3 ml of clove oil mixed in 10 ml of ethanol which was diluted in 1litre of water. Whole blood was collected from caudal vein puncture of each fish using 3 ml syringes (27-gauge needles) that were rinsed with heparin (5000 IU/ml) and transferred into 4 ml lavender top Vacuum EDTA K3 Tube (Henso Medical, China) for hematological assay. Blood hematology analyzer (Beckman Coulter, USA) was used to determine the values of red blood cell (RBC), white blood cell (WBC), hematocrit or packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Different white blood cell counts, including neutrophil (NE), lymphocyte (LY), monocyte (MO), eosinophil (EO), and basophils (BA) were measured. All operations on fish were conducted according to the Guidelines of the European Union (Directive 2010/63/UE).

Gut morphology study

At the end of feeding trial two fish from each triplicate aquarium were anesthetized in 1–3 ml of eugenol/clove oil mixed in 10 ml of ethanol which was diluted in 1 litre of water. For preparation of intestinal sample firstly whole gastrointestinal tract was removed and proximal, middle and distal portion of intestine was collected. Then each tissue was placed in 10 ml sample vials half filled with 10% formalin fixative. Slide preparation and scanning took place at Pathology Department of St. Paul's Millennium Medical College, Addis Ababa. Each sample was embedded in paraffin wax, sliced (5 µm thick), mounted on glass slides and stained with hematoxylin and eosin. Two longitudinal slices were prepared from each sample. The sample slides were documented photographically with a digital camera connected to a microscope (MoticEasyScan Pro 6 (USA) (Resolution: 40X: 0.26 µm/pixel-20X: 0.52 µm/pixel; Scanning Camera: 5.0 MP (2/3″ high speed Sensor); Scanning Mode: three-dimensional stacking). Counting of the number of goblet cells/villus (GC), measuring of villus height (VH), and villus width (VW) was made using a Dell OptiPlex 7450 computer connected to the scanner. For each sample, 6 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. Mean values were considered significantly different at $p<0.05$. Tukey's test was used to compare all parts of a column and to rank the groups.

RESULTS

Growth performance

The growth performance parameters of Chamo strain Nile tilapia fish fry fed different types of prebiotics are shown in Table 2. Although fish fed the diets supplemented with prebiotic MOS had highest growth performance parameters, no statistically significant differences were observed between final weight, weight gain, SGR, DGR and FCR of fry fed control or prebiotics supplemented diets ($p>0.05$). Fish fed 6 g kg^{-1} of MOS had higher weight gain, DGR, SGR and FCR than fish fed the basal diet and the diet supplemented with inulin alone or mixture of inulin and MOS. The second highest final body weight, SGR, and weight gain were recorded in fish fed basal diet.

Table 2. Growth performance of Nile tilapia fry fed experimental diets for 12 weeks.

	Diets				
	Diet-T_1	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**	**p-value**
Initial weight (g)	1.37 ± 0.159	1.36 ± 0.100	1.36 ± 0.133	1.37 ± 0.070	0.996
Final weight (g)	8.10 ± 0.931	7.41 ± 0.295	8.36 ± 0.648	8.06 ± 1.202	0.578
Weight gain (g)	497.1 ± 109.7	448.9 ± 58.5	517.29 ±14.51	488.7 ± 99.6	0.766
DGR (g/day)	0.087 ± 0.078	0.079 ± 0.055	0.091± 0.067	0.088 ± 0.067	0.976
SGR (% day^{-1})	2.310 ± 0.948	2.214 ± 0.509	2.365 ± 0.644	2.304 ± 0.724	0.969
FCR	2.846 ± 1.277	2.635 ± 0.618	2.544 ± 0.897	2.789 ± 1.543	0.920

Fish carcass composition

Proximate composition of the carcass is given in Table 3. Whole body moisture, crude protein, crude lipid, and ash were not affected significantly by prebiotic supplementation ($p>0.05$). However, fish fed basal diet (Diet-T_1) had higher crude protein (51.26 ± 0.88) and ash content (7.79 ± 0.28) than experimental diets. Fish fed MOS and combination of inulin and MOS had higher value of crude lipid than other treatments. The highest crude lipid value of 36.96 ± 0.09 and 36.89 ± 0.17 were recorded in fish fed on MOS alone and combination of inulin and MOS supplemented diets, respectively.

