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Short communication

Dietary sodium citrate improved oxidative stability in red hybrid tilapia (*Oreochromis* sp.) but reduced growth, health status, intestinal short chain fatty acids and induced liver damage

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ABSTRACT

Among organic acids, citric acid and their salts are currently the most studied as a supplement in aquafeeds to potentially improve growth and nutrient utilization in fish. The results have been generally beneficial but no studies have been performed on tilapia. A 50 day experiment was conducted on the effects of dietary sodium citrate at 0, 1, 2 and 4% on the growth, feeding efficiency, body indices, muscle proximate composition, muscle lipid peroxidation, some plasma and blood parameters, intestinal short chain fatty acids (SCFA), and liver histopathology of red hybrid tilapia (*Oreochromis* sp.). Triplicate groups of 60 tilapia fingerlings (initial weight of 1.86 ± 0.01) were in each treatment. Results showed that, while not significant, increasing dietary sodium citrate reduced tilapia growth ($p > 0.05$). However, muscle crude protein ($r^2 = 0.931$), lipid ($r^2 = 0.962$), and ash ($r^2 = 0.834$) significantly decreased at increasing dietary sodium citrate levels ($p < 0.05$). Plasma ALT significantly increased ($p < 0.05$; $r^2 = 0.357$) with increasing dietary sodium citrate treatments along with histopathological liver damage that included hemorrhages, necrosis and inflammatory responses. Many of the cell differential counts were significantly ($p < 0.05$) altered by increasing dietary sodium citrate levels. Among the intestinal SCFA in tilapia, acetic acid was the highest, followed by propionic and butyric acid, and these all significantly decreased ($p < 0.05$) with increasing dietary sodium citrate. In all dietary sodium citrate treatments, muscle lipid peroxidation was significantly less ($p < 0.05$; $r^2 = 0.211$) indicating increased oxidative stability. While dietary sodium citrate was toxic to tilapia at the levels used, and is not recommended as a supplement, the decreased lipid peroxidation warrants further investigation with other species. Such research may have important implications for filet quality over prolonged storage.

Statement of relevance: Sodium citrate reduced growth but may increase shelf-life.

1. Introduction

Organic acids and their salts are increasingly being researched as dietary supplements in aquafeeds as a potential means to improve nutrient utilization, growth and/or disease resistance to aquatic animals (Lim et al., 2015). It is believed that this is largely due to their acidifying properties that can improve various parameters such as nutrient utilization, the gastrointestinal health of the host animal and/or digestive enzyme activity. While there are various organic acid types, perhaps the most studied as a dietary supplement to aquatic animals is citric acid and their salt (e.g. sodium citrate), which has been demonstrated to have good success with a variety of aquatic species (Sugiura et al., 1998; Khajepour and Hosseini, 2012; Sarker et al., 2012). However, research suggests that the effects of dietary organic acids are highly species-specific (Ng and Koh, 2011)

In an early study, it was shown that a supplementation of 5% citric acid or sodium citrate in fishmeal based diets of rainbow trout increased phosphorus, calcium, strontium, magnesium and manganese utilization, however citric acid tended to improve such mineral utilization over sodium citrate (Sugiura et al., 1998). It has since been demonstrated that dietary citric acid supplementations improved the growth and/or nutrient utilization to various species including carp (Baruah et al., 2007), red sea bream (*Pagrus major*) (Hossain et al., 2007), Beluga (*Huso huso*) (Khajepour and Hosseini, 2012), rainbow trout (Hernández et al., 2012), yellowtail, (*Seriola quinqueradiata*) (Sarker et al., 2012), red drum (*Sciaenops ocellatus*) (Castillo et al., 2014) and yellow catfish (*Pelteobagrus fulvidraco*) (Zhu et al., 2015). On the other hand, dietary citric acid led to decreased feed intake and no growth

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improvement to rainbow trout (Fauconneau, 1988) and recently Silva et al. (2015) found that dietary sodium citrate decreased hepatopancreatic digestive enzyme activity of white shrimp (*Litopenaeus vannamei*) while there was no growth benefit.

For tilapia, the use of dietary organic acids appears to be relatively successful. When dietary sodium diformate was supplemented at 0.3%, this significantly improved the protein efficiency ratio and retention to Nile tilapia fingerlings (Liebert et al., 2010). Meanwhile, the use of dietary potassium diformate (KDF) (between 1 to 9%) or an organic acid blend (at 2%) slightly, but not significantly, increased their growth (Ng et al., 2009; Zhou et al., 2009; Koh et al., 2016). The aim of the current study was to fed red hybrid tilapia (*Oreochromis* sp.) diets with increasing amounts of sodium citrate (0, 1, 2 and 4%) for 50 days and then were measured for growth, feeding efficiencies, body indices, muscle proximate composition, muscle cholesterol, muscle lipid peroxidation, some innate immunological responses and plasma parameters, intestinal short chain fatty acids (SCFA), and liver histopathology. The genetic background of red hybrid tilapia is somewhat unclear, but likely was a cross between *Oreochromis niloticus* and *Oreochromis mossambicus* yielding a distinct red coloration preferred by many Malaysians and now dominants aquaculture production in Malaysia (Ng et al., 2013).

