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Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*

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ABSTRACT

In aquaculture species, organic acids and their salts have been shown to trigger beneficial effects on mineral absorption, nutrient digestibility and growth performance by reducing the pH in the digestive tract through H^+ ion deposition. Therefore, the aim of this study was to determine if calcium lactate (CaLac), citric acid (CA) and potassium diformate (KDF) can improve growth performance of juvenile red drum (*Sciaenops ocellatus*) and if improved performance is in part due to an increase in digestive enzyme activities. A basal diet was formulated to contain 40% crude protein and six experimental diets were prepared by supplementing the basal diet with 1.5% or 3.0% CaLac, 0.75% or 1.5% CA and 0.75% or 1.5% KDF. One hundred eighty juvenile red drum were stocked in 110-L glass aquaria (20 fish/aquarium), and diets were fed to fish in triplicate aquaria at a rate approaching apparent satiation, twice daily, for 8 weeks in two separate trials. At the end of each feeding trial, growth performance indicators were obtained and four fish per aquarium were euthanized to measure pH of the digestive tract contents as well as to evaluate the digestive enzyme activities. The pH of the experimental diets was lowered by the addition of 0.75% CA (pH 5.63), 1.5% CA (pH 5.36) and 1.5% KDF (pH 5.79) when compared with the basal diet (pH 6.15). Moreover, the pH values of the stomach contents were correlated with the pH of the experimental diets. Additionally, based on the weight gain results, it seems that organic acids at a 1.5% dose can improve growth performance in juvenile red drum, although only the weight gain in the 1.5% CA treatment (1547%) was significantly higher than the basal treatment (1357%). This enhanced performance may be in part due to an increment in the activity of digestive enzymes, because pepsin activity, pancreatic enzyme activities (trypsin and lipase) and intestinal enzyme activities (leucin-aminopeptidase and phosphatases) were higher with the inclusion of organic acids in the diet.

1. Introduction

Given the state of the world's fisheries, where most of the fish stocks are fully exploited and, therefore, have no potential for increases in production, future demand for seafood and fisheries products can only be met by expanded aquacultural production. Such production will likely become more intensive and increasingly depend on nutritious prepared feeds to efficiently produce the cultured organism (FAO, 2012).

One of many strategies to improve fish health and performance in aquaculture is by including feed additives to produce functional feeds. Feed additives are non-nutritive ingredients that are included in diet formulations to influence physical and/or chemical properties of the feed for improving fish performance (NRC, 2011). Organic acids and their salts have been used as feed additives functioning as acidifiers of animal feeds. Such organic acids, including acetic, butyric, citric, formic, lactic, malic, propionic and sorbic acid have been shown to improve health and growth performance in livestock and poultry by altering the gastrointestinal tract function and energy metabolism, increasing the availability of nutrients and inhibiting the growth of pathogenic bacteria (Luckstadt, 2008).

In aquaculture species, organic acids have been shown to trigger beneficial effects on mineral absorption (Baruah et al., 2005; Hossain et al., 2007; Khajepour and Hosseini, 2012; Sugiura et al., 1998, 2001; Vielma and Lall, 1997), nutrient digestibility (Morken et al., 2011; Ringo et al., 1994) and, in some cases, growth performance (Cuvin-Aralar et al., 2011; Hossain et al., 2007; Khajepour and Hosseini, 2012; Ringo et al., 1994) by reducing the pH in the digestive tract through H^+ ion deposition. It is known that during food consumption, the concentration of hydrochloric acid in the stomach is reduced, and therefore the pH increases. This pH increment negatively affects the activation of pepsin and pancreatic enzyme secretions, decreasing the digestion capability. In pigs, providing acidifiers in the diet diminished this problem, such that positive effects of organic acids on digestive enzyme activities, hydrolysis of proteins, nitrogen retention and nutrient digestibility have been demonstrated (Kim et al., 2005; Suryanarayana et al., 2012).

The effects of organic acids on growth performance and metabolism have not been as extensively studied in fish as other terrestrial animals. However, based on promising results obtained by several authors with

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different fish species (Cuvin-Aralar et al., 2011; Hossain et al., 2007; Khajepour and Hosseini, 2012; Ringo et al., 1994; Trabizi et al., 2012), it is important to continue elucidating the effect of organic acids on fish performance; and to evaluate, as in this study, if improved performance is in part due to an increase in digestive enzyme activities, as has been found in pigs (Kim et al., 2005; Suryanarayana et al., 2012) but has not been evaluated in aquatic animals.

Therefore, the aim of this study was to determine if different organic acids or their salts, specifically calcium lactate (CaLac), citric acid (CA) and potassium diformate (KDF) can improve growth performance and digestive enzyme activities in juvenile red drum (*Sciaenops ocellatus*). Red drum is a euryhaline sciaenid that is native to the Gulf of Mexico and Atlantic Ocean and is considered a good model marine carnivore species for experiments in aquaculture nutrition. This fish historically supported commercial and recreational fisheries for many decades, however, commercial overfishing provoked a decline in natural populations. Since then, research efforts related to the aquacultural production of this species for enhancement of wild populations as well as for food production were accelerated (Gatlin, 2002).

