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FULL LENGTH ARTICLE

Physiological and microbiological indices as indicators of evaluating dietary fungi degraded date pits as a probiotic for cultured Nile tilapia *Oreochromis niloticus* fingerling and its effect on fish welfare

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KEYWORDS

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Probiotic

Abstract The main goal of this study is to explore the potentiality of using blood haemoglobin, plasma glucose, plasma triglycerides, plasma cholesterol, growth rate and intestinal bacterial count as biomarkers to evaluate the dietary supplementation of fungi-degraded date pits (FDDP) as growth promoters as well as probiotic for fingerling Nile tilapia *Oreochromis niloticus* reared in a recirculating system. Five isoenergetics–isonitrogenous diets containing 0, 0.5, 1.0, 1.5 and 2.0 g/100 g of fungi *Trichoderma reesei*-degraded date pits (FDDP), as a replacement for dietary α-cellulose, were used to feed triplicate groups of fish fingerlings (2.0 g initial weight), for 9 weeks. Tilapia weight gain (4.27 g/g), was superior only in fish fed on diets containing 0.5 g/100 g FDDP, when compared with those fish fed on other diets. Blood haemoglobin was found to be unchanged in fish fed on different percentages of FDDP diets. Plasma glucose was high in fish fed on 0.5% FDDP, the accompanied plasma hypertriglyceridemia, could possibly be due to hepatic neo lipogenesis. Cholesterol levels in fish plasma fed on experimental FDDP diets at all concentrations were highly and significantly decreased which may indicate the attenuated ability for transporting lipid out of liver. Although significant correlation among the physiological indices and the dietary FDDP have been observed, blood haemoglobin, plasma triglycerides and cholesterol are shown as more sensitive indicators followed by plasma glucose and then growth rate. The present study showed a possible potential of using FDDP to lower the bacterial population densities in *O. niloticus* intestine without affecting the fish weight or health welfare condition, that could be an excellent approach in designing subsequent probiotic researches on FDDP. Oestradiol was measured in all tested diets and was found to increase linearly with FDDP content in diets, which indicates that a FDDP may has phytoestrogenic roll.

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Introduction

The human demand of fish consumption increased all over the world. Fish is about to become the main alternative source of animal protein. Nile tilapia, *Oreochromis niloticus*, is the predominant cultured species worldwide (Thodesen et al., 2012; Gjerde et al., 2012; El-Sayed et al., 2012). Tilapia aquaculture represents about 42% of world total aquaculture fish production, and about 75% of the total fish production in Egypt (GAFRD, 2012). In addition, tilapia culture has been gradually shifted from traditional semi-intensive systems to more intensive systems, which rely exclusively on artificial feeds. Therefore, the major challenge facing tilapia culture industry is the production of cost effective and environmentally performing feeds for farmed tilapia, using inexpensive, locally available ingredients. Several studies have been conducted to evaluate the incorporation of different unconventional animal and plant proteins and energy sources for farmed tilapia with varying results (El-Sayed, 2006).

Middle East Egypt is one of the largest date-producing countries (1.373.57 MT/year, FAO, 2011). Date pits are by products of the date industry. Depending on the variety and grade quality of the ripe date, date pit comprises from 60 to 120 g/kg of its total weight (Aldhaheri et al., 2004). In Egypt, approximately 120 tons of date pits are available every year as a by-product from date processing plants.

Date pits were tested as a feed ingredient on fish feeds (Belal and Al-Owaifer, 2005; Belal, 2008) but fish production was not significantly affected. High content of indigestible carbohydrates may limit the use of date pits in fish ration (Rahman et al., 2007). In his study, Belal (2008) discussed the use of Xylanase enzymes in feed formulations to liberate nutrients either by hydrolysis of non-degradable cellulose and hemicellulose fibres or by liberating nutrients blocked by these fibres.

Because of the high cost of enzyme utilization in animal feed, Belal (2008) tested and recommended the use of the soft-rot fungus, *Trichoderma reesei* (also known as *Hypocrea jecorina*), which was able to produce the Xylanases enzymes, as an economical alternative for dietary fibre degradation.