Table 3. Proximate composition (%) in carcass of Nile tilapia in the 12-week feeding trial (Mean ± SD).

	Diets				
Parameters	**Diet-T_1**	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**	**p-value**
Moisture	6.23 ± 0.30	6.35 ± 0.32	7.03 ± 0.05	6.30 ± 0.18	0.081
Crude protein	51.26 ± 0.88	50.55 ± 0.32	50.96 ± 0.67	50.40 ± 0.26	0.521
Crude lipid	34.13 ± 0.12[b]	36.14 ± 44[a]	36.96 ± 0.09[a]	36.89 ± 0.17[a]	0.001
Ash	7.79 ± 0.28	7.75 ± 0.07	7.10 ± 0.79	6.99 ± 0.02	0.258

Fatty acid profiles

Fatty acid analyses of Nile tilapia fed on different types and levels of prebiotic supplemented feed are shown in Table 4. The fatty acid profiles of total lipid of Nile tilapia were influenced by dietary supplementation of prebiotics. Σn-6 and *n*-3 PUFAs as well as the percentage of ΣPUFAs and PUFAs/SFAs ratio were higher in fish fed Diet-T_3 and Diet-T_4 than other feeding groups. Fish fed Diet-T_3 and Diet-T_4 had higher level of PUFAs such as 18:3*n*-3, 20:3*n*-6, 20:4*n*-6, 22:4*n*-6, 20:5*n*-3 and 22:6*n*-3 compared with fish fed Diet-T_2 and control group.

Table 4. Fatty acid profiles of Nile tilapia carcass fed experimental diets for 12 weeks.

	Final % of area			
Fatty acid	**Diet-T_1**	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**
12:0	0.09	0.08	0.07	0.05
14:0	1.88	1.91	1.85	1.93
15:0	0.71	0.82	0.86	0.81
16:0	18.59	18.42	16.88	17.11
17:0	0.55	0.57	0.43	0.46
18:0	5.49	5.24	5.16	5.15
ΣSFAs	**27.31**	**27.04**	**25.25**	**25.51**
16:1*n*-9	4.10	4.23	3.94	4.52
18:1*n*-9	23.29	23.96	22.55	22.28
18:1*n*-13	3.51	3.39	2.41	3.11
20:1*n*-9	0.74	0.72	0.62	0.78
ΣMUFAs	**31.64**	**32.3**	**29.52**	**30.69**
18:2*n*-6	24.72	24.69	25.54	25.65
18:3*n*-3	3.81	3.42	4.15	4.23
20:2*n*-6	1.42	1.54	1.19	0.85
20:3*n*-6	1.03	1.24	1.33	1.26
20:4*n*-6	1.55	1.48	1.99	1.72
20:4*n*-6	0.36	0.31	0.47	0.44
20:5*n*-3	0.36	0.39	0.53	0.47
22:4*n*-6	0.59	0.57	0.69	0.63
22:5*n*-6	0.68	0.61	0.78	0.67
22:5*n*-3	1.74	1.58	1.88	1.79
22:6*n*-3	3.47	3.89	4.69	4.17
DHA/EPA	10.64	12.31	13.98	12.2
Σn-6 & n-3 PUFAs	39.73	39.72	43.24	41.88
Percent (%) PUFA	40.26	40.1	44.12	42.7
Percent (%) MUFA	32.06	32.61	30.12	31.29
Percent (%) SFA	27.68	27.3	25.76	26.01
PUFA/SFA ratio	1.45	1.47	1.71	1.64

Hematology

The effect of dietary prebiotics supplementation on Chamo strain Nile tilapia on some hematological parameters is presented in Table 5. The hematological parameters were significantly affected by the dietary treatments in this study. Supplementing additives on Chamo strain Nile tilapia feed improved RBC, MCV, MCH, MCHC, hemoglobin, neutrophil, and monocyte level. RBC, PCV, HGB, neutrophil, and monocyte levels were higher in the fish fed 6 g MOS kg^{-1} compared to other experimental and the control group. MCH, MCHC, platelets, and eosinophil levels were higher in fish fed 5g kg^{-1} inulin supplemented diet.

