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Effects of Dietary Mannan Oligosaccharides (MOS) on Growth, Body Composition, and Intestine and Liver Histology of the Hybrid Tilapia *(Oreochromis niloticus* **x** *O. aureus)*

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Abstract

This is the first study on the effects of dietary mannan oligosaccharides (MOS) on growth, body composition, and intestine and liver histology of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). Experimental diets were prepared from commercial trout diet, supplemented with MOS at levels of 0, 1.5, 3.0, or 4.5 g MOS/kg feed and randomly assigned to triplicate groups. At the end of the trial, there were no significant differences between treatment groups ($p>0.05$) in growth parameters (live weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio) or body indices (hepatosomatic and viscerosomatic). Dry matter and protein contents increased with increasing rates of dietary MOS ($p<0.05$) while the mean villi length of fish fed the diet containing 1.5‰ MOS was significantly longer ($p<0.05$) than that of the fish fed 4.5‰ dietary MOS. The different levels of dietary MOS had no detrimental effects on liver tissue or general fish health.

Introduction

Rapid growth and disease resistance are two of the most important concerns in aquaculture. In some animal species, antibiotics can enhance growth and feed efficiency by killing intestinal micro-flora and thereby increasing amino acid utilization by the host (Rawles et al., 1997). However, the use of antibiotics as additives in fish diets is banned or restricted in many countries because of the development of antibiotic resistance in micro-organisms. Therefore, there is an increase in research on alternative feed additives. Policy changes

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impact aquaculture, prompting interest in development of alternative strategies for healthy growth. Beside vaccine development, dietary supplements including probiotics, prebiotics such as mannan oligosaccharides (MOS), and immunostimulants have received heightened attention. Development of prebiotics (non-digestible food ingredients that beneficially affect the host by stimulating growth and/or activity of a limited number of bacteria in the intestine) is in its infancy with fishes, compared to progress in the development of prebiotics for poultry (Patterson and Burkholder, 2003).

Brewers yeast has been included as a protein feedstuff in commercial diet formulations for several fish species including salmonids (NRC, 1993). Mannan oligosaccharide (MOS), a glucomannoprotein complex derived from the cell wall of the yeast *Saccharomyces cerevisiae*, was introduced as a feed additive for broiler chickens in 1993. MOS prevent attachment and colonization of pathogenic bacteria in the digestive tract and reduce adverse effects of micro-flora metabolites (Newman, 1994; Savage et al., 1997). MOS has been shown to improve body weight, feed conversion ratio, livability (Hoodge, 2004), and intestinal function or 'gut health' (villi height, uniformity, and integrity; Loddi et al., 2002) in poultry. Immune modulation stimulates gut-associated and systemic immunity by acting as a non-pathogenic microbial antigen, producing adjuvant-like effects (Ferket et al., 2002). Several studies concentrated on growth, feed efficiency, and resistance to pathogenic bacteria in fish fed diets containing prebiotics (Li and Gatlin, 2004, 2005), but only one focused on the effects of MOS in Gulf of Mexico sturgeon performance (Pryor et al., 2003).

Tilapias have a great impact on warm-water fish culture throughout the world. The worldwide harvest of farmed tilapia has surpassed 800,000 tons (Popma and Masser, 1999). Therefore, the objective of this study was to investigate the effects of dietary MOS on growth, body composition, and intestine and liver histology of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*).

Materials and Methods

The feeding trial was conducted at the Fisheries Faculty of Mustafa Kemal University in Hatay, Turkey, with hybrid tilapia *Oreochromis niloticus* x *O. aureus* fingerlings obtained from the hatchery of the Fisheries Faculty of Cukurova University in Adana, Turkey. Upon arrival, the fish were acclimatized in a 1000-l fiberglass tank and, after an adaptation period of 10 days under laboratory conditions, 15 fish were stocked into each of twelve 96-l aquaria. The aquaria were housed inside a room with a natural photoperiod and received continuous aeration. A static system was used and 80% of the water in each aquarium was changed daily. Water temperature was 25±1ºC throughout the feeding trial. Oxygen, pH, and total alkalinity were 6.2-6.5 mg/l, 7.82-8.33, and 250-255 mg/l $CaCO_3$, respectively.