2. Materials and methods

2.1. Experimental diets

A total of four isonitrogenous diets were formulated to contain different levels of sodium citrate (Sigma 71497) at 0% (control), 1%, 2% and 4%, at the expense of cellulose. Local fishmeal (13.57%) and soybean meal (54.87%), which were fined ground and sieved, were the main sources of protein while soybean oil (11.09%) was the main lipid source (Table 1). After thoroughly mixing the dry ingredients, soybean oil was then slowly added, followed by distilled water (20% of the ingredient weight), and mixed again for 30 min. This mash was then pelleted through a 1.0 mm diameter die and extruded through a single-screw extruder (Brabender KE19; Brabender GmbH, Germany). The three barrel temperatures were maintained at 60–100–120 °C and the die head temperature was set at 160 °C. The extruded pellets were then oven-dried at 55 °C overnight, kept in air-tight plastic bags and stored at −20 °C until use. The proximate composition, which was measured according to standard AOAC (1997) methods, was shown to be similar among treatments. Dietary pH was measured according to Romano et al. (2015), and was shown to significantly decrease with increasing sodium citrate levels ($p < 0.05$; $r^2 = 0.843$). Phytic acid was measured according to Roohani et al. (2012) and no significant relationship was shown with dietary phytic acid and sodium citrate levels ($p > 0.05$; $r^2 = 0.015$).

Table 1
Ingredient formulation and proximate composition (% dry matter) of the experimental diets with increasing concentrations of sodium citrate.

Ingredients	Experimental diets			
	Control	1%	2%	4%
Fishmeal[a]	13.57	13.57	13.57	13.57
Soybean meal[b]	54.87	54.87	54.87	54.87
Soybean oil[c]	11.09	11.09	11.09	11.09
Tapioca starch	8.50	8.50	8.50	8.50
Vitamin premix[d]	4.00	4.00	4.00	4.00
Mineral premix[e]	3.00	3.00	3.00	3.00
L-Methionine[f]	0.50	0.50	0.50	0.50
Sodium citrate[g]	0.00	1.00	2.00	4.00
α-Cellulose[h]	4.47	3.47	2.47	0.47
Proximate composition				
Dry matter	93.39	93.59	91.77	93.64
Crude protein	32.01	31.89	31.70	32.21
Crude lipid	9.51	9.95	9.46	9.62
Crude ash	9.90	9.32	9.21	9.47
Crude fiber	5.87	5.56	5.43	5.24
pH	6.05	5.73	5.38	5.22
Phytic acid[i]	0.84	1.45	1.06	0.96

[a] Local fishmeal (mixed species) (moisture, crude protein, lipid, ash of 16.5, 54.07, 4.93 and 23.2% on as is basis, respectively).
[b] Soybean meal (moisture, crude protein, lipid and ash of 10.79, 54.48, 1.75 and 6.75% on as is basis, respectively).
[c] Soybean oil was purchased from a local grocery store.
[d] The same composition as Kamarudin et al. (2012).
[e] The same composition as Kamarudin et al. (2012).
[f] L-Methionine (Sigma M9625).
[g] Sodium citrate (Sigma 71497).
[h] α-Cellulose (Sigma C8002).
[i] Expressed as mg/100 g.

2.2. Source of experimental animals and experimental design

Tilapia fingerlings (2–4 cm) were obtained from the Puchong Aquaculture Experimental Station, Universiti Putra Malaysia (UPM), and brought to Wet Laboratory, Department of Aquaculture, Faculty of Agriculture, UPM. The fish were acclimated in a 1000 L fiberglass tank and fed a commercial tilapia diet for one week. The fish were then randomly selected, individually weighed (0.01 g) and measured for length (0.1 cm) and a total of 20 fish (initial weight = 1.86 ± 0.01; mean ± SD) were placed in glass aquaria filled with 45 L. The fish were further acclimated in the aquaria for one week and fed the control diet. After one week, the fish were individually weighed and measured again, and the aquaria were randomly designated one of the four treatments to yield a total of three replicates in each treatment, and the fish were then fed their respective diets to satiation twice each day for 50 days.

In each aquarium, gentle aeration and individual pre-conditioned biofilters were used. However, each day a 20% water exchange was performed and once each week a 100% water exchange was performed. The ammonia and nitrite levels were tested once per week prior to the water exchanges from each aquarium using a commercial test kit (Aquarium Pharmaceuticals®) and never exceeded 0.5 mg L^{-1}. The dissolved oxygen and pH were also measured once per week using a digital probe and these ranged from 5.3–5.6 ppm and 7.6–7.9, respectively. The temperature was ambient (25–28 °C) and the water source was from the city mains and sodium thiosulfate was used to neutralize any residual chlorine.

After 50 days, the tilapia were mildly anesthetized with clove essential oil and the final length and weights were measured. The blood from the fish was then obtained from the caudal vein and the fish was dissected for further analysis.

2.3. Phagocytic activity, differential cell counts and plasma biochemistry

Three replicate fish from each treatment were quickly dissected for the spleen to measure the phagocytic index, which was carried out using a protocol modified from Perticarari et al. (1994). Briefly, spleen suspensions were co-cultured with fluoresceinated-yeast cells (*Saccharomyces cerevisiae*), in a ratio of 1 fish leukocyte:10 yeast cells in a 96-well microplate. The microplate was incubated at 28 °C for 60 min and the unbound cells were removed by washing with PBS. The adherent phagocytes were recovered using 0.1% trypsin in PBS. Spleen cell suspensions without any yeast cells served as negative control to set the background noise. The assay was acquired by flow cytometry with ten thousand events per sample to measure the percentage of spleen phagocytes that phagocytosed yeast cells. The mean fluorescence intensity (MFI), which was a measure of the number of yeast cells internalized per reactive phagocyte, was measured and expressed as the phagocytic index by multiplying the percentage phagocytic leukocyte with the MFI.

Blood smears from three replicate fish in each treatment were made onto glass slides. The air-dried blood smears were then fixed with absolute methanol for 1 min, followed by 3 min of incubation in a May–Grünwald solution (BDH Chemicals Ltd., England). Each slide