Table 5. Hematological parameters of Nile tilapia fry fed experimental diets for 12 weeks (Mean ± SD).

	Diets			
Parameters	**Diet-T_1**	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**
RBC	1.43 ± 0.03^a	1.35 ± 0.06^a	1.62 ± 0.11^a	1.49 ± 0.01^a
Hb	8.00 ± 3.54^b	8.90 ± 1.13^a	9.85 ± 0.07^a	9.60 ± 0.14^a
PCV	26.55 ± 0.21^b	24.75 ± 0.78^c	29.85 ± 1.91^a	26.80 ± 0.28^b
MCV	185.75 ± 1.91^a	183.20 ± 3.39^a	185.0 ± 0.57^a	179.95 ± 0.21^a
MCH	55.80 ± 23.5^b	66.30 ± 5.66^a	61.35 ± 4.60^a	64.30 ± 1.56^a
MCHC	30.10 ± 13.01^b	36.06 ± 3.46^a	33.20 ± 2.40^a	35.75 ± 0.92^a
PLT	215.0 ± 38.2^b	478.0 ± 26.90^a	159.50 ± 27.60^b	405.00 ± 185^a
NE	10.10 ± 4.67^c	7.70 ± 0.00^d	18.05 ± 2.76^b	21.90 ± 3.39^a
LY	60.90 ± 14.6^a	20.20 ± 0.00^d	44.65 ± 3.61^c	53.65 ± 13.93^b
MO	1.05 ± 0.21^b	1.20 ± 0.00^b	3.10 ± 0.14^a	2.55 ± 0.50^a
EO	28.0 ± 19.50^c	70.90 ± 0.00^a	34.05 ± 6.43^b	21.90 ± 16.80^d
BA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Intestinal morphology

Intestinal morphology parameters (villi length, villi width and goblet cell number) of Nile tilapia fed on experimental diets are shown in Fig. 1, Fig. 2, and Table 6. The result indicated that dietary administration of different prebiotics significantly increased villi length and villi width compared to the control diet. Mean villi length in the proximal and middle portion of Nile tilapia intestine was affected significantly ($p<0.05$) by diet (Fig. 1A and 1B). Fish fed Diet-T_3 and Diet-T_4 had significantly higher villi length in the proximal and middle portion of the intestine compared to other feeding groups. There was also significant difference ($p<0.05$) recorded in villus width in fish fed Diet-T_3 and other feeding groups.