2. Materials and methods

2.1. Diet formulation

The basal diet was formulated and analyzed to contain, on a dry-matter (DM) basis, 40% crude protein from menhaden fishmeal and cooked soybean meal, 10% total lipid primarily from menhaden fish oil, and dextrin as the soluble carbohydrate to provide approximately 3.1 kcal digestible energy g^{-1} diet. Six experimental diets were prepared by supplementing the basal diet with 1.5% or 3.0% calcium lactate (CaLac), 0.75% or 1.5% citric acid (CA) and 0.75% or 1.5% potassium diformate (KDF) in place of cellulose (Table 1).

2.2. Feeding trials

Two feeding trials (FT1 and FT2) were conducted sequentially in this study. Based on dietary levels of organic acids used in previous studies with fish, which range from 0.2% to 3.0% (Baruah et al., 2005; Gislason et al., 1996; Hossain et al., 2007; Khajepour and Hosseini, 2012; Ng et al., 2009; Trabizi et al., 2012), in FT1 two different dietary levels of CaLac supplementation (1.5% and 3.0%) were evaluated. Based on the growth performance results obtained in FT1, the lower inclusion level tended to support better growth performance. With this in mind, a second feeding trial (FT2) was conducted in order to evaluate the effects of a 1.5% inclusion level of CaLac and a 1.5% and 0.75% inclusion of CA and KDF to test additional organic acids and a broader range of supplementation. Moreover, in FT2, smaller fish were used so that higher weight gains (in the same time period), might be achieved and increase the potential to observe effects of the acidifiers tested.

FT1 and FT2 were conducted at the Texas A&M University Aquacultural Research and Teaching Facility in 110-L aquaria configured as a recirculating system (1 L min^{-1}) with biological/mechanical filtration to maintain adequate water quality. Salinity was maintained at 6–8 g L^{-1} by mixing synthetic seawater mixture (Fritz Industries) with well water. Low-pressure electrical blowers provided aeration via air stones to maintain dissolved oxygen levels near air saturation. Water temperature was maintained at (26.8 $\pm$ 0.5 °C) by controlling ambient temperature with dual air-conditioning units. A 12 h light–12 h dark photoperiod was maintained with fluorescent lights controlled by automatic timers.

Red drum juveniles with no visual signs of disease were selected (8.01 $\pm$ 0.29 g for FT1; 1.26 $\pm$ 0.01 g for FT2), and groups of 20 fish were stocked into each aquarium. Fish were subjected to a 1-week conditioning period during which the basal diet was fed to apparent satiation based on visual cues of the fish. After the conditioning period, triplicate aquaria were randomly assigned to each dietary treatment. The fish were fed close to satiation with pre-weighed rations based on growth and visual cues of the fish, twice daily (morning and evening) and 7 days per week. The feeding regime for FT1 was 5% of body weight during weeks 1–3, 4% of body weight during weeks 4–6, and 3% of body weight during weeks 7–8; the feeding regime for FT2 was 7% of body weight during weeks 1–2, 6% of body weight during weeks 3–4, 5% of body weight during weeks 5–6 and 4% of body weight during weeks 7–8. Fish in each aquarium were group-weighed every week and feed rations adjusted accordingly. Both feeding trials continued for a total of 8 weeks.

2.3. Growth performance and body condition indices

Four representative fish per aquarium were randomly sampled at the end of week 8 in both trials. Prior to all sample collection, fish were euthanized via tricainemethane sulphonate overdose. Whole-

Table 1
Formulation and proximate composition of the basal diet and organic acid-supplemented diets in FT1 and FT2.*

Ingredients	FT1			FT2					
	Basal diet	1.5% CaLac	3.0% CaLac	Basal diet	1.5% CaLac	0.75% KDF	1.5% KDF	0.75%CA	1.5% CA
Menhaden fishmeal[1]	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69
Soybean meal[2]	34.76	34.76	34.76	34.76	34.76	34.76	34.76	34.76	34.76
Dex. starch[3]	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Menhaden oil[1]	6.14	6.14	6.14	6.14	6.14	6.14	6.14	6.14	6.14
Vitamin premix[4]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[4]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Carboxymethylcellulose[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calcium lactate[5]	0.00	1.50	3.00	0.00	1.50	0.00	0.00	0.00	0.00
Potassium diformate[6]	0.00	0.00	0.00	0.00	0.00	0.75	1.50	0.00	0.00
Citric acid[5]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75	1.50
Alphacel[3]	5.41	3.91	2.41	5.41	3.91	4.66	3.91	4.66	3.91
Proximate composition (g/100 g DM)									
Crude protein	40.96	40.98	41.54	40.42	40.54	40.40	40.48	40.40	40.32
Crude lipid	10.99	11.57	11.53	8.26	8.33	8.28	8.54	8.17	8.14
Crude ash	11.07	11.56	12.56	10.62	10.73	10.64	11.24	10.44	10.36

* Values represented in g/100 g, dry weight.
[1] Special Select, Omega Protein Corporation.
[2] Rangen, Inc.
[3] USB Corporation.
[4] Same as Moon and Gatlin, 1991.
[5] Sigma-Aldrich.
[6] ADDCON Nordic AS.