Journal of Advanced Research (2015) 6, 621–629

Cairo University

Journal of Advanced Research

ORIGINAL ARTICLE

Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*

Nermeen M. Abu Elala [a,*], Naela M. Ragaa [b]

[a] Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, Egypt
[b] Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Egypt

ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form 26 February 2014
Accepted 27 February 2014
Available online 4 March 2014

Keywords:
Acidifiers
Growth performance
Eubiosis
Gut probionts
Innate immunity
Challenge test

ABSTRACT

In connection with the global demand for safe human food and the production of environmentally friendly aquaculture products, acidifiers are natural organic acids and salts that have received considerable attention as animal-feed additives. The current study was designed to evaluate the effects of potassium diformate (KDF) on the growth performance and immunity of cultured *Oreochromis niloticus (O. niloticus)*. Four iso-nitrogenous and iso-caloric rations containing graded levels of KDF, including 0% (control basal diet), 0.1%, 0.2% and 0.3%, were fed separately to four equal fish groups (30 fish/group with an initial body weight of 53.49 ± 6.15 g) for sixty days. At the end of the experimental period, the fish groups fed on 0.2% and 0.3% KDF exhibited significant improvements in their feed intake, live weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio, with concomitant improvement of their apparent protein digestibility ($p < 0.05$). Dietary supplementation of 0.3% KDF appeared to stimulate the beneficial intestinal flora; a proliferation was observed of indigenous probionts (Eubiosis) associated with the relative activation of cellular and humeral innate immunity (phagocytic activity/index, nitroblue tetrazolium reduction test and serum/gut mucous lysozyme activity). The cumulative mortality of the fish groups fed on KDF and challenged orally with *Aeromonas hydrophila* was lower than that of the control group. The resistance against diseases increased with dietary KDF in a dose-dependent manner. Thus, we conclude that the use of acidifiers can be an efficient tool to achieve sustainable, economical and safe fish production.

Introduction

The long-term administration of antibiotic growth promoters, AGPs, in aquafeeds creates an optimal environment to enable antibiotic resistance genes to multiply [1]. The treated animals become "reservoirs" for the production and distribution of antibiotic-resistant bacteria. A wide variety of natural growth

* Corresponding author. Tel.: +20 1067114455; fax: +20 2 35725240.
E-mail address: nermeen_abuelala@cu.edu.eg (N.M. Abu Elala).
Peer review under responsibility of Cairo University.

Production and hosting by Elsevier

2090-1232 © 2014 Production and hosting by Elsevier B.V. on behalf of Cairo University.
http://dx.doi.org/10.1016/j.jare.2014.02.008

promoters (NGPs), including plant extracts, prebiotics, probiotics and organic acids, have been broadly applied worldwide with reasonable success. Organic acids and their salts have been used as a potential replacement of AGPs to improve the performance and the health of livestock [2]. Formic, acetic, propionic, and citric acid are the most commonly used dietary organic acids in aquaculture. Particularly, the salts of formic acid KDF have been recently used in tropical and cold-water fish. Formic acid KDF was the first substance approved as a possible non-antibiotic growth promoter by the European Union [Commission Reg (EC) number 1334/2001] [3].

Dietary acidifiers have demonstrated effectiveness in enhancing the growth performance and the nutrient availabilities in various aquatic species. They reduce the pH of the digesta of the stomach and the foregut, which in turn stimulates the pepsin activity, improving protein digestibility and mineral absorption [4,5]. Dietary inclusion of citric acid/formic acid enhances the bioavailability of minerals, including phosphorus, magnesium, calcium and iron in rainbow trout (*Oncorhynchus mykiss*), sea bream (*Pagrus major*) and Indian carp (*Labeo rohita*) [5,6]. These short-chain organic acids are generally absorbed through the intestinal epithelia by passive diffusion, providing energy for renewing the intestinal epithelia and maintaining the gut health [6]. Despite the reported improvement in the nutrient availabilities of aquatic animals fed on dietary acidifiers, contradictory results have been reported on the growth promoting effects. Oral administration of potassium diformate (KDF) significantly improves the feed intake, the live weight gain, the feed conversion ratio and the protein efficiency ratio of various tilapia species [7–11]. In contrast, Petkam et al. [12] and Zhou et al. [3] reported no significant improvement in the growth performance of tilapia fed on organic acids/salt blend or KDF, respectively, at various dietary levels.

