Effects of two different organic acid blands in olive flounder

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Abstract

This experiment was conducted to evaluate the effect of two different organic acid products as antibiotic replacement in olive flounder paralichthys olivaceus . Fish averaging 3.5 ± 0.05 g(mean ± SD) were fed one of the ten semi-purified diets : Diet 1 ; Control , Diet 2 ; add antibiotics - 50mg OTC / kg body weight / day(OTC), Diet 3 ; Add organic acid bland A(OABA) - 4g/kg diet, Diet 4 ; add organic acid bland B(OABB) - 4g/kg diet for 10 weeks. Total gut microflora counts were significantly higher in the control group compared to the OTC and organic acid groups(P ‹0.05). Fish fed OABA, OABB and OTC had lower gut Vibrio counts compared to the control, but were not significantly different. Results from the challenge study indicate that mortality in the different treatment groups (50%) was significantly lower than those observed for the control group (100%). There were no differences in mortality between the OTC and organic acid groups. Overall findings from this study indicate that the organic acid blends A and B were as effective as oxytetracycline, an antibiotic, in regulating total gut bacterial numbers, Vibrio counts and providing protection against a pathogen such as Edwardsiella tarda.

Introduction

The worldwide application of antibiotics in aquaculture for prophylactic and therapeutic purposes has resulted in an increase in bacterial resistance in exposed microbial ecosystems, impacting both animal and public health. International agencies recommend that antibiotics should be restricted to therapeutic purposes only, and that preventative approaches to disease management should be preferred over costly post effect treatments. Therefore, to make the aquaculture industry more sustainable, new strategies to control infection are urgently needed. One potential substitute for antibiotic in aqua feeds is organic acids. The mechanism of action of these weak acids in limiting microbial growth had been reviewed by Booth and Stratford (2003). The undissociated form of an organic acid is lipophilic and can passively diffuse through a bacterium's cell wall. Once inside the more alkaline cytoplasm, it dissociates and causes the internal pH to decrease. This inhibits bacterial cell metabolism. Categories of bacteria that do not tolerate changes in trans-membranous pH gradients will undergo cellular stress and eventually die. Currently, there is considerable interest in the commercial use of organic acids in fish diets, both to control disease and to enhance growth performance. Olive flounder (*Paralichthys olivaceus*) is one of the most economically important fish species farmed in eastern Asia including Korea, Japan and China. Its production is top most among the Korean mariculture finfish species. Therefore, the present study was conducted to effect of two different organic acid products as antibiotic replacement on growth performance in olive flounder *paralichthys olivaceus*

Materials and methods

This study was conducted in an indoor re-circulating system at the Feeds and Foods Nutrition Research Center at Pukyong National University, Busan, Korea. A basal diet was formulated to contain approximately 52% crude protein and was composed mainly of fish meal, squid liver powder, dehulled soybean meal and corn starch. The basal diet served as the control diet and three other diets were formulated that were similar in all respects other than that they contained either an antibiotic (oxytetracyline) at 50 mg/kg body weight per day, 4 g/kg of OABA (Formic acid + Propionic acid) or 4 g/kg of OABB (2-Hydroxy-4-Methylthio Butanoic acid) respectively . Replicate groups (N=5) of juvenile flounder (3.5g initial weight) were fed one of various dietary treatments at a rate of 3% body weight per day for a period of 10 weeks. Water temperature was maintained at 20°C for the duration of the trial. At the end of the 10 weeks fish were anesthetized and weighed for estimates of performance. Intestinal samples were taken from each of the groups of fish, pooled homogenized and plated on either plate count agar (PCA) or TCBS agar for total gut bacteria or gut vibrio counts respectively. A subsample of fish from each tanks (n=5) was used for a challenge test and were injected intraperitoneally (0.1 ml) with *Edwardsiella tarda* ($1x10^6$ CFU/ml). Mortality of fish from each group was recorded over a 10 day period.