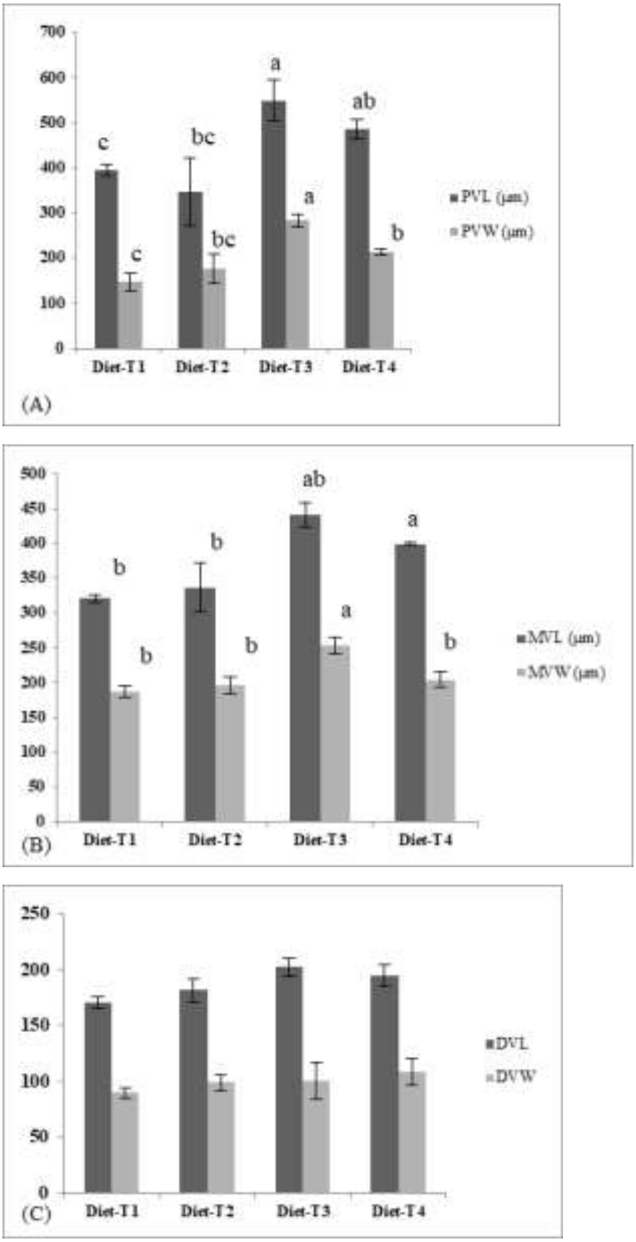

Fig. 1. Graph showing proximal villi length (PVL) and villi width (PVW) (A), middle villi length (MVL) and villi width (MVW) (B), and distal villi length (DVL) and villi width (DVW) (C) of intestine of Nile tilapia in µm.

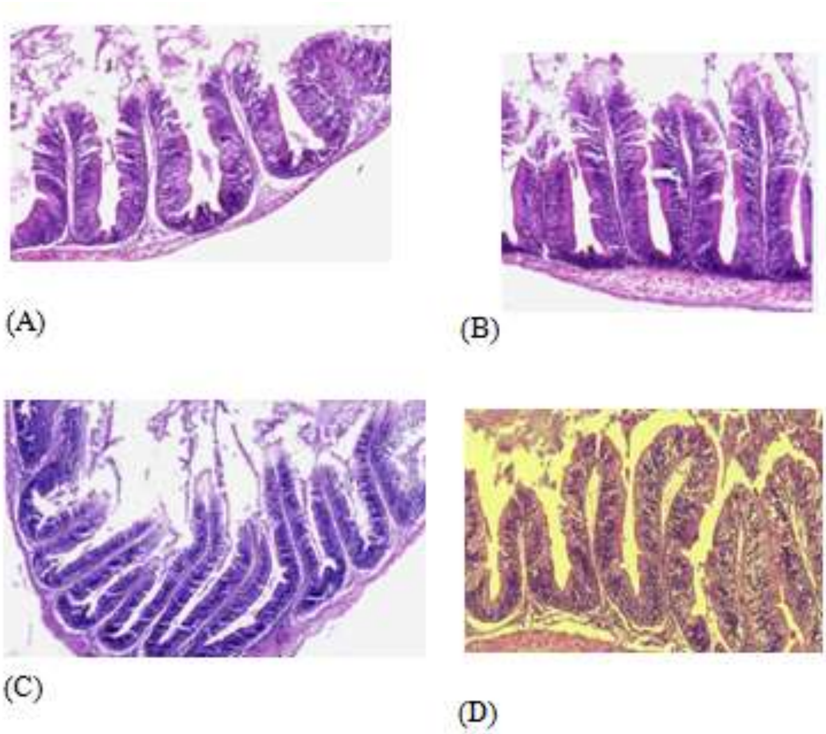

Fig. 2. Gut morphology slide samples scanned by MoticEasyScan Pro 6 (USA): Middle villi length and width of (A) Diet-T_1; (B) Diet-T_2; (C) Diet-T_3 and (D) Diet-T_4.