Different levels of phosphorylated mannan oligosaccharides (MOS; AQUA-MYCES, Vitomix, Colombia) from the outer portion of the cell wall of the yeast *Saccharomyces cerevisiae* were used as the feed additive (prebiotic). Experimental diets were prepared by supplementing a commercial trout diet by 0, 1.5, 3.0, or 4.5 g MOS per kg diet and randomly assigned to triplicate groups. Dietary MOS doses were determined according to the commercial recommendation of AQUA-MYCES and Pryor et al. (2003). The diets were given to the fish by hand to visible satiation twice a day (at 10:00 and 16:00) for 80 days. All fish were weighed individually every 20 days, measured following anesthetization in an aerated water bath containing 5 mg/l quinaldine sulphate (Sigma Chemical Company, Germany) and 1 mg/l diazepam (Deva Company, Turkey; Yanar and Genc, 2004).

On completion of the feeding trial, fish were starved for 24 h, killed by over-anesthetization, and measured for growth parameters. Liver and viscera (except kidney) from five fish per aquarium were weighed to determine hepatosomatic and viscerosomatic index values. The remaining fish were pooled and stored frozen for proximate analysis. Proximate compositions of diets and fish fillets were analyzed according to AOAC (1997) procedures as follows: moisture was deter-

mined by oven-drying at 105°C for 24 h, crude protein (N x 6.25) by the Kjeldahl method, and crude ash by combustion in a muffle furnace at 550°C for 16 h. Total lipid concentration was determined by extract with the chloroform-methanol method described by Bligh and Dyer (1959).

Fixed second segments of the proximal major coil of the intestine (Tengjaroenkul et al., 2000) and liver specimens (4% buffered formaldehyde) were processed automatically (Thermo Shondon) and embedded in paraffin wax for histology. Sections (5 µ) were cut (Laica) and mounted on glass slides before staining with Mayers hematoxilin and eosin. Stained sections were examined and photographed under light trinocular (Olympus BX50) microscopy (Takashima and Hibiya, 1995; Genc et al., 2005). Villi lengths were measured with micrometric ocular (1-100 µm) under light microscopy.

Final fish weights, live weight gains, specific growth rates, feed conversion ratios, protein efficiency ratios, carcass compositions, hepatosomatic indices, viscerosomatic indices, and villi lengths were subjected to one-way analysis of variance to determine if significant differences occurred between dietary treatments. Data were statistically analyzed with one-way ANOVA and Duncan's multiple range tests. Effects with a probability of $p<0.05$ were considered significant. Statistical analyses were performed using SPSS for Windows (Standard Version 9.0 SPSS Inc. Chicago, Illinois). Data were expressed as mean values ± SEM.

Results

There were no significant differences in live weight gains and specific growth rates between treatments during the feeding trial (each sampling period of 20 days). At the end of the experiment, live weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, and hepatosomatic and viscerosomatic indices did not differ (Table 1). The mean villi length of fish fed the diet containing 1.5‰ MOS was longer than that of fish fed the diet containing 4.5‰ dietary MOS. Dry matter and protein contents of fish fillets increased with the rate of dietary MOS (Table 2). Different levels of dietary MOS had no detrimental effects on liver or intestine tissues (Fig. 1).

Discussion

This study was the first attempt to investigate the effects of MOS on growth, body composition, intestine structure, and liver histology of hybrid tilapia. Results showed no differences between groups of fish fed 0, 1.5, 3.0, or 4.5‰ MOS diets with respect to growth and body indices even though MOS enhances growth in some terrestrial vertebrates (Savage et al., 1997; White et al., 2002; Fritts and Waldroup, 2003). Lara-Flores et al. (2003) investigated the effects of whole brewers yeast, *Saccharomyces cerevisiae,* as a probiotic on growth, body composition, and digestibility of diets in fry of Nile tilapia (*Oreochromis niloticus*) and found that the addition of 1‰ yeast to a diet with an optimum protein content (40%) administered in low density tanks (10 fry/20 l) produced the best growth (individual weight gain, specific growth rate), with values higher than those of the 1‰ bacterial probiotic mix and control treatments ($p<0.05$). While whole yeast improved growth in Nile tilapia, our use of only the outer cell wall of the yeast (MOS) had no effect on growth in hybrid tilapia.

