Aquaculture 291 (2009) 89–94

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of dietary potassium diformate (KDF) on growth performance, feed conversion and intestinal bacterial community of hybrid tilapia (*Oreochromis niloticus* ♀ × *O. aureus* ♂)

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ARTICLE INFO

Article history:
Received 30 December 2008
Received in revised form 24 February 2009
Accepted 24 February 2009

Keywords:
Oreochromis niloticus ♀ × *O. aureus* ♂
Potassium diformate (KDF)
Flavomycin
Quinocetone
Intestinal bacterial community

ABSTRACT

The aim of the present study was to investigate the effect of dietary inclusion of potassium diformate (KDF), a possible non-antibiotic growth promoter, and two widely-used antibiotics, flavomycin and quinocetone, on growth performance, feed conversion ratio and gut microbiota of hybrid tilapia. An 8 week feeding trial was conducted with five levels of KDF: 0 (C), 3.0 (KDF3), 6.0 (KDF6), 9.0 (KDF9), and 12.0 (KDF12) g kg^{-1} diet and three antibiotic treatments: flavomycin (8 mg kg^{-1}, AF), quinocetone (100 mg kg^{-1}, AQ), and flavomycin (4 mg kg^{-1}) + quinocetone (50 mg kg^{-1}) (AFQ). At the end of the experiment, fish were starved for one day and bulk weighed. Pooled gut contents sampled from four replicate tanks were analyzed for bacterial community by 16 S rDNA PCR, denaturing gradient gel electrophoresis (DGGE) and Bio-1D++ software. The results indicate that the addition of dietary KDF and antibiotics had no significant effect on tilapia growth performance, feed conversion ratio or survival compared to the control group. Among the experimental groups, however, fish fed the KDF3 and KDF6 diets showed improved growth performance and feed conversion ratio with higher final body weight and specific growth rate and lower feed conversion ratio compared to those fed the AFQ diet. Dietary KDF and antibiotics showed effects on the gut microbiota. Dietary KDF3 and KDF6 improved the relative richness of some intestinal allochthonous bacteria such as *Mycobacterium* sp. partial MHSD12-like, *Mycobacterium peregrinum*-like, *Pseudomonas* sp. HMPB4-like and six uncultured bacterium-like species. However, *alpha Proteobacterium* IMCC1702-like, *Rhodococcus* sp. P14-like, and three uncultured bacterium-like species were depressed in the gut. Based on these results, the possible beneficial effects of KDF on gut bacteria will be discussed.

1. Introduction

Potassium diformate (K-diformate, KDF) is odorless, low-corrosive, flowable (Hebeler et al., 2000) and dietary inclusion has demonstrated effectiveness in enhancing growth performance of terrestrial animals (Paulicks et al., 1996, 2000; Roth et al., 1996, 1998; Kirchgessner et al., 1997; Øverland et al., 2000; Canibe et al., 2001; Mroz et al., 2002). Consequently, KDF was the first substance approved as a possible non-antibiotic growth promoter by the European Union [Commission Reg (EC) number 1334/2001]. KDF improves the general health status of cultured animals by its stronger antimicrobial effect towards coliform bacteria than toward *Lactobacilli* (Lueck, 1980; Kirchgessner et al., 1992; Février et al., 2001), leading to a more favorable microbiota with lower population levels of *Escherichia coli* and *Salmonella* and higher population level and diversity of *Lactobacilli* (Hebeler et al., 2000). To our knowledge only one preliminary study on the inclusion of KDF in aquatic feed has been reported (Ramli et al., 2005) and its anti-microbial mode of action in aquatic animals are therefore unclear.

In aquaculture, supplementations of flavomycin and quinocetone either alone or in combination are used as growth promoters for farmed fish in China (Xiong et al., 2007; Li et al., 2008; Liu et al., 2008a). Flavomycin is a phosphoglycolipid antibiotic produced by various species of *Streptomycetes* and has been used as a feed additive for the improvement of the feed conversion ratio in farm animals since its discovery in the mid-1950s (Wallhausser et al., 1965). It has been most extensively used in swine and poultry production (Bolder et al., 1999; Denli et al., 2003; Juśkiewicz et al., 2004), but information on the use of flavomycin as a growth promoter in aquatic animals is less available (Xu et al., 2007; Li et al., 2008). Quinocetone (3-methyl-2-styrene-based-quinoline dioxin-1, 4-dioxide) is regarded as a safe and efficient growth promoter in the production of swine, poultry, and aquatic animals in

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doi:10.1016/j.aquaculture.2009.02.043

China (Xiong et al., 2007). It has been reported that Gram negative bacteria are more sensitive to quinocetone than Gram positive bacteria (Hu and Zhang, 2006). Currently, the use of antibiotics as growth promoters in aquaculture is under public scrutiny and criticism (WHO, 1999; OIE, 2003; FAO, 2006). Development of resistance of bacteria to antibiotics and cross-resistance of human and animal pathogens associated with sub-therapeutic and/or improper use of antibiotics are major concerns (WHO, 1996). During the last two decades, the use of antibiotics in aquaculture has become more and more restricted, and the European Union ratified a ban in 2006 for the use of all sub-therapeutic antibiotics for use as growth promoting agents (Regulation 1831/2003/EC). Therefore, alternatives to antibiotic growth promoters are currently being investigated and deployed (Ramli et al., 2005; Barnes et al., 2006; Lv et al., 2007; Zhou et al., 2007, in press).

The aims of the present study were to study the effects of dietary KDF on growth performance, feed conversion ratio and the intestinal bacterial community of hybrid tilapia. Since conventional culture-based methods are time consuming, selective and do not present a full picture of the bacterial community (see reviews, Cahill, 1990; Ringø et al., 1995), molecular methods such as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) were used to evaluate microbial diversity due to the advantages of reliability, rapidity, sensitivity and easy to operate (Liu et al., 2008b, Zhou et al., 2009, in press).

