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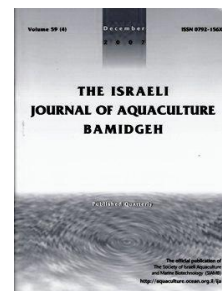
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Effects of Cumin-Supplemented Diets on Growth and Disease (*Streptococcus iniae*) Resistance of Tilapia (*Oreochromis mossambicus*)

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Abstract

The effects of dietary cumin (*Cuminum cyminum*) as a feed additive on growth performance and disease resistance of tilapia (*Oreochromis mossambicus*) were studied. Five isonitrogenous (37% crude protein) and isocaloric (18.6 kJ/g) diets were formulated to contain 0% (control), 0.5%, 1.0%, 1.5%, or 2.0% cumin. Fifteen aquaria (80 l) were stocked with 15 fish (0.56±0.02 g), each, and fish were fed one of the five diets for 75 days. The cumin supplementation did not affect the feed conversion rate (FCR) or specific growth rate (SGR). Cumulative mortality was 60% in fish fed the 0% control diet and challenged with *Streptococcus iniae*. However, in fish fed the 1.0%, 1.5%, or 2.0% supplemented diets, mortality was only 10.42%, 31.25%, and 37.50%, respectively. In conclusion, a dietary cumin level of 1% provides the best survival rate for tilapia, *O. mossambicus*, without adversely affecting growth performance or feed utilization.

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Introduction

Streptococcal disease causes significant economic losses to the fish culture industry. The disease has been reported in cultured rainbow trout, yellowtail, striped bass, seabream, and tilapia (Kitao, 2001). *Streptococcus iniae* is commonly isolated from diseased tilapia in aquaculture farms in Israel and is controlled mainly by antibiotics (Abutbul et al., 2004). However, the use of antibiotics is environmentally unacceptable because of the possible establishment of resistant microbial populations (Citarasu, 2010). Medicinal plants and spices are natural alternatives to antibiotics because they have active compounds such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectines, and polypeptides (Citarasu, 2010). Herbs and spices can successfully replace antibiotics in fish aquaculture and bacterial disease control (Abutbul et al., 2005; Bhuvanewari and Balasundaram, 2006; Zakes et al., 2008; Harikrishnan et al., 2009; Immanuel et al., 2009; Ganguly et al., 2010; Zilberg et al., 2010; Harikrishnan et al., 2011; Yilmaz, 2011).

Cumin (Apiaceae; *Cuminum cyminum*) has been used as a spice since ancient times (Azeez, 2008). It is cultivated in Mediterranean countries, Saudi Arabia, Iran, India, Mexico, China, Sicily, and Malta (Amin, 2001). India is the world's largest producer and consumer of cumin, with annual production ranging 0.1-0.2 million tons, and Turkey also cultivates cumin (Azeez, 2008). Cumin has high antimicrobial and antioxidant activity with major compounds of 29.1% α -pinene and 17.9% 1.8-cineole (Gachkar et al., 2007; Singh et al., 2002). It is used in medicines as a stimulant of gastrointestinal, immune systems, and tyrosinase inhibitor activity, and has hypoglycaemic, hypolipidaemic, and chemoprotective effects (Azeez, 2008). Therefore, cumin can possibly be used for disease prevention and growth promotion in fish. The objective of this study was to determine the effects of cumin on the feed conversion rate (FCR), specific growth rate (SGR), and treatment against *S. iniae* in tilapia (*Oreochromis mossambicus*).

Materials and Methods

Fish and experimental conditions. Healthy cultured *Oreochromis mossambicus* (0.56±0.02 g) were produced in the Faculty of Fisheries of Çanakkale Onsekiz Mart University. The fresh water used in the experiment was measured daily with a YSI 85 oxygen, conductivity, and temperature hand-held meter and a Hanna C 200 (HI 83200) photometer. Temperature ranged 28.6±0.1°C, pH 7.4±0.3, dissolved oxygen 7.02±0.5 mg/l, and conductivity 580±12 μ S.

Experimental herb and diets. Cumin (*Cuminum cyminum*) seed meal was obtained from a local market and added to the feed at 0.5%, 1.0%, 1.5%, or 2.0% (Table 1). The control diet contained no supplementation (0%). The ingredients were mixed in a blender, the feeds were pressed through a 2-mm die in a pelleting machine, and the pellets were dried in a drying cabinet (40°C) until the moisture dropped to around 10%. It was then stored in bags and deep frozen at -20°C until use.