A few studies investigated the effects of different prebiotics on fish growth (Li and Gatlin, 2004, 2005). In these studies, the effects of partially autolyzed brewers yeast (Brewtech^{R}) and Grobiotic™ AE (a commercial prebiotic) on growth, immune response, and resistance of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) were investigated. Although enhanced growth was generally observed, there were no significant differences in growth between treatments with 1% and 2% Grobiotic™ AE compared to treatments with the basal diet after the first feeding periods (for 7 and 16 weeks). Only one study reported the effects of dietary MOS on growth and digestive tract (intestine) morphology in Gulf sturgeon, *Acipenser oxyrinchus desotoi* (Pryor et al., 2003). In that study, there were no differences in growth performance (condition factor, specific growth rates for weight

Table 1. Growth performance and body indices of hybrid tilapia fingerlings fed diets containing different levels of mannan oligosaccharides (MOS).

	MOS (‰)			
	0	1.5	3.0	4.5
Initial weight (g)	9.79±0.28	9.76±0.27	9.83±0.28	9.86±0.28
Final weight (g)	39.31±2.07	37.61±1.92	39.11±1.80	37.95±1.73
Live weight gain (g)	29.52±3.34	27.84±4.64	29.29±1.13	28.10±1.66
SGR[1]	1.73±0.11	1.67±0.16	1.73±0.04	1.68±0.06
FCR[2]	1.35±0.11	1.42±0.11	1.42±0.06	1.37±0.06
PER[3]	1.75±0.14	1.66±0.14	1.64±0.07	1.70±0.08
HSI[4]	2.48±0.12	2.41±0.08	2.43±0.10	2.24±0.12
VSI[5]	9.19±0.34	8.62±0.16	9.02±0.25	8.52±0.31
Villi length (μm)	598.20±27.90^{ab}	639.70±19.80^{b}	625.20±27.90^{ab}	550.40±26.50^{a}

Different superscripts in rows indicate significant differences (p<0.05).
[1] Specific growth rate (%/day) = 100(log final body wt - log initial body wt)/days
[2] Feed conversion ratio = feed consumption/wt gain
[3] Protein efficiency ratio = live wt gain/consumed protein
[4] Hepatosomatic index = 100(liver wt/body wt)
[5] Viscerosomatic index = 100(viscera wt/body wt)

Table 2. Proximate composition (% dry wt) of diet and hybrid tilapia fingerlings fed diets containing different levels of Mannan oligosaccharides (MOS).

		MOS (‰)			
	Diet	0	1.5	3.0	4.5
Moisture	10.21±0.13	76.69±0.23^{a}	75.51±0.13^{b}	74.33±0.18^{c}	72.94±0.08^{d}
Crude protein	42.83±0.19	21.49±0.99^{a}	21.92±0.59^{a}	23.12±0.05^{ab}	24.49±0.27^{b}
Lipid	19.64±0.36	1.35±0.06^{a}	1.39±0.03^{a}	1.42±0.13^{a}	1.51±0.02^{a}
Crude ash	9.64±0.11	1.36±0.01^{a}	1.31±0.24^{a}	1.36±0.02^{a}	1.11±0.22^{a}

Values (triplicate composite samples of five fish each) in rows with different superscripts differ significantly (p<0.05).

and fork length, feed conversion ratio), gross gastrointestinal morphology (gut and spiral valve lengths), or spiral valve villi structure between fish fed the control and the 3‰ MOS-supplemented diets. The current study indicates that villi lengths in the intestine were 1.5‰>0‰=3‰>4.5‰ with the only significant difference being between fish fed 1.5‰ and 4.5‰ MOS. Therefore, our results were similar to Pryor's (2003) findings that the control and treatment groups did not significantly differ with respect to villi length.