Mean scores of goblet cell number per villus at proximal, middle and distal portion of intestine are presented in Table 7. There was significant ($p<0.05$) difference observed in the number of goblet cells among different feeding groups within different intestinal portions. Fish fed Diet-T_3 showed significantly higher goblet number at proximal and middle portion of the intestine. However, there was no significant difference observed in all feeding groups in distal portion of the intestine.

Table 7. Goblet cells/villi of proximal, middle and distal parts of intestine of Nile tilapia fed with diets containing different types and levels of prebiotics (Mean ± SD).

	Diets				
	Diet-T_1	**Diet-T_2**	**Diet-T_3**	**Diet-T_4**	**p-value**
Proximal	8.11 ± 0.30^c	13.55 ± 0.59^b	17.05 ± 0.52^a	14.89 ± 1.10^{ab}	0.005
Middle	14.03 ± 0.80^{bc}	15.89 ± 0.36^b	20.60 ± 0.83^a	11.04 ± 1.03^c	0.001
Distal	9.27 ± 0.47^a	11.40 ± 1.03^a	12.02 ± 1.65^a	10.57 ± 0.06^a	0.170

Means that do not share a letter are significantly different.

DISCUSSION

Growth performance

Dietary prebiotics have been tested in different life stages of tilapia (Abd El-Gawad *et al*., 2016; Van Doan *et al*., 2018; Dawood *et al*., 2020a; Yones *et al*., 2020; Poolsawat *et al*., 2021). Most of these studies have agreed that prebiotics have a positive effect on Nile tilapia performances. The current study displayed that dietary MOS supplementations could improve growth performance of fish, suggesting that dietary MOS supplementations were conducted on the growth of Nile tilapia. Nile tilapia fed 6 g kg^{-1} MOS-supplemented diet displayed increased WG, SGR, and decreased FCR than control group and other feeding groups. However, there were no significant differences between treatments during the feeding trial. This result was similar to Genc *et al*. (2007) findings that the control and treatment groups did not significantly differ with respect to growth parameters. Dawood *et al*. (2020a) reported that Nile tilapia fed MOS supplemented diet showed higher growth, survival rates, and lower FCR than control group.

The study result was similar with Poolsawat *et al*. (2021) report on xylooligosaccharide supplementation on tilapia nutrition. According to Al-Wakeel *et al*. (2019), adding MOS into Nile tilapia feed and rearing water significantly increased weight gain and SGR. Abdel-Latif *et al*. (2020) study showed that feeding tilapia with raffinose at 1 g kg^{-1} increased the WG and SGR and decreased the FCR with regards to the control. Azevedo *et al*. (2016) reported that supplementing Nile tilapia feed with MOS alone or with probiotic *Bacillus subtilis* could improve growth parameters. Wang *et al*. (2022) also found that juvenile Nile tilapia fed with high carbohydrate diet supplemented with MOS increased growth performance.

According to Momeni-Moghaddam *et al*. (2015), supplementing common carp (*Cyprinus carpio)* fingerlings feed with MOS improves FCR compared with control group. Dietary supplementation of juvenile striped catfish (*Pangasianodon hypophthalmus*) with 6 g kg^{-1} or 8 g kg^{-1} MOS significantly influenced final weight, SGR, and feed utilization in terms of FCR compared to the remaining treatment groups and control group (Akter *et al*., 2016). Dietary MOS supplementation also improved growth performance in grass carp. However, Sado *et al*. (2015) reported that supplementing MOS feed has no effect on growth parameters in Nile tilapia.

In this study, Nile tilapia fed on inulin supplemented diet showed lowest growth performance compared with other feeding groups. Contrary to this, Tiengtam *et al*. (2017) and Yones *et al*. (2020) reported that adding inulin 5 g kg^{-1} and 2.5 g kg^{-1} to the Nile tilapia fingerlings feed improved growth performance and efficiency, respectively. According to Hoseinifar *et al*. (2017), common carps fed on galacto-oligosaccharide supplemented feed displayed improved ($p<0.05$) growth performance, including final weight, SGR, and FCR compared to the with-inulin group.