From another point of view, KDF can improve the general health status of cultured animals by its stronger antimicrobial effect towards coliform bacteria, *Escherichia coli* and *Salmonella* sp., than towards lactobacilli [3]. It was reported that the total bacteria per gram of faeces was significantly reduced in the fish fed with an organic acid blend and KDF diets [10]. Similarly, Da Saliva et al. [13] indicated that propionate, butyrate and acetate salts exhibit the highest inhibitory capacity against vibrio species in marine shrimp. These acids can penetrate through the cell wall of gram-negative bacteria and release protons into the cytoplasm. Thus, the bacteria consume a large amount of ATP to excrete protons in trying to maintain a balanced intracellular pH, resulting in the depletion of cellular energy with eventual cell death [14]. Although the scientific publications focused on the antimicrobial effects of organic acids are numerous, very few publications have tackled their effects on the indigenous beneficial flora, lactic acid bacteria (LAB), which has become a major source of concern as one of the most common probiotic bacteria used in aquafeeds [15]. To our knowledge, there have been no previous reports about the ability of acidifiers to influence the humoral and cellular non-specific immunity of cultured tilapia. As a result, the current study was planned to assess the effect of potassium diformate, KDF (Aquaform)® on the growth performance, protein digestibility, gastrointestinal pH, gut beneficial flora, innate immunity and survival of *Oreochromis niloticus* challenged with pathogenic *Aeromonas hydrophila*.

Material and methods

Experimental fish

One hundred and twenty apparently healthy *O. niloticus* were obtained from a private fish farm. Fish acclimated to the laboratory conditions for two weeks before being randomly divided into four groups (30 fish/treatment, three replicates/tank) representing four nutritional groups. One group served as the control, and the other three groups represented the feed additives tested. The experimental fish (mean individual initial weight of 53.49 ± 6.15 g) were fed to satiation, 2% of a total body weight two times/day (at 0800 and 0400) for 60 days and weighed biweekly to adjust the daily requirements [16]. *All Institutional and National Guidelines for the care and use of fisheries were followed.*

Experimental unit

The present study was conducted in the Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University. The experimental fish were stocked in 12 glass aquaria (80 cm × 30 cm × 40 cm) supplied with de-chlorinated tap water. The water was aerated continuously by using an air compressor (BOYU S 2000 Air pump, Malaysia). The photoperiod was 12 h light/12 h dark. The water temperature was maintained at (24 ± 1 °C) using a 250-Watt immersion heater with a thermostat. The water temperature and the dissolved oxygen level were recorded daily (by Metteler Toledo, model 128, s/No 1242), and the average range of dissolved oxygen was greater than 5.8 mg/l. Other water quality parameters, including pH and ammonia level, were measured every two days with a pH meter (Orion model 720A, s/No 13062) and ammonia meter (Hanna ammonia meter); the average range of the total ammonia was 0.12–0.23 mg/l, and the pH was in the range of 7.2 ± 0.5 during the experiment.

Experimental diet

Four iso-nitrogenous and iso-caloric diets were formulated from practical ingredients to satisfy the nutrient requirements of *O. niloticus* according to NRC [16] (Table 1). The control (basal diet) and the other diets were supplemented by 0.1%, 0.2% and 0.3% (KDF) Aquaform®, which contains 35% free formic acid, 35% formate and 30% potassium (ADDCON, NordicAS, Porsgrunn, Norway). The experimental diets were formulated to contain nearly 28% crude protein. The diets were prepared by individually weighing each component and thoroughly mixing the minerals, vitamins and additives with corn. The organic acid powder was mixed thoroughly in the stated quantities into a small amount of feed (1 kg) in a premixer. Water was added until the mixture became suitable for making pellets. The wet mixture was passed through a pellet machine with a 2-mm diameter. The produced pellets were dried at room temperature and kept frozen until the beginning of the experiment. The tested diets were analysed for crude protein (CP %), ether extract (EE %), crude fibre (CF %), ash (%) and moisture %, according to the procedures described by the standard A.O.A.C. methods [17]. The nitrogen free-extract (NFE %) was calculated by the differences.