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FULL LENGTH ARTICLE

Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia (*Oreochromis niloticus*)

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Abstract Twelve practical diets were formulated to contain four levels of *Bacillus licheniformis* (0.0, 0.24×10^6, 0.48×10^6 and 0.96×10^6 CFU g^{-1}), respectively, with three yeast extract levels (0%, 0.5% and 1%), respectively. Each diet was randomly assigned to duplicate groups of 50 Nile tilapia (*Oreochromis niloticus*) (5.99 ± 0.03 g) in 24 concrete ponds (0.5 m^3 and 1.25 m depth) for 12 weeks. Increasing dietary *B. licheniformis* levels in *O. niloticus* and yeast extract levels significantly ($P < 0.01$) improved growth performance and nutrient utilization. Supplementation of the experimental diets with, 0.48×10^6 CFU/g^{-1} and 1.0% yeast extract showed the best nutrient utilization compared to other treatments. All probiotic levels significantly ($P < 0.01$) increased chemical composition ($P < 0.05$) compared to the control group, while increasing yeast extract did not significantly alter chemical composition. Hematological indices, total protein and albumin of *O. niloticus* significantly increased while aspartate aminotransferase and alanine aminotransferase significantly ($P < 0.01$) decreased with an increase in *B. licheniformis* level up to 0.48×10^6 CFU g^{-1}. Increasing levels of yeast extract had no effect on hematological parameters and the diets supplemented with 0.48×10^6 CFU g^{-1} and 0.5% yeast extract showed the highest hematological values.

Introduction

Probiotics are a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring an improved use of the feed or enhancing its nutritional value, by increasing the host response towards disease, or by improving the quality of its

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environment (Verschuere et al., 2000). Nowadays, probiotics are also becoming an internal part of aquaculture practices to obtain high production. Although considerably low information is available on probiotic application for fish, they offer benefits with regard to improving immune status and fish production (Cerezuela et al., 2011). Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of specific health-promoting bacteria, which can improve host's health (Gibson et al., 2003).

Based on the studies of Mahious and Ollevier (2005) and Gibson et al. (2004) foodstuff that reaches the colon (e. g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids) is a candidate prebiotic (Yousefian and Amiri, 2009). However, most of the studies have focused on non-digestible carbohydrates, mainly oligosaccharides. Synbiotics are nutritional supplements that combine probiotics and prebiotics, enhancing their beneficial effects (Cerezuela et al., 2011).

The use of probiotics and prebiotics has been regarded during recent years as an alternative viable therapy in fish culture, appearing as a promising biological control strategy and becoming an integral part of aquaculture practices for improving growth and disease resistance (Rombout et al., 2010). This strategy offers innumerable advantages to overcome the limitation and side effects of antibiotics and other drugs and also leads to high production (Sahu et al., 2008).

In recent years there has been a growing interest in understanding the mechanism of action of probiotics and prebiotics, especially in humans and other mammals. Probiotic activity is mediated by a variety of effects that are dependent on the probiotic itself, the dosage employed, treatment duration and route and frequency of delivery. Some probiotics exert their beneficial effects by elaborating antibacterial molecules such as bacteriocins that directly inhibit other bacteria or viruses and, activity participating in the fight against infections; whereas, others inhibit bacterial movement across the gut wall (translocation), enhance the mucosal barrier function by increasing the production of innate immune molecules or modulate the inflammatory/immune response (Cerezuela et al., 2011).

On the other hand, the potential mechanism of prebiotics includes a selective increase/decrease in specific intestinal bacteria that modulate local cytokine and antibody production, an increase in the intestinal short chain fatty acid production, an enhanced binding of these fatty acids to G-coupled protein receptors on leucocytes, an interaction with carbohydrate receptors on intestinal epithelial and immune cells, and partial absorption resulting in a local and systemic contact with the immune system (Seifert and Watzl, 2007).

The alternative methods of disease prevention have been used as a means of reducing the presence of opportunistic pathogens and simultaneously stimulating the host immune responses. However, other effects related have been observed, as improved growth performance, feed utilization, digestive enzyme activity, antioxidant enzyme activity, gene expression, disease resistance, larval survival and gut morphology alter the gut microbiota, mediate stress response, improve nutrition, reduce risk of certain cancers (colon, bladder), produce lactase, alleviate symptoms of lactose intolerance and malabsorption (Rombout et al., 2010; Dimitroglou et al., 2011; Yousefian and Amiri, 2009; Ringø et al., 2010).

Synbiotic is defined as a combination of probiotic and prebiotic. It is presumed to impart the beneficial effects of both ingredients. Few data are available regarding the application of synbiotics in aquaculture (Li et al., 2009; Rodriguez-Estrada et al., 2009; Zhang et al., 2010). Synbiotics can help to improve health status, disease resistance, growth performance, feed utilization, carcass composition, gastric morphology, and digestive enzyme activities. As such; many commercial dietary formulations now routinely include probiotics or prebiotics.

Therefore, the aim of the present study is to investigate the effects of supplementation of a probiotic (*Bacillus licheniformis*) and the prebiotic (yeast extract) and their synbiotic interaction on growth performance, chemical composition, hematological and biochemical blood parameters of the Nile tilapia (*Oreochromis niloticus*).

Materials and methods

Experimental design and culture technique

A 4×3 factorial experiment was designed to study the effect of probiotic (*B. licheniformis*) levels, prebiotic (yeast extract) levels, and their synbiotic interactions on growth performance, feed utilization, proximate chemical analysis of whole fish body, hematological and biochemical blood parameters of the Nile tilapia (*O. niloticus*).

Nile tilapia, were obtained from the Abbassa hatchery, Abou-Hammad, Sharkia Governorate, Egypt and were acclimated for two weeks at the El-Kanater El-Khayria Fish Research Station, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt. During this period, fish were fed with a commercial diet (30% crude protein) twice a day to be adapted to pelleted feed according to Hassaan et al. (2013). The experiment was conducted in 24 concrete ponds (0.5 m^3 and 1.25 m depth). The ponds were supplied with freshwater from the Darawa irrigation Baranch, Kalubiya, Governorate by a pump machine and a fine net was put in the inlet of each pond. Each pond was stocked with 50 fish with initial weight ranging between 5.69–6.05 g. Two replicates were randomly assigned to each treatment, prior to the start of experiment. During the experiment, fish were hand-fed their respective diets at a level of 3% of body weight, 6 days/week. The daily ratio was divided into three equal amounts and offered three times a day (09:00, 12:00 and 15:00 h). Fish for each pond were weighed biweekly and the amount of daily diet was adjusted accordingly. About one-third of water in each pond was daily renewed by the outlet at the bottom of the pond before feeding. All ponds were provided with continuous aeration to maintain the dissolved oxygen level near saturation and fish were held under natural light.

Water temperature and dissolved oxygen were measured every other day using a YSI model 58 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). Total alkalinity and chloride were monitored twice weekly using the titration; pH was monitored twice weekly using a pH meter (Orion pH meter, Abilene, Texas, USA) (APHA, 1992). The water temperature was 26.17 ± 0.8 °C: dissolved oxygen, 5.6 ± 0.8 mg L^{-1}: total