Egyptian Journal of Aquatic Research xxx (2018) xxx–xxx

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Contents lists available at ScienceDirect

Egyptian Journal of Aquatic Research

journal homepage: www.sciencedirect.com/locate/ejar

Full length article

The influence of dietary supplementation of cinnamaldehyde and thymol on the growth performance, immunity and antioxidant status of monosex Nile tilapia fingerlings (*Oreochromis niloticus*)

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ARTICLE INFO

Article history:
Received 25 March 2018
Revised 12 July 2018
Accepted 24 July 2018
Available online xxxx

Keywords:
Nile tilapia
Cinnamaldehyde
Thymol
Growth performance
Antioxidant status
Immune response

ABSTRACT

This study investigated the effects of two essential oils (Eos), cinnamaldehyde and thymol on the growth performance, antioxidant status of the meat and the immunity of Nile tilapia fingerlings, *Oreochromis niloticus*. A total of 375 *O. niloticus* (10.2 ± 0.06 g) were randomly assigned to 5 dietary treatments (3 replicates for each). Diet 1 was the control with no additives. Diets 2 and 3 were supplemented with 1 and 2 ml cinnamaldehyde /kg diet (CINN1 or CINN2), respectively. While diets 4 and 5 were supplemented with 1 and 2 ml thymol /kg diet (THYM1 or THYM2), respectively. Fish feeding was done by hand until satiation 3 times daily for 75 days. The results showed that dietary supplementation with 1 ml thymol/ kg diet increased the growth performance significantly than other groups. Dietary supplementation with cinnamaldehyde or thymol significantly reduced ($P \leq 0.05$) the malondialdehyde (MDA) formation and increased glutathione reductase (GR) in the muscle, and increased lysozyme activity, IgM, IgG levels and catalase activity in the serum thus improved the antioxidant protective capacities and the immune status of the fish. Furthermore, thymol supplementation can stimulate more growth and immune responses than cinnamaldehyde as essential oils in Nile tilapia nutrition.

Introduction

A number of antibiotics were used in animal diets as growth promoters (AGPs) to avoid disease, improve growth performance, and increase beneficial intestinal microbial population (Bento et al., 2013). However, because of the emergence of bio-resistance along with the transmission of resistance from animals to humans and with the increasing demand for environmentally friendly aquaculture, nutrionists are searching for other feed additives alternative to antibiotics. Giannenas et al. (2013) reported that, all over the world, the animal industry is facing strong political and social pressure to produce safe food products with minimal use of antibiotics or substances of synthetic origin in animal feeds, and with low impact on environmental pollution. Essential oils are aromatic compounds of herbs and spices and are one of the natural growth promoters alternative to antibiotics in animal diets due to their promising effect on growth performance, gut health and wellbeing (Bento et al., 2013). Besides, essential oils EOs add flavors and aroma to feeds leading to improvement of the palatability and feed consumption, also they improve feed digestibility, and immune response (Krishan and Narang, 2014). EOs increase fermentation of undigested fibrous feed particles due to the improving growth and multiplication of the beneficial microflora in the gut (Giannenas et al., 2013). EOs improve the storage quality of fish by improving the antioxidant status of fish fillet (Gatlin et al., 1992). Thymol (from thyme and oregano) and cinnamaldehyde (from cinnamon) are examples for these natural growth promoters. They are different in their structures and chemical properties; cinnamaldehyde is an aliphatic aldehyde, whereas thymol is a phenolic compound. These differences in their chemical structure led to differences in their antimicrobial action (Di Pasqua et al., 2007).

Essential oils have been used in growth promotion and enhancement of immune responses in rabbit, (Soultos et al., 2009), pigs (Janz et al., 2007; Jugl-Chizzola et al., 2006), poultry (Khan et al., 2012) and in ruminants (Benchaar et al., 2008; Benchaar et al., 2009). There are limited researches on the application of essential oils in fish nutrition. Therefore, this study investigates the effect of supplementing the diets of Nile tilapia fingerlings with cinnamaldehyde and thymol on the growth performance, whole body composition, serum catalase activity, and the levels of lysozyme, IgG, IgM and antioxidant status of fish muscle.

Peer review under responsibility of National Institute of Oceanography and Fisheries.

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https://doi.org/10.1016/j.ejar.2018.07.004
1687-4285/

Please cite this article in press as: Amer, S.A., et al. The influence of dietary supplementation of cinnamaldehyde and thymol on the growth performance, immunity and antioxidant status of monosex Nile tilapia fingerlings (*Oreochromis niloticus*). Egyptian Journal of Aquatic Research (2018), https://doi.org/10.1016/j.ejar.2018.07.004

Material and methods

Fish, diet and experimental design

This study was done at Fish Research unit, faculty of Veterinary Medicine, Zagazig University, Egypt. All procedures of the experiment were carried out with reference to the Committee of Local Experimental Animal Care and approved by the ethics of our Nutrition and Clinical Nutrition Department institutional committee, Veterinary Medicine College, University of Zagazig, Egypt. The fish were purchased from a reputable fish hatchery. The fish were stocked in five clean concrete tanks ($4 \times 1 \times 1$ m); and 3 cages ($1.33 \times 1 \times 1$ m) made from plastic mesh material of 1 mm mesh; were placed in each tank, representing three replicate groups for each treatment. Then 25 fingerlings were stocked in each cage. The ponds were supplied with dechlorinated water and oxygen was supplied by a large air compressor. Throughout the experimental time; natural light was available providing nearly 12 hrs light day^{-1}. Each pond was scrubbed and thoroughly cleaned every two weeks, after sampling to avoid natural food formation such as algal growth and to protect fish in the ponds from the adverse effect of these wastes. According to the methods of Clesceri et al. (1996), the quality of water was checked 3 times weekly. The parameters were reserved within the best range for Nile tilapia; temperature 24 ± 2 °C, pH 8.0 ± 1, ammonia 0.03 ± 0.01 mg L^{-1} and dissolved oxygen (DO) 6 ± 1 mg L^{-1}.