Belal (2008) had investigated the effect of FDDP in the diet of *O. niloticus* and concluded that FDDP improved the growth performance of the fish. In his study, he tested different concentrations of FDDP in the formulated diet and he depended only on growth parameters as indicators of FDDP effects.

Since fish peripherical blood analysis is used as a diagnosis to assess its physiological state and the effect of hazardous substances, to determine the nonspecific resistance of progenitors and its descendants, to establish the quality of food, the genetic variability and the effect of stress (Assem et al., 2013; Hassan et al., 2013), an objective of the present study was to investigate the potential use of some physiological health parameters of *O. niloticus* as indices in evaluations of fish diets supplemented with different concentrations of fungi *T. reesei* degraded date pits (FDDP). The concentrations we used were so far lower than those used by Belal (2008).

In aquaculture, infectious diseases are the major cause of economic losses. Probiotic supplementation may change the microbiota of the digestive tract and modulate the immune defences and nutritional performance (Ramos et al., 2013). The use of bacterial count in the intestine of the fish as indicator in evaluating the effect of fungi degraded date pits as feed ingredient in diminution of bacterial infection and hence leading to health benefits to *O. niloticus*, was another goal of our present study. The possibility of using FDDP as probiotic was also discussed.

Aldhaheri et al. (2004), proposed that date pits have an oestrogen-like substance, which acts as a phytoestrogen in the body of animal fed date pits, also El-Sayed et al. (2012) discussed the possible effect of phytoestrogen on sex reversal on Nile tilapia *O. niloticus* larvae fed diets treated with 17α ethyltestosterone. In order to investigate, if any, the possible role of hormone in FDDP, we measured the concentration of testosterone and oestradiol in the diet supplemented with increasing percentage of FDDP (0.5–2%).

Materials and methods

Preparation of the fish for experiments

Nile tilapia *O. niloticus* fingerlings (1.95 g average initial weight) were collected from local hatcheries. The fish were stocked into 20 L fibreglass tanks in a closed, recirculating indoor system. The tanks were provided with a continuous flow of aerated dechlorinated tap water at 25 °C (hardness 5.2 mg/L as $CaCO_3$; Ca^{2+} 0.045–0.069 mM/L; Na^+ 0.024 mM/L; dissolved oxygen 80% saturation; pH 7.2–7.5).

Experimental design

Groups of ten fish were randomly stocked into each aquarium, with four replicates per each treatment. In order to avoid the influence of any systematic stress factors, the fish groups were randomly redistributed half way through the experiment. In each trial, one aquarium was allocated to fish fed on the diet without FDDP to serve as control. Each aquarium was considered as an experimental unit. The fish was fed ad lib at 09.00, 12.00, and 15.00 h. Approximately 15 to 20 min after all feeding activity had subsided, the uneaten feed was removed and weighed to determine the amount of eaten feed. Faecal waste was siphoned from each aquarium every day. Group weight measurements were done at weekly intervals. Experimental periods lasted for 9 weeks for all tested diets.

Culture system

The feeding trials were conducted in 3 L plastic aquaria. Water was recirculated through biological and mechanical filters. The recirculating system used a 200 L vertical screen filter system utilizing high-density polyester screens (FIAB fish technic, GmbH, Germany) to remove particulate matter and provide substrate for *Nitrosomonas* and *Nitrobacter* bacteria. A blower and air stone provided continuous aeration. Water exchange rate for the system was approximately 5% of the total volume per day. Each aquarium was supplied with water at a rate of 200 mL/h and cleaned daily. A PVC screen covered the aquarium bottom to prevent the experimental fish from eating their faeces. A black plastic screen covered the back and sides of all aquaria to minimize disturbances caused by personnel present in the laboratory. Illumination was supplied by fluorescent ceiling lighting, controlled by an automatic electric timer to maintain a photoperiod of 18:6 h light:dark per day cycle.