Experimental design and feeding trial.

The experiment was performed in triplicate with 225 fish divided into 15 aquaria (15 fish/aquarium). Fish were fed a diet containing 37.0% protein and 10.0% fat before the start of the experiment. After conditioning for two weeks, the fish were randomly allocated into 80-l aquaria, aerated

Table 1. Ingredients and proximate composition of diets for tilapia containing cumin at different rates.

	0%	0.5%	1.0%	1.5%	2.0%
<i>Ingredients (% dry matter)</i>					
Fishmeal	30.0	30.0	30.0	30.0	30.0
Soybean meal	33.0	33.0	33.0	33.0	33.0
Wheat flour	16.2	16.2	16.2	16.2	16.2
Fish oil	6.5	6.5	6.5	6.5	6.5
Vitamin-mineral mix	4.0	4.0	4.0	4.0	4.0
Starch	10.3	9.8	9.3	8.8	8.3
Cumin	0	0.5	1.0	1.5	2.0
<i>Chemical analyses (% dry matter)</i>					
Protein	37.20	37.33	37.45	37.57	37.69
Fat	10.02	10.03	10.04	10.05	10.05
Ash	9.47	9.57	9.67	9.77	9.87
NFE ¹	34.73	34.50	34.27	34.04	33.81
Energy (kJ/g) ²	18.64	18.63	18.62	18.62	18.61

¹ Nitrogen-free extracts = matter - (crude lipid + crude ash + crude protein)

² Energy calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid, and 17.0 kJ/g NFE.

by an air pump. Water was exchanged daily at a rate of ~10% of the total volume. The fish were fed the experimental diets three times a day at 08:00, 12:00, and 16:00 for 75 days.

Growth. Growth performance and feed utilization were calculated every 25 days as wt gain = $100(\text{final fish wt} - \text{initial fish wt})/\text{initial fish wt}$, specific growth rate (SGR) = $100(\ln \text{ final fish wt} - \ln \text{ initial fish wt})/\text{experimental days}$, and feed conversion ratio (FCR) = feed intake/wt gain. Proximate analyses of the diets were performed using standard methods (AOAC, 1998). Dry matter was analyzed by drying at 105°C in an oven to a constant weight, crude fat by ether extraction, crude protein by the Kjeldahl method, and crude ash by incineration at 525°C in a muffle furnace for 12 h.

Challenge experiment. A bacterium (*S. iniae*) was isolated from the brain of diseased tilapia. API-strep (Biomeurex, Turkey) and the biochemical properties of the isolate confirmed the identity. Aliquots of *S. iniae* were kept frozen in 15% glycerol, 85% brain heart infusion (BHI) broth (Merck), at -70°C until use. After 75 days, fish (15 fish/aquarium) were stocked in 50-l aquaria, containing 49.5 l water, and kept at 28°C throughout the challenge experiment. Tenfold serial dilutions of *S. iniae* culture with an initial concentration of 10^{10} CFU/ml BHI broth were prepared and a 500-ml injection of the dilution was added to each experimental aquarium. The same amount of culturing broth without bacteria was added to the control aquaria. Dead fish were removed from the aquaria daily and mortality was recorded daily for four days.

Statistics. Values are expressed as means±standard error of mean (SEM) for each measured variable. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by a Tukey multicomparison test, using SPSS 17.0 software. Statistical significance was established at $p < 0.05$.

Results

The five diets were equally accepted by the fish and there was no mortality or disease in any treatment. There were no significant differences in average weight gain, SGR, or FCR (Table 2). The cumulative mortalities of fish fed the control (0%) or 0.5% cumin diets and challenged with *S. iniae* were 60% and 58.33% respectively (Fig. 1). Mortality was 10.42%, 31.25%, and 37.50% in the groups fed the 1.0%, 1.5%, and 2.0% cumin diets.

Table 2. Fish performance and feed utilization for *O. mossambicus* fish fed diets containing different levels of cumin levels for 75 days.