2. Materials and methods

2.1. Experimental diets

In the present study, 8 isonitrogenous and isoenergetic diets were prepared (see Table 1). The diets were made by pulverizing the feedstuffs into 250 μm particles to produce 3 mm pellets with a pelletiser (MYZL180, Muyan Inc., Jiangsu, China). Diets were fan-dried and stored in sealed bags prior to use. The following diets were used; 0 (C), 3.0 (KDF3), 6.0 (KDF6), 9.0 (KDF9), 12.0 g potassium diformate (KDF12) per kg diet, 8 mg flavomycin (AF), 100 mg quinocetone per kg diet (AQ), and 4 mg flavomycin + 50 mg quinocetone per kg diet (AFQ). KDF, flavomycin and quinocetone were supplied by Beijing Challenge Bio-Technology Co., Ltd, Beijing, China. Contents of moisture, crude protein, crude lipid, and ash of the different feedstuffs were analyzed according to Xie et al. (1997). Chemical analyses for crude protein, crude lipid, carbohydrate and ash contents in the diets were ~389.3, 61.2, 122.7, and 426.8 g kg^{-1} as dry matter, respectively.

2.2. Feeding trial

Juvenile hybrid tilapia (*Oreochromis niloticus* ♀ × *O. aureus* ♂) were obtained from a local commercial farm in Haikou, Hainan, China. The experiments were carried out in our laboratory at Hainan, China. Prior to the feeding trial, the fish were acclimated in a recirculation system for two weeks and fed the control diet. After one day of starvation, uniform fish (2.67 ± 0.01 g) were randomly stocked into 32 glass tanks (0.5 m × 0.5 m × 0.5 m; water volume, 110 l) in a recirculation system. Each dietary group had four replicates, each containing 20 fish per tank. The feeding trial lasted for 8 weeks. During this period, the fish were hand-fed to apparent satiation within half an hour, four times per day (07:30, 11:00, 15:00 and 18:00 h). Water temperature was 24.5 ± 1.9 °C with a dissolved oxygen level of 6.90 ± 0.20 mg O l^{-1}, pH of 7.53 ± 0.04, NH_3-N of 0.35 ± 0.02 mg N l^{-1} and NO_2^--N of 0.046 ± 0.005 mg N l^{-1}.

At the end of the feeding trial, the fish were weighed after one day of starvation. The growth and feed utilization parameters evaluated were as follows: specific growth rate (SGR, % d^{-1}) = 100 × ln (FBW / IBW) / days, feed conversion ratio (FCR) = diet consumed (g) / (FBW − IBW), feed intake (FI, g d^{-1} $fish^{-1}$) = diet consumed (g) / days / fish number, and % survival (%S) = 100 × survival fish number / total fish number (FBW and IBW are the final body weight (g) and initial body weight (g), respectively).

All data were subjected to one-way ANOVA analysis and are presented as mean plus standard deviation (S.D.). When appropriate, Duncan's multiple test ($P<0.05$) was applied to evaluate the differences among means. All statistical analysis was carried out using SPSS version 10.0.

2.3. Sample collection and extraction of DNA

The gut contents of five randomly selected fish from each tank were gently squeezed out under sterile conditions as previously described by Zhou et al. (2007). Since one objective of this study was to compare the allochthonous (transient) microbial community in the different fish groups fed the different diets, and not to analyze fish-to-fish variation, an approximately equal amount of intestinal content from each fish in each dietary group was pooled to provide a representative sample.

Genomic DNA was extracted by the method described by Yu and Morrison (2005) with some modifications as described elsewhere (Zhou et al., 2007, 2009; Liu et al., 2008b).

2.4. 16S rDNA PCR and DGGE

Universal primers were used for amplification of the variable V3-region on 16S rDNA. Primers for PCR were: the forward primer 338f (5′-ACT CCT ACG GGA GGC AGC AG-3′) including a 40 base GC clamp at the 5′ end, and the reverse primer 519r (5′-ATT ACC GCG GCT GCT GG-3′) (Muyzer et al., 1993). The PCR and DGGE were performed as described by Liu et al. (2008a,b). Briefly the method consists of PCR

Table 1
Ingredients (g kg^{-1}) of the experimental diets.

Ingredients	C	KDF3	KDF6	KDF9	KDF12	AF	AQ	AFQ
Red fish meal (Zhejiang, China)[a]	207.6	207.6	207.6	207.6	207.6	207.6	207.6	207.6
Soybean meal (USA)[a]	240.0	240.0	240.0	240.0	240.0	240.0	240.0	240.0
Cottonseed meal (Xianjiang, China)[a]	190.8	190.8	190.8	190.8	190.8	190.8	190.8	190.8
Wheat middling (Henan, China)[a]	280.0	280.0	280.0	280.0	280.0	280.0	280.0	280.0
Zeolite (Hubei, China)[a]	20.0	17.0	14.0	11.0	8.0	19.8	19.9	19.85
Choline chloride[b]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
$Ca(H_2PO_4)_2$[b]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Mineral mixture[c]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin mixture[c]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin C[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Soybean oil[a]	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6
Potassium diformate[b]	0.0	3.0	6.0	9.0	12.0	0.00	0	0
Flavomycin (4%)[b]	0	0	0	0	0	0. 2	0	0. 1
Quinocetone[b]	0	0	0	0	0	0	0. 1	0.05

[a] Supplied by Puai Feed Mill, Hubei, China.
[b] Supplied by Beijing Challenge Bio-Technology Co., Ltd, Beijing, China.
[c] According to Lv et al. (2007).