Aquaculture and Fisheries xxx (2018) 1–7

Contents lists available at ScienceDirect

Aquaculture and Fisheries

journal homepage: www.keaipublishing.com/en/journals/aquaculture-and-fisheries/

Effects of three positively buoyant dietary supplements on the buoyancy of feces, growth and intestinal health of Tilapia, *Oreochromis niloticus*×*O. aureus*

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ARTICLE INFO

Article history:
Received 27 August 2017
Received in revised form
4 January 2018
Accepted 8 January 2018
Available online xxx

Keywords:
Tilapia
Buoyant materials
Growth
Floating feces
Intestinal histology

ABSTRACT

Two trials were conducted to evaluate the effects of positively buoyant dietary materials on growth, intestinal health, and fecal properties of tilapia. In trial 1, ten diets containing 0 (control) 1%, 2% or 3% cork, expanded and vitrified microball or expanded vermiculilite fine particles (250–450 μm) were fed to tilapia fry (5.0 ± 0.1 g) for 30 days to evaluate the effects on the growth and feeding efficiencies. In trial 2, the three buoyant materials with coarse particles (450–830 μm) were included in diets at 0 (control), 3%, 4% or 5% supplementation levels to feed tilapia juveniles (55.0 ± 1.0 g), and the growth, feed efficiencies, evacuation velocity, fecal floatability and intestinal histology were examined after 21 days feeding. In trial 1, the weight gain (WG) of the fish significantly decreased with the supplementation of 2%, 3% cork, 3% microball or 3% vermiculite ($P < 0.05$), and the feed conversion ratio (FCR) was significantly increased by 3% cork and 3% vermiculite ($P < 0.05$) when compared to the control. In trial 2, cork-fed groups were observed to have more floating feces than microball- and vermiculite-fed groups. Dietary cork significantly decreased the sinking velocity of diets and feces ($P < .05$), but microball only decreased the sinking velocity of diets, and vermiculite (4%, 5%) decreased the sinking velocity of feces ($P < 0.05$). All buoyant materials supplemented groups showed lower WG and higher FCR than the control ($P < .05$). The intestine evacuation velocity and villus height, crypt depth, muscle thickness of intestinal walls were decreased by the increasing buoyant materials in diets, and 5% microball group showed the lowest values among all groups. In conclusion, dietary cork, microball and vermiculite (3%–5%) negatively affect the growth performance and intestinal histology of tilapia, and diets with cork supplementation could decrease the density of feed and feces to produce floating feces.

1. Introduction

In intensive aquaculture, fishes excrete lots of feces that cause a tremendous pressure on the health of the water treatment system and environment. Quickly and efficiently removing the feces from the water is an effective way to stabilize the farming system and ensure successful production. In the culture of Atlantic salmon (*Salmo salar*), Hillestad, Åsgård, and Berge (1999) reported that the percentage of excreted fecal nutrients into water system, accounting for the ingested nutrients were 13% (protein), 8% (fat), 40% (carbohydrate), 17% (organic matter), 50% (ash) and 23% (dry matter). The property of feces, including the density, stability and size, significantly affects the treatment efficiency of feces (Unger & Brinker, 2013a). These physical characteristics of feces are closely related with the feed characteristics and composition. Dias, Huelvan, Dinis, and Métailler (1998) found that dietary cellulose increased the feces firmness of European seabass (*Dicentrarchus*

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https://doi.org/10.1016/j.aaf.2018.01.004

Please cite this article in press as: Yang, H., et al., Effects of three positively buoyant dietary supplements on the buoyancy of feces, growth and intestinal health of Tilapia, *Oreochromis niloticus*×*O. aureus*, Aquaculture and Fisheries (2018), https://doi.org/10.1016/j.aaf.2018.01.004

labrax). In Nile tilapia (*Oreochromis niloticus*), a high inclusion of starch in diet increased the viscosity of digesta and the removal efficiency of feces (Amirkolaie, Verreth, & Schrama, 2006). Guar gum has been found to increase the water content in digesta of rainbow trout (*Oncorhynchus mykiss*) (Storebakken, 1985).

In a small-scale feeding system, the excreted feces by fish can be easily removed by siphon, fecal collectors and nets, while a cone bottom clarifier is usually designed to remove the wastes in a large-scale feeding system. These methods are designed to clean sinking feces and are not convenient for the rearing enterprise. Unger and Brinker (2013b) found that supplementing 2% cork powder in rainbow trout diets could generate floating feces, which made the feces removal easier and may be a new alternative method to treat water quality in intensive aquaculture.

Buoyant materials can decrease the density of fish feces, but should not affect fish growth, feeding efficiencies and intestinal health. To evaluate the effects on intestinal health and digestion, intestinal histology and evacuation velocity are usually conducted. Thus, the buoyant materials should be indigestible and non-toxic with a low density and high floating rate. Cork, expanded and vitrified microball and expanded vermiculite could be considered as the possible candidates for this supplemental material. Cork is the bark of the cork oak (*Quercus suber* L.), and it has a peculiar cellular structure with low density and good elasticity, and is impermeable to gases and liquids (Anjos, Pereira, & Rosa, 2008; Gil, 2009; Silva et al., 2005). Vermiculite is a natural mineral, and its volume can expand 8–20 times after heating. Expanded vermiculite (abbreviated as vermiculite) is a chemically inert and fire resistant material with low density (El-Gamal, Hashem, & Amin, 2012; Sutcu, 2015). Expanded and vitrified microball (abbreviated as microball) is made from a special type of perlite mineral with porous structure and low density, which is also chemically stable and resistant to fire (Fang, Mukhopadhyaya, Kumaran, & Shi, 2011; Ping, Yi, & Gong, 2009).

