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Collaborating effects of rearing density and oregano oil supplementation on growth, behavioral and stress response of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

To investigate the impacts of rearing density and oregano essential oil (OEO) supplementation on growth, behavioral and stress response of Nile tilapia, a factorial design ($3 \times 3 \times 2$) was assembled using 18 (100 L) glass aquaria where each aquarium had a rearing density and an OEO supplemental level. Six aquaria had a density of 10 fish aquarium^{-1} (low rearing density; LRD), another six had a density of 20 fish aquarium^{-1} (medium rearing density; MRD) and the remaining six aquaria had a density of 40 fish aquarium^{-1} (high rearing density; HRD). For each rearing density, fish were fed three diets with variable OEO levels (0.0, 1.0, and 2 ml kg^{-1} diet) and were assayed in two replicas for 10 weeks. OEO improved the growth performance of fish maintained at LRD and MRD. Tilapia exhibited behavioral changes in all densities with a reduced aggression in the HRD group. The OEO reduced the oxidative stress and the aggressiveness of juvenile tilapia as deduced from stress and welfare indicators' levels (cortisol antioxidant enzymes). In addition, a density-stress related effect as well as a nonspecific immune stimulant effect of OEO was detected from the increased antioxidant and NO activity in this study.

Introduction

Increasing rearing density in fish aquaculture is an effective approach to moderate the operational costs and thus guaranteeing sustainable and profitable culture. However, intensively cultured fish are highly vulnerable to variable levels of stress through crowding or adversarial social interactions. These stressors weaken tilapia growth (Sánchez-Muros et al., 2016), increase disease susceptibility of fish (Alexander et al., 2010; Ashley, 2007) and the tank mate competition as well, and thereby minimize the aggression behavior among fish (North et al., 2006; Turnbull et al., 2005). Furthermore, the impact of rearing density on fish welfare seems to incorporate several interrelating factors. Studying these factors especially through investigating the behavior of fish maintained under high rearing density and its subsequent related stress and aggressiveness will help to improve fish welfare and performance (Ashley 2007). Likewise, crowding stress can cause oxidative stress, mirrored by an elevated production of free radicals in the form of reactive oxygen species (ROS) (Ahmad et al., 2000). The quick accumulation of ROS augments cellular oxidative damage (Evans and Cooke, 2004). In addition, various regulatory functions of the immune system are eventually dependent upon an oxidant/antioxidant balance. Thus, the immune responsiveness is highly correlated to ROS accumulation with subsequent reduced immune responses (Knight, 2000).

Recently, antioxidants containing supplements provided a good solution to the impaired growth and the stress responses related to intensive culture conditions as well as the oxidative stress-related immune deficiencies (Adhikari et al., 2018; Knight, 2000; Kucukbay et al., 2009; Sahin et al., 2014). Of these dietary additives; essential oils (EOs) constitute a generous source of natural active constituents, hence credited to be an economical and a harmless plausible option to ordinary therapeutics and additives in aquaculture (Aanyu et al., 2018; Sutili et al., 2017; Yang et al., 2015; Yitbarek, 2015). Among the EOs with such potentiality are the oregano essential oils (OEO) with their high concentrations of carvacrol and thymol (phenols classified as monoterpenoids). They mainly exist in high concentration in those EOs extracted from thyme and oregano plants and have strong biological effects

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(Ouwehand et al., 2010; Bassole and Juliani, 2012). Hence, they are praised as a potential feed supplement for triggering growth and immune response in fish (Aanyu et al., 2018; Shehata et al., 2013; Zheng et al., 2009).

Therefore, this study aims to give a compendious evaluation of the interactive effects of rearing density as a crowding stress and dietary OEO supplementation on the density-related growth and stress, as well as the behavioral response of juvenile Nile tilapia as welfare indicators.