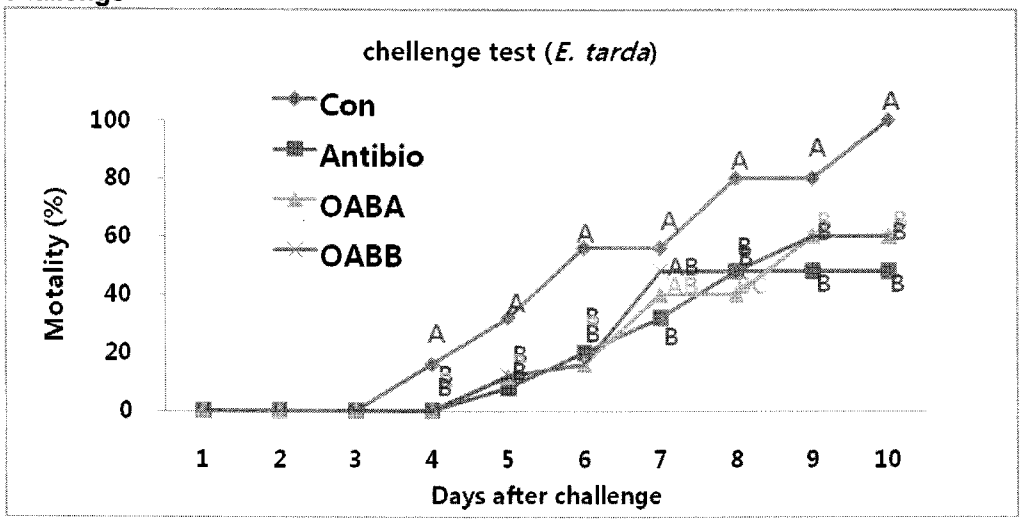
Results

At the end of 10 weeks, no differences in growth performance were observed between dietary treatments. However, total gut microflora counts were significantly higher in the control group compared to the OTC and organic acid groups ($P < 0.05$). There were no differences in total bacterial counts between the OTC and organic acid (OABA, OABB) groups. Fish fed OABA, OABB and OTC had lower gut *Vibrio* counts compared to the control, but were not significantly different. Results from the challenge study indicate that mortality in the different treatment groups (50%) was significantly lower than those observed for the control group (100%)($P < 0.05$). There were no differences in mortality between the OTC and organic acid groups.

Tab. 1 : Effects of the basal diet and five test diets on total bacterial count & *Vibrio* spp count in intestine

Experimental diets	Total bacterial count CFU X 10^6/g	*Vibrio* spp count CFUX10^4/g
CONTROL	1.79 ± 0.14 [a]	2.31 ± 0.53
OTC	1.13 ± 0.10 [c]	1.47 ± 0.40
OABA	1.20 ± 0.04 [c]	1.60 ± 0.48
OABB	1.14 ± 0.07 [c]	1.61 ± 0.47

Figure 1. Cumulative mortality in olive flounder following *Edwardsiella tarda* challenge



Discussion

There was no significant differences in weight gain(WG), feed efficiency(FE), specific growth rate(SGR) and protein efficiency ratio(PER) among fish fed all diet. Results on the growth-promoting effects of organic acids and their salts have been reported in the literature for other fish species, with some studies showing a significant impact (Gislason et al.1996; DeWet 2005; Baruah et al.2007) and others reporting no significant effect (Owen et al. 2006; Hossain et al. 2007).The undissociated form of an organic acid is lipophilic and can passively diffuse through a bacterium's cell wall. Once inside the more alkaline cytoplasm, it dissociates and causes the internal pH to decrease. This inhibits bacterial cell metabolism. In this study, total bacterial count in intestine showed that fish fed OABA, OABB, OTC diet has a significantly lower than fish fed Control diet ($P < 0.05$). The growth inhibition of enteric bacteria in the gastro-intestinal tracts of terrestrial livestock animals fed organic acid-added diets has been well documented (Alp et al. 1999; Kluge et al. 2006). Kluge et al. (2006) suggested that the improved growth, feed conversion and nitrogen balance observed in piglets fed benzoic acid-supplemented diets might be associated with a reduction in the bacterial population in the gastrointestinal tract, thereby causing less nutrient degradation by microbial enzymes and making these nutrients more available for the piglets. The impact of fish intestine bacterial population on growth remains to be defined. *Vibrio* spp. was identified as one of the bacterial disease pathogens. Fish fed organic acid or OTC supplemented diets in this experiment tended to decrease count of *Vibrio* spp. compared with fish fed control diet without any added additives. *Edwardsiella tarda* was identified as one of the major bacterial disease pathogens in olive flounder farm. Challenge test with *Edwardsiella tarda* in the present study showed that fish fed OTC diet, OABA diet and OABB has a significantly lower cumulative mortality rate than fish fed control diet($P < 0.05$). Ramli et al. (2005) reported that the addition of organic acid(potassium diformate) in the diet of hybrid tilapia significantly increased the survival rates of fish after challenge with *V. anguillarum* and the effects were dose-dependent. Also W-K NG et al. (2009) reported that dietary organic acids may have a positive effect in protecting tilapia against *S. agalactiae.*

Conclusions

This study indicates that the organic acid blends A and B were as effective as oxytetracycline, an antibiotic, in regulating total gut bacterial numbers, *Vibrio* counts and providing protection against a pathogen such as *Edwardsiella tarda* in olive flounder.

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