As the second most farmed fish group worldwide, tilapia has provided the increasing worldwide demand for protein sources (Ng & Romano, 2013). So, in this study, three buoyant materials including cork, microball and vermiculite with fine particles (a diameter of 250–450 μm, 40–60 mesh, trial 1) or coarse particles (a diameter of 450–830 μm, 20–40 mesh, trial 2) were supplemented with varying levels into a basal diet to investigate the effects on growth performance, intestinal histology, and fecal properties of tilapia, to inform a possible strategy for improving the waste treatment efficiency in intensive aquaculture.

2. Materials and methods

2.1. Experimental materials

Three materials, cork, microball and vermiculite of two sizes, fine particles with a diameter of 250–450 μm (40–60 mesh) and coarse particles with a diameter of 450–830 μm (20–40 mesh), were used in trial 1 and trial 2, respectively. The cork was a brown color with a bulk density of 0.12 g/cm^3 for coarse particle and 0.20 g/cm^3 for fine particle. The microball had a gray color with a bulk density of 0.10 g/cm^3 (coarse particle) and 0.20 g/cm^3 (fine particle). The vermiculite had a brown color with a bulk density of 0.15 g/cm^3 (coarse particle) and 0.30 g/cm^3 (fine particle). Cork, microball and vermiculite were supplied by Xinxin Cork Products Factory (Zhejiang), Wangda Building Materials Factory (Jiangxi) and Yanxi Minerals Processing Plant (Hebei), China, respectively (Fig. 1).

2.2. Experimental design and diets

The study consisted of two trials: Trial 1 was designed for the fine particles of buoyant materials and tilapia fry and trial 2 for the coarse particles and tilapia juveniles.

Trial 1: Fine particles (250–450 μm, 40–60 mesh) of cork, microball and vermiculite were supplemented into basal diet (control) with 1%, 2%, and 3% levels, respectively, and then 10 diets were obtained. The soybean meal and wheat bran inclusion was appropriately adjusted to balance the formula composition. Feed ingredients were ground and then screened through a 40-mesh sieve. The mixture of all ingredients was granulated into pellets with a diameter of 2.0 mm by a single-screw extruder (SLP-45, Fishery Machinery and Instrument Research Institute of the Chinese Academy of Fishery Sciences, Shanghai, China). The pelleting temperature was 90–95 °C. All diets were air-dried and stored at 4 °C until use. The diet formulation and proximate composition are shown in Table 1.

Trial 2: Larger particles and higher supplementation levels of buoyant materials than in trial 1 were conducted in trial 2. Coarse particles (450–830 μm, 20–40 mesh) of cork, microball and vermiculite were supplemented into basal diet (control) with 3%, 4%, and 5% levels, respectively. The diets were prepared as detailed in trial 1. The diet formula and proximate composition are shown in Table 2.

2.3. Experimental fish and feeding management

Trial 1: Nine hundred tilapia (*Oreochromis niloticus*×*O. aureus*) fry with an initial body weight of 5.0 ± 0.1 g were allocated randomly into 30 cages (1.5 m × 1.0 m × 1.2 m) with 30 fish per cage. Ten cages from the ten diets were randomly placed in one indoor cement pool without direct sunshine, and three pools were used. The fish were fed with one of the ten diets three times a day (8:00, 12:30 and 17:00) with a daily feeding rate of 5%–10% of body weight. The feed consumption was recorded daily. The feed intake was adjusted appropriately according to the feeding behavior and water temperature to ensure to satiation and no feed residue was left after feeding (in 5 min). All cages were maintained with a similar amount of feed intake. During the feeding period, about one third of the cultured water was renewed with pond water after filtration and dark sedimentation every 7 d. The waste in the pools was cleared by siphon every 7 d and water was continuously aerated. Water temperature, dissolved oxygen and ammonia nitrogen levels were 26–32 °C, >5 mg/L, and <0.2 mg/L, respectively. The feeding trial was conducted at Binhai Aquaculture Station of Shanghai Ocean University (Pudong New District, Shanghai, China) and lasted for 30 days.

Trial 2: Four hundred and fifty fish with an initial body weight of 55.0 ± 1.0 g were allocated randomly into 30 cages with 15 fish per cage. The feeding trial lasted for 21 days. Water temperature, dissolved oxygen and ammonia nitrogen were 24–26 °C, >5 mg/L, and <0.2 mg/L, respectively. The cages distribution and feeding management were the same as in trial 1 except that the daily feeding rate was 3%–5% of body weight.

2.4. Measurement indicators and methods

2.4.1. Growth performance and physical indices

At the end of the feeding trial, the total weight of fish per cage were weighed after withholding feed for 24 h, to calculate weight gain (WG) and feed conversion ratio (FCR). Three fish per cage were selected randomly to measure body weight and body length individually to calculate condition factor (CF) and then were dissected to weigh the whole intestine (including stomach, but not including liver and other organs) without chyme in digestive tract, to calculate intestine body index (IBI).