Material and methods

Fish, experimental diets, and design

This experiment was accomplished during the production season 2016 using juvenile monosex Nile tilapia. The experimental fish were sourced from a captive population kept at a private fish farm located in Kafr El-Sheikh governorate. Fish were transferred to the Fish Breeding and Production Research Laboratory, Faculty of Veterinary Medicine, Damanhur University, El-Behira Province, Egypt. Juveniles were retained in indoor plastic tanks (350 L) for a 14 days adaptation period. Thereafter, a 3×3 factorial design was assembled with three rearing densities and three diets (2 supplemented diets and one control) and the experiment extended for 70-days. Juvenile monosex tilapias (*Oreochromis niloticus*) with an initial weight of (13.21 ± 1.71 to 14.24 ± 1.18 g; Means ± SE) were distributed into 18 glass aquaria (100 L) and stocked at three densities. Each aquarium was assigned a rearing density and an OEO dietary supplemental level. Six aquaria had a rearing density of 10 fish aquarium^{-1} or ~1.4 kg m^{-3} (low rearing density; LRD), another six had a rearing density of 20 fish aquarium^{-1} or ~2.8 kg m^{-3} (medium rearing density; MRD) and the remaining six aquaria had a rearing density of 40 fish aquarium^{-1} or ~5.6 kg m^{-3} (high rearing density; HRD). For each rearing density, fish were fed three isonitrogenous (300 g crude protein kg^{-1}) diets with different inclusion levels of OEO (0.0, 1.0, and 2 ml kg^{-1} diet) and assayed in duplicates for 10 weeks (3 stocking densities × 3 experimental diets × 2 replicates). The commercial diet was obtained from the AQUA International for food industries. The OEO used in this experiment was an *Origanum vulgare* L. steam extract containing 60% carvacrol and 5% thymol (i.e. 1 ml OEO kg^{-1} is equivalent to carvacrol 0.012% or 120 ppm and thymol 0.001% or10 ppm). It was purchased from Al-Abgy for Plant Oil Extraction Co., Ltd., Al Basatin, Cairo, Egypt. The OEO was sprayed over the diet to prepare the experimental diets (Yilmaz et al., 2014). A simplified recirculating system with sufficient aeration was applied to each aquarium using a commercially available biological filter. The water quality was checked weekly; dissolved oxygen (DO) was checked by portable oxygen meter (Microprocessor Oxygen Meter HI 9143, HANNA instruments). Also, a portable pH-temperature meter (pH/EC/TDS/Temperature Meter, Model HI 991300). The ammonia nitrogen (TAN), nitrate-nitrogen, nitrite-nitrogen, organic matter, were analyzed using analytical kits (Lovebird®, Multidirect, Co 210070 England).

No critical values were detected for dissolved oxygen (6.05 ± 0.13 mg l^{-1}), pH (7.8 ± 0.14), NO_2 (0.19 ± 0.03 mg l^{-1}) and NO_3 (1.06 ± 0.26 mg l^{-1}) through the study period with an average water temperature 27.9 ± 0.48 °C.

The feeding rate was justified at 4% of the biweekly-recorded weights and offered twice daily. Fecal matters were siphoned out once daily together with daily replacement of one-third of each aquarium's water.

Behavioral observations

For all experimental groups, sampled behavioral categories were scanned by one observer (Table 1). The tilapia juvenile behavior was weekly recorded (two observations day^{-1} twice a week) in each aquarium using instantaneous sampling method according to Martin and Fraser (1986). The proportion of fish engaged in each behavior response was calculated during all scan samples in each aquarium.

Growth performance and survival rate

At the termination of the 10-week trial, tilapia juveniles in each tank were weighed individually and the following parameters were calculated.

$$\text{Total body gain (TBG, g fish}^{-1}) = W_2 - W_1$$

$$\text{Specific growth rate (SGR, \% day}^{-1}) = \frac{\text{Ln}(W_2) - \text{Ln}(W_1)}{T} \times 100$$

where W_2 is the final body weight (g), W_1 is the initial body weight (g), T is the trial period (days).

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}} \times 100$$

$$\text{Survival rate (SR, \%)} = \frac{\text{Number of survived fish}}{\text{Total number at stocking}} \times 100$$

Measurement of stress indicators

Blood samples (6 fish group^{-1}) were gathered from the caudal vein 10 weeks after the start of the trial. The blood samples were relocated in Eppendorf tubes (left to clot at 4 °C) and then centrifuged at 5000 rpm, for 10 min. The collected serum was stored at −20 °C for further assaying of the cortisol level calorimetrically using commercial kit. Also, further measurements were done, the superoxide dismutase based on the prescript techniques, glutathione reductase (GR) according to the method described by

Table 1
The behavior responses of juvenile male Nile tilapia (*O. niloticus*) recorded during the experiment.

Behavioral patterns	Description	Reference
Chafing (scratching)	Rubbing of any part of the body against an object (wall, floor and equipment of aquarium). Fish appear as if they are eliminating sources of irritations from their external skin surface	Reebs (2009)
Resting	Fish are immobile and rest on the bottom of the aquaria.	Reebs (2008)
Surfacing	Fish are gulping air at the water surface.	Ferey and Miller, 1972
Schooling	Fish are swimming or gathering with each other.	Bond (1979)
Eliminative	Fish are showing dropped or hanged feces from the anus.	Bond (1979)
Chasing	Fish are swimming vigorously to follow another fish.	Ferey and Miller (1972)
Fin tugging (biting)	Fish bit any part of body regions of another fish.	De Boer (1980)
Mouth pushing	Fish were standing face to face with their opened mouth against each other i.e. snapping simultaneously at the presented mouth of the other.	De Boer (1980)

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