	0%	0.5%	1.0%	1.5%	2.0%
Initial wt (g)	0.56±0.01	0.57±0.03	0.57±0.01	0.56±0.03	0.56±0.02
Final wt (g)	6.32±0.05	6.49±0.13	6.36±0.11	6.31±0.06	6.50±0.03
Wt gain (g)	5.76±0.06	5.91±0.16	5.79±0.11	5.75±0.04	5.94±0.02
SGR	4.52±0.01	4.55±0.02	4.53±0.01	4.52±0.01	4.55±0.01
Feed intake (g)	6.52±0.37	6.49±0.03	6.43±0.03	6.38±0.14	6.19±0.17
FCR	1.13±0.06	1.10±0.03	1.11±0.03	1.11±0.03	1.04±0.03

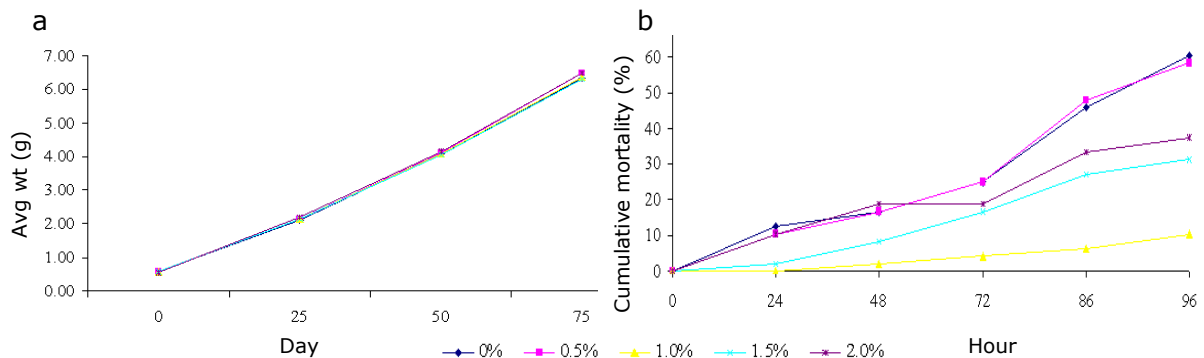


Fig. 1. (a) Average weight gain and (b) cumulative mortality of *Oreochromis mossambicus* fed cumin-supplemented diets and challenged with *Streptococcus iniae* (n = 45).

Discussion

Supplementation of cumin at 0.5%, 1.0%, 1.5%, and 2.0% did not affect growth performance in *O. mossambicus*, similar to results with black cumin seed (*Nigella sativa*) in *O. niloticus* (Diab et al., 2008). Effects of dietary herbal additives on fish growth are contradictory. While growth improved with *Carum carvi* and *Allium sativum* in *O. niloticus* (Metwally, 2009; Ahmad and Tawwab, 2011) and with *Ocimum basilicum* in *O. niloticus* x *O. aureus* (Dakar et al., 2008), growth performance decreased in fish fed *Ferula coskunii* (Yilmaz et al., 2006) and camu-camu fruit *Myrciaria dubia* (Palacios et al., 2006). Negative effects of medicinal plants may be related to toxic constituents, excessive doses, or allergic conditions, but they generally have no effects on health when used in the proper dosage and application (Bandaranayake, 2006). This is in agreement with the findings in the present study, where the mortality rates in fish fed less than or more than 1% cumin was drastically higher than in fish fed 1% cumin.

An inhibitory effect of cumin against *Streptococcus* species was reported by Keskin and Toroglu (2011). These results are in fair agreement with the administration of herbal supplemented diets showing resistance against streptococcal disease in tilapia fed *Rosmarinus officinalis* (Abutbul et al. 2004; Zilberg et al., 2010), *Cinnamomum verum* (Rattanachaikunsopon and Phumkhachorn, 2010), and *Andrographis paniculata* (Rattanachaikunsopon and Phumkhachorn, 2009). Previous reports support our findings in terms of inhibitory effects of cumin on *Streptococcus iniae*.

In conclusion, a dietary cumin level of 1% provides the best survival rate for tilapia, *O. mossambicus*, without adversely affecting growth performance or feed utilization. Such treatment could have economical benefits for tilapia farming and be used in organic tilapia culture. Further investigation on the potential effects of cumin on control of *S. iniae* and other *Streptococcus* species is encouraged.

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