Fish carcass composition

In this study, fish fed prebioics supplemented diets had higher value of body crude lipid and lower value of crude protein compared to control groups. This is true in common carp fry (Eshaghzadeh *et al*., 2015) and rainbow trout (Ortiz *et al*., 2013) fed on inulin supplemented diet (Eshaghzadeh *et al*., 2015). On the contrary, Genc *et al*. (2007) and Azevedo *et al*. (2016) found that supplementing MOS to tilapia feed increased crude protein content. Tiengtam *et al*. (2015) reported that Nile tilapia fed 5 g kg^{-1} inulin supplemented feed had higher body fat than other feeding groups. This is also true in tilapia fed on FOS supplemented diet (Poolsawat *et al*., 2020).

Fatty acid profiles

In this study, fish fed MOS or combination of inulin and MOS supplemented diet had higher level of PUFAs such as 18:3*n*-3, 20:3*n*-6, 20:4*n*-6, 22:4*n*-6, 20:5*n*-3 (EPA) and 22:6*n*-3 (DHA) and lower contents of saturated and monosaturated fatty acids compared with fish fed inulin alone or control group. This result is in agreement with Lu *et al*. (2023) study on grass carp and Eryalçin *et al*. (2017) study on gilthead sea bream (*Sparus aurata*) larvae. According to Eryalçin *et al*. (2017), adding MOS in this species feed during larval stage significantly ($p<0.05$) reduced larval content in saturated fatty acids, particularly 16:0 and 18:0, monounsaturated, such as 18:1*n*-9 and *n*-9 fatty acids. But larval total *n*-3, *n*-3 HUFA, EPA and DHA were increased by the elevation of MOS dietary levels in gilthead sea bream. According to Gelibolu *et al*. (2018a), PUFA and ∑n-3 fatty acids were found at higher levels in groups fed MOS-added (3 g kg^{-1}) diet compared to the 0 g kg^{-1} MOS-added group in gilthead seabream. Dietary incorporation of MOS at 1.6 g kg^{-1} promotes increasing LC-PUFA (long chain PUFA) accumulation in the muscle and liver of European sea bass and promoting b-oxidation (Torrecillas *et al*., 2015). Contrarily, total PUFA of lipids was reduced in sharp snout seabream (*Diplodus puntazzo*) when inulin and MOS added in feed with soyabean meal was the main protein source compared with control diet without prebiotics (Piccolo *et al*., 2013).

Hematology

In fish, hematological parameters like RBC, hematocrit, hemoglobin concentration (Hb), and erythrocyte indexes (MCV, MCH, and MCHC) are used to provide information about the health and physiological status of fish, feeding conditions and water quality in which they live (Hrubec and Smith, 2010; Fazio, 2019). In the present study, feed additives improved some hematological parameters and maintained at normal reference level for tilapia (Hrubec and Smith, 2010), as also observed in tellate sturgeon (*Acipenser stellatus*) juvenile (Akrami *et al*., 2013b); juvenile great sturgeon (*Huso huso*) (Akrami *et al*., 2013a); snakehead (*Channa striata*) fingerlings (Talpur *et al*., 2014); juvenile pacu (*Piaractus mesopotamicus*) (Sado *et al*., 2014); and common carp fingerlings (Abdulrahman and Ahmed, 2016).

Supplementing Nile tilapia feed with MOS increased RBC and hemoglobin level in this study. This might be because prebiotics enhance iron absorption since fermentation of prebiotics by natural microflora present in the colon may decrease the pH of the luminal content, promote reduction of Fe (III) to Fe (II), stimulate proliferation of epithelial cells to expand the absorptive surface area, and potentially stimulate expression of mineral-transport proteins in epithelial cells (Yeung *et al*., 2005; Lauzon *et al*., 2014). Increased hemoglobin level was also reported on Asian seabass (*Lates calcarifer*) fingerlings fed on MOS supplemented diet (Ali *et al*., 2015).

