

Original article

## The development of a new generation of functional commercial tilapia feeds to reduce the use of harmful antibiotics in intensive aquaculture systems

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### Introduction

The rapid expansion of the global tilapia production is due in large part to the intensification of culture systems made possible with the use of commercial pelleted feeds [1]. Tilapia is currently the second most produced fish in the world, after carps. Intensification of culture systems often leads to increased incidence of disease. The spread of diseases in the aquaculture industry had often times led to the misuse and overuse of antibiotics both as a prophylactic as well as a therapeutic agent [2]. The development of antibiotic resistant bacteria strains is a potential public health concern other than being harmful to the host animal and the environment [2]. With the current global trend in banning and/or restricting the use of antibiotics, especially as antibiotic growth promoters, suitable alternatives to antibiotics need to be investigated. Functional feeds are the new generation of aquafeeds with additional function, usually with health-promoting or disease resistance properties beyond their nutritional value. Our research into the use of organic acids as a functional feed additive in tilapia feeds as an alternative to the use of harmful antibiotics have shown very promising results [3,4].

Organic acids are compounds with one or more carboxyl groups in their structure and include short-chain fatty acids, volatile fatty acids or weak carboxylic acids such as formic, citric, benzoic, lactic acid, etc. Organic acids are categorized as “Generally Regarded as Safe” and have been extensively used in terrestrial livestock feeds. Despite many reports on the beneficial effects of dietary organic acids in improving nutrient utilization, its use as an anti-microbial and functional feed additive is relatively new to aquafeeds. We have recently reviewed the utilization and mode of action of organic acids in the feeds of cultured aquatic animals [5]. Despite promising results, the effectiveness of dietary organic acids are affected by the farmed species, age, diet composition, type and concentration

of organic acids as well as the culture conditions [5].

As far as we know, the potential benefits of dietary organic acids in improving nutrient utilization and disease resistance have not been previously reported in a tilapia commercial farm setting. It is the aim of this study to validate the promising results obtained in our laboratory and in other laboratories around the world as to whether dietary organic acids can impart similar benefits in commercial feeds used in a commercial tilapia farm. The adoption of functional tilapia feeds by the fish farming industry would contribute to the continued sustainability and scalability of the industry.

### Materials and methods

Four 20-meter diameter and 12 m deep semi-rigid circular floating cages (2 replicates per treatment) in a commercial tilapia farm in Malaysia were used in the study. All male Nile tilapia, *Oreochromis niloticus*, fingerlings (GenoMar Supreme strain, average initial weight 29 g) were stocked at a density of 60,000 fish per cage (16 fish per m<sup>3</sup>). The extruded commercial tilapia feeds, containing no added organic acids (control) or 2% of a prototype organic acids blend (OAB) were produced and purchased from Cargill Feed Ltd., Malaysia. The prototype OAB were manufactured in collaboration with Sunzen Feedtech Ltd. (Malaysia) and consisted of four organic acids microencapsulated in a specialized lipid matrix using centrifugal spray cooling technology. With the exception of the added organic acids, both feeds were similarly formulated. Dietary protein levels for the starter and grower feeds are 37-38% and 34-35%, respectively. Feeds underwent a comprehensive risk assessment by Cargill International to ensure compliance with Global Gap and Aquaculture Stewardship Council certification for this new functional feed additive. Feeds were transported daily to the tilapia cages and mechanically dispersed twice a day for 203 days of culture.

Two to four weeks before the final harvest, 85 large

fish were randomly sampled using a dip net from each cage. All fish were dissected and fecal samples extracted from the last 5% of the total gut length for nutrient digestibility determination. Endogenous acid-insoluble ash was used as a marker for dry matter, protein, lipid, ash and phosphorus digestibility determination. Another 10 fish per cage were sampled and the digesta removed from various gut segments for microbial counts and pH measurements. Blood serum was collected for lysozyme activity determination. Liver, viscera and visceral fat was excised to measure various body indices and the fish carcasses filleted to determine fillet yield and proximate composition.