In contrast to growth parameters, the protein contents and dry matter of the fish

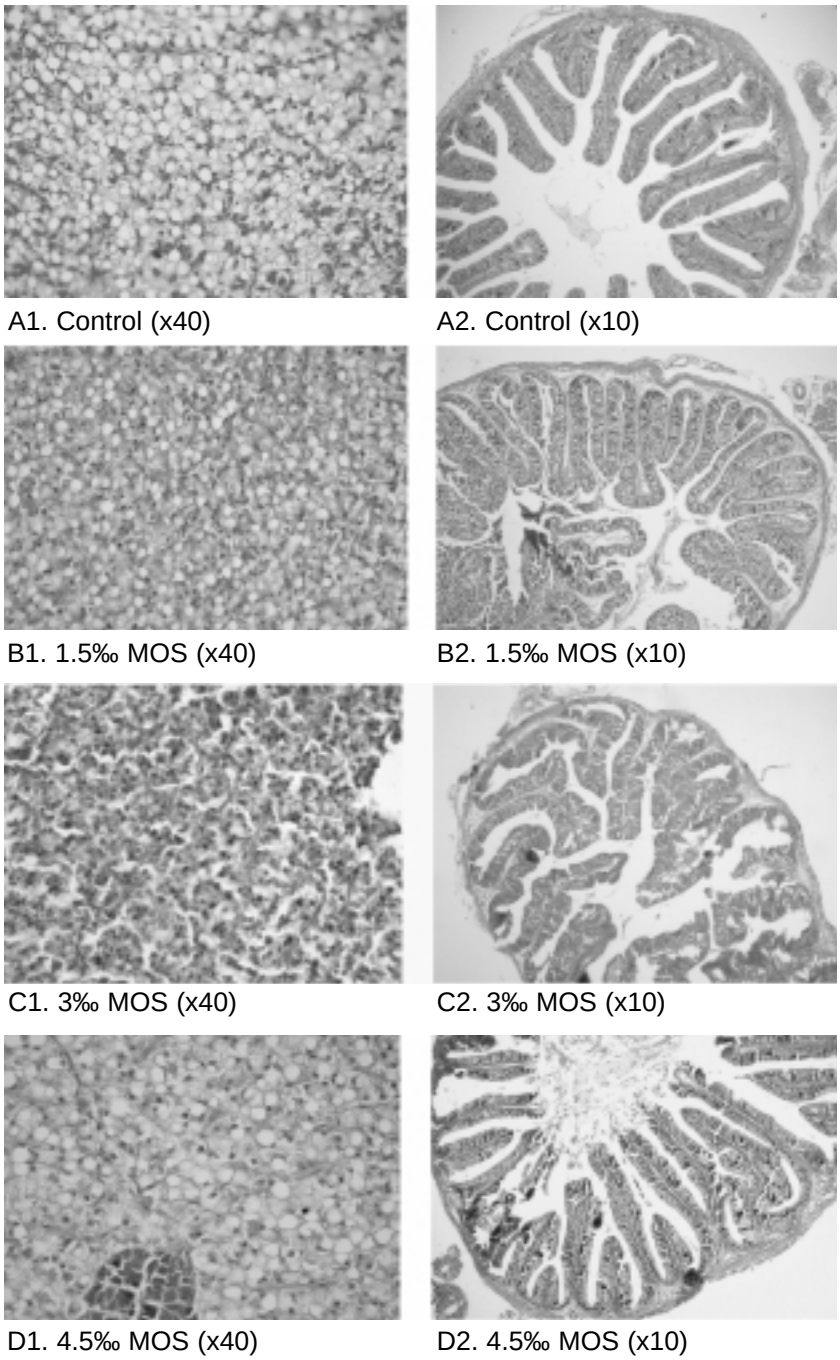

Fig. 1. Light photomicrograph of (A) liver and (B) intestine sections (H&E) of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) showing normal diffuse macro-vesicular lipid accumulation in liver tissue and normal villus structure in intestine in all treatments.

increased as dietary MOS increased. The role of MOS in amino acid utilization has not yet been explored in fish, but antibiotics have been reported to enhance amino acid utilization by killing intestinal micro-flora, thereby increasing amino acid utilization in the host, as in other animal species (Rawles et al., 1997). Also, Lara-Flores et al. (2003) indicated that supplementing diets with probiotics significantly improved protein utilization in tilapia fry. Therefore, in future research, the relationship between the effects of dietary MOS on intestinal micro-flora and amino acid utilization should be investigated.

Despite the fact that the current study did not produce the expected positive results on growth in hybrid tilapia, the differences in villi lengths and protein contents show the need for further research on the effects of MOS on diet digestibility in tilapia and other cultured fish. Likewise, the effects of dietary MOS in aquaculture should be researched with respect to other aspects related to growth and diet digestibility.

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