Similar to this study, Ahmdifar *et al*. (2010) reported that adding inulin to juvenile great sturgeon diet increased MCH but decreased red blood cell count and hematocrit level compared to the control group. However, Ibrahem *et al*. (2010) reported that adding inulin in Nile tilapia feed improved hematocrit level. Reza *et al*. (2009) found that supplementing juvenile beluga (*Huso huso*) feed with different levels of inulin had no significant effect on MCH and RBC levels. However, Sado *et al*. (2008) report on Nile tilapia; Razeghi Mansour *et al*. (2012)'s report on giant sturgeon juvenile; and Gültepe *et al*. (2012)'s finding on gilthead seabream, showed that supplementation of MOS had no significant effect on hematological parameters.

Supplementing Nile tilapia feed with inulin alone or with combination of MOS improved platelet level in this study. In the study by Gelibolu *et al*. (2018b), dietary MOS inclusion did not affect platelet level in sea bream.

Our results of the present study showed that adding inulin or MOS in Nile tilapia feed increased eosinophils level. Dietary MOS alone or combined with inulin also increased neutrophil and monocyte levels, which are primary phagocytic cells in fish (Hrubec and Smith, 2010). Razeghi Mansour *et al*. (2012) found that administration of MOS increased eosinophils level in giant sturgeon juvenile.

Intestinal morphology

In this study, fish fed 6 g kg^{-1} MOS and combination of inulin and MOS had significantly higher villi length in the proximal and middle portion of the intestine compared to other feeding groups. According to Sado *et al*. (2015), Nile tilapia fed 4 g kg^{-1} of MOS for 30 days had higher villi length than other feeding groups. Cechim *et al*. (2012) also reported that Nile tilapia fed diet containing 0.4% MOS supplementation showed increased ($p<0.05$) villi length compared with control group. Mucosal, villi lengths, and muscle thickness significantly increased in GIFT tilapia fed on β-Glucan supplemented diet compared to control group (Dawood *et al*., 2020b). Supplementing juvenile striped catfish feed with 6 g kg^{-1} MOS increased villi length and growth performance compared to control groups (Akter *et al*., 2016). Al-Wakeel *et al*. (2019) reported that adding MOS into Nile tilapia feed and rearing water increased villi length and enhanced growth performances.

In the current study, fish fed 5 g kg^{-1} inulin alone also had higher villi length in middle portion of the intestine compared to control group. This result was similar to that of Boonanuntanasarn *et al*. (2018) and Tiengtam *et al*. (2015) investigations on Nile tilapia fed 5 g kg^{-1} inulin. Supplementing trans-galactooligosaccharide in common carp increased the height, width, and surface of the villi (Ziółkowska *et al*., 2020).

In this study, fish fed 6 g kg^{-1} MOS supplemented diet showed significantly higher goblet number at proximal and middle portion of the intestine compared with other feeding groups. Nile tilapia fed on 5 g kg^{-1} inulin supplemented diet also had better goblet cell number than control group. This result was similar to Tiengtam *et al*. (2015) and Boonanuntanasarn *et al*. (2018) investigation on Nile tilapia diet fed inulin supplemented diet.

CONCLUSION

Supplementation of fish feed with 6 g kg^{-1} MOS or combination of 2.5 g kg^{1} inulin and 3 g kg^{-1} MOS improved growth performance, some hematological parameters, villi length, PUFA (especially DHA; 22:6 *n*-3 and EPA; 20:5 *n*-3) and lowered the contents of saturated and monosaturated fatty acids of Chamo strain Nile tilapia. MOS supplementation also improved the goblet cell number in the villi which produces mucus coating of the small intestine. Hence, addition of prebiotics MOS alone or with combination of inulin have beneficial effects on the performance of Chamo strain Nile tilapia and could be used as sustainable natural additive in tilapia nutrition.

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