The used essential oils were thymol synthetically manufactured and provided by Oxford Laboratory Company, Mumbai India with purity of 99% while cinnamaldehyde was supplied by Flaka Chemical Switzerland with purity of (≥98%). A total of 375 *O. niloticus* (10.2 ± 0.06 g) were randomly assigned to 5 dietary treatments in 3 replicates. Diet 1 was the control diet with no additives. Diets 2 and 3 were supplemented with 1 and 2 ml cinnamaldehyde /kg diet (CINN1 or CINN2), respectively. While diets 4 and 5 were supplemented with 1 and 2 ml thymol /kg diet (THYM1 or THYM2), respectively. Thymol and Cinnamaldehyde were mixed with fish oil then mixed with other feed ingredients. Fish were randomly assigned to the 5 groups at 25 fingerlings per group and in triplicates. Dietetic feed ingredients were prepared in pelleted form (4 mm). The pellets were kept in plastic bags at −30 °C until usage. The proximate analyses of the used feedstuffs, and the experimental diets (Table 1) were calculated according to the standard measures of (NRC, 2011) methods for dry matter, crude protein, ether extract, crude fiber and ash. Digestible energy (DE) contents of the experimental diets were calculated based on values of protein 3.5 kcal gm^{-1}, fat 8.1 kcal gm^{-1}, NFE 2.5 kcal gm^{-1} according to (Santiago et al., 1982). Fish were submitted to 14 days adaptation period before the beginning of the experiment. Fish feeding was done by hand until satiation 3 times daily for 75 days. Fish were individually weighed every 2 weeks following a 14-h starvation period.

Table 1
The proximate chemical composition and analysis of the basal diet (g kg^{-1}).

Ingredients	Fish diet (g kg^{-1})
Fish meal[1]	150
Soybean meal	276
Yellow corn	260
Corn gluten	120
Wheat bran	127
Fish oil	55
Vit. & mineral premix[2]	12
Chemical analysis[3] (g kg^{-1}) as fed basis	
DM	916.7
Crude protein	334.1
Crude lipid	93.5
Crude ash	64.8
DE[4] (kcal/kg)	2900.423

[1] Argentinean fish meal by Coomarpes Ltda, Argentina.
[2] Each 1 kg of premix contain: vit A 550000 IU, vit D 110000 IU, vit E 11000 mg, vit K 484 mg, vit C 50 gm, vit B1 440 mg, vit B2 660 mg, vit B3 132oo mg, vit B5 1100 mg, vit B6 1045 mg, vit B9 55 mg, Choline 110,000 mg, Biotine 6.6 mg, iron 6.6 gm, copper 330 mg, Mn 1320 mg, Zn 6.6 gm, Se 44 mg, iodine 110 mg.
[3] According to NRC (2011).
[4] Digestible energy (DE) contents of the experimental diets were calculated based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm. According to (Santiago et al. 1982).

Growth performance

The body weight, body weight gain, feed intake and feed conversion ratio FCR were recorded every 2 weeks and at the end of the experimental period. Fish are observed daily for any disease or mortality. Total weight gain, average daily gain, specific growth rate and FCR were calculated according to (Castell and Tiews, 1980). Protein efficiency ratio (PER) was determined according to (Stuart and Hung, 1989). No mortality was recorded during the experimental period.

$$\text{Total gain}\left(\text{g fish}^{-1}\right) = (\text{WT - WI}),$$

where : WT : Final weight of fish in grams and
WI : Initial weight of fish in grams.

$$\text{Average daily gain(ADG)}\left(\text{g fish}^{-1}\,\text{day}^{-1}\right) = \text{total gain/duration period in day.}$$

$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100 \times (\ln \text{WT} - \ln \text{WI})/\text{duration period/day.}$$

Where, Ln is the natural log.

$$\text{Feed conversion ratio (FCR)} = \text{total feed intake(g)/total gain (g).}$$

$$\text{Protein efficiency ratio (PER)} = \text{total gain (g)/protein intake (g).}$$

Sample collection and laboratory analysis

At the end of the experiment, blood samples were collected from 12 fish/treatment by heart puncture then serum was obtained by blood centrifugation at 3500 rpm for 15 min for the determination of the catalase and lysozyme activity, as well as the levels of IgG and IgM. 12 fish per treatment were frozen at (−25 °C) for the determination of the whole body composition. Muscle samples from 12 fish per treatment were stored for a month in the refrigerator at −25 °C immediately after sampling for determination of antioxidant activity.

Whole body composition

Frozen whole fish were thawed, dried in hot air oven, blended and analyzed for the determination of moisture, crude protein, ether extract, and ash content according to (Horwitz and Latimer, 2000).

Determination of antioxidant status:

Muscle samples were analyzed for the determination of malondialdehyde (MDA) and glutathione reductase (GR) levels according to the methods of (Giannenas et al., 2011).

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