

PERFORMANCE OF *Centropomus parallelus* FINGERLINGS FED A DIET SUPPLEMENTED WITH CITRATE AND ACETATE

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ABSTRACT

This study aimed to evaluate the addition of sodium acetate and sodium citrate in the diets of fingerling fat snook (*Centropomus parallelus*), through growth performance and modification of the intestinal microbiota. In the experiment, 3,000 fat snook fingerlings were used, with an initial average weight of 0.57 ± 0.10 g. The fish were stocked into 12 tanks (50 L) at a density of 5 fingerlings L-1 per tank distributed in three treatments with four replicates. The treatments were as follows: control group (without supplementation), one group supplemented with sodium acetate, and another group supplemented with sodium citrate. The diets supplemented with organic salts were sprayed with cod liver oil previously homogenized with 3% sodium acetate or sodium citrate. The fish supplemented with sodium acetate showed higher final weight, length, and yield, compared with the control group fish. The intestine of fingerling fat snook showed lower counts of total heterotrophic marine bacteria in the fish supplemented with acetate and citrate in relation to fish of the control group. Lower counts of sucrose non-fermenting vibrios were also observed in fish supplemented with acetate. Through this study, it was possible to evaluate for the first time the dietary inclusion of organic salts in the diets of marine fish in Brazil, with the dietary inclusion of 3% sodium acetate showing benefits for performance of fingerling fat snook.

Keywords: fat snook; organic salts; growth performance; vibrio

DESEMPENHO DE ALEVINOS DE *Centropomus parallelus* ALIMENTADOS COM DIETAS SUPLEMENTADAS COM CITRATO E ACETATO

RESUMO

Este estudo teve como objetivo avaliar a adição de acetato de sódio e citrato de sódio na dieta de alevinos robalo-peva (*Centropomus parallelus*), através do desempenho zootécnico e modificação da microbiota intestinal. No experimento, foram utilizados 3000 alevinos de robalo peva, com peso médio inicial de $0,57 \pm 0,10$ g. Os peixes foram estocados em 12 tanques (50 L) a uma densidade de cinco alevinos L-1 por tanque, distribuídos em três tratamentos com quatro repetições. Os tratamentos foram: grupo controle (sem suplementação), um grupo com suplementação dietética de acetato de sódio, e outro grupo suplementado com citrato de sódio. As dietas suplementadas com sais orgânicos foram pulverizadas com óleo de fígado de bacalhau previamente homogeneizadas com 3% de acetato de sódio ou citrato de sódio. Os peixes suplementados com acetato de sódio apresentaram maior peso final, comprimento e produtividade, em comparação com o peixe do grupo controle. Os intestinos dos alevinos de robalo apresentaram menores contagens de bactérias marinhas heterotróficas totais quando foram suplementados com acetato e citrato em relação aos peixes do grupo controle. Menores contagens de víbrios não fermentadores de sacarose também foram observadas nos peixes suplementados com acetato. Através deste estudo, foi possível avaliar pela primeira vez a inclusão na dieta de sais orgânicos para peixes marinhos brasileiros, com a inclusão na dieta de acetato de sódio 3% mostrando benefícios para o desempenho de robalo-peva.

Palavras-chave: robalo-peva; sais orgânicos; crescimento; víbrio

Artigo Científico: Recebido em 27/11/2014 - Aprovado em 27/10/2015

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Bol. Inst. Pesca, São Paulo, 42(1): 65-72, 2016

Doi: 10.5007/1678-2305.2016v42n1p65

INTRODUCTION

In recent decades, extractive fishing has severely reduced the fish stocks of marine ecosystems. An alternative to reduce fishing activity toward preserving the natural stocks of fish is fish farming (WARD and MYERS, 2005).

The snook genus *Centropomus*, with its present high commercial value, stands out for its refined quality of meat and resistance to adversity in culture (CERQUEIRA, 2002). The species *Centropomus parallelus*, popularly known as fat snook, consists of diadromous and euryhaline marine fishes that can be found in coastal areas, bays, estuaries and brackish lagoons, freshwater environments, and occasionally in hypersaline lagoons (CERVIGÓN *et al.*, 1992). They are carnivorous fish in the natural environment, feeding mainly on fish and crustaceans (CERQUEIRA, 2004).

As with other marine fish, the most critical step in fat snook production is the hatchery, because high mortality makes it difficult to obtain juveniles, and a major factor of mortality is bacterial disease (SOUZA *et al.*, 2010).

Several studies on the ecology (TONINI *et al.*, 2007), genetics (KIRSCHBAUM *et al.*, 2009), maturation and reproduction (ALVAREZ-LAJONCHEGRE *et al.*, 2002; FERRAZ *et al.*, 2002; CERQUEIRA *et al.*, 2005; TIBA *et al.*, 2009), hatchery (CERQUEIRA and BRÜGGER, 2001; TEMPLE *et al.*, 2004), management (OSTINI *et al.*, 2007; CORRÊA and CERQUEIRA 2007), and physiology (ROCHA *et al.*, 2005; TSUZUKI *et al.*, 2007) of fat snook have already been made, but few studies have been aimed at health. Only SOUZA *et al.* (2010) and BARBOSA *et al.* (2011) evaluated the use of probiotic bacteria to *C. parallelus*.

Antibiotics in aquaculture have traditionally been used as a prophylactic treatment. However, extensive use of antibiotics causes the selection of resistant bacterial strains and environmental pollution. Thus, there is a need to search for alternative feed additives that aid in the nutrition and health of cultivated aquatic animals (DEFOIRDT *et al.*, 2011). In this context, research with organic acids and their salts has been highlighted up (LÜCKSTÄDT, 2008; NG and KOH, 2011).

Organic acids and their salts affect the growth performance of aquatic animals through distinct mechanisms. In