All data were subjected to one-way analysis of variance ANOVA using the SPSS statistical software (SPSS version 14.0 for Windows). Differences between means were determined by independent sample t-test and effects with a probability of  $P < 0.05$  were considered statistically significant.

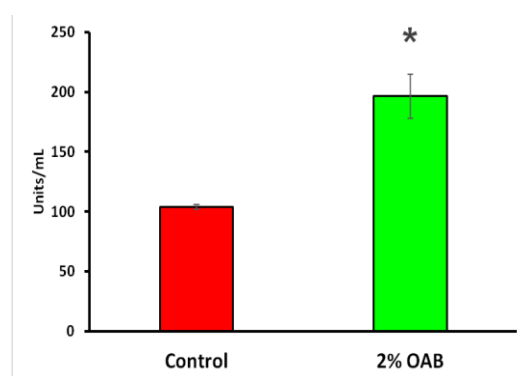
## Results and discussion

The analyzed nutrient composition of the control and 2% OAB feed were similar. A slight decrease in diet pH was observed, from pH 6.0 to 5.6 when 2% OAB was added in the feed.

Results indicated that dietary organic acids did not significantly ( $P > 0.05$ ) affect growth performance with fish registering an average daily weight gain of  $4.18 \pm 0.14$  and  $4.10 \pm 0.32$  g/day for fish fed the control or 2% OAB feed, respectively. Feed conversion ratio was not significantly better for the OAB-fed fish compared to control (1.24 versus 1.27). The high growth rates and good FCR are characteristic of this fast growing tilapia strain. Under controlled laboratory conditions, OAB-fed tilapia has shown growth improvement in a dose-dependent manner [3,4]. Over the 203 days of culture, total average fish mortality was slightly lower ( $30.4 \pm 8.2\%$ ) in cages fed the OAB-added feed compared to control ( $33 \pm 4.6\%$ ). The relatively high mortalities encountered were mostly due to *Streptococcus agalactiae* infections that sometimes occur during the hot season in the lake. It was observed that fish in cages fed the control feed had more incidences of hemorrhages on the skin and fins compared to fish fed the organic acid-added feed.

Biological indices, including various organ-body indices, hematocrit and fillet yield were not negatively affected by the added organic acids. With the exception of ash apparent digestibility, a general trend of improved nutrient utilization efficiency was observed in the OAB feed. This might have accounted for the slightly better FCR observed in tilapia fed this diet. Decreased total viable bacteria and increased lactic acid bacteria counts in the gut of tilapia fed organic acids-added feeds was observed. This indicated that the organic acids might have a beneficial effect in

inhibiting the colonization of harmful bacteria in the gut. It is also noteworthy to observe that beneficial lactic acid bacteria populations were increased in the terminal segment of the gut in OAB-fed fish. It would seem that dietary organic acids may have an additional beneficial effect on gut health over that offered by antibiotics which will indiscriminately kill all bacteria. Tilapia fed organic acids-added feeds had significantly ( $P < 0.05$ ) enhanced serum lysozyme activity (Fig. 1), almost doubled that of the control. Lysozyme is part of the non-specific humoral defense mechanism against invading microbes in fish. Antibiotic use in OAB-fed tilapia was significantly reduced. Functional feed additives such as organic acids can be used as an immuno-stimulant to offer a more environmentally friendly strategy for intensive tilapia farming.



**Fig. 1.** Serum lysozyme activity (lysozyme units/mL) of tilapia fed commercial feeds with (2% OAB) or without (Control) organic acids supplementation. Values are mean  $\pm$  SE of duplicate groups of 10 fish. Asterisk indicates statistical significance between the two treatment groups ( $P < 0.05$ ).

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## References

1. Ng WK, Romano N (2013) *Aquaculture* 5: 220–254
2. Marshall BM, Levy SB (2011) *Clinical Microbiol Rev* 24: 718–733
3. Ng WK et al. (2009) *Aqua Res* 40: 1490–1500
4. Koh CB et al. (2016) *Aqua Res* 47: 357–369
5. Ng WK, Koh CB (2016) *Rev Aqua* doi: 10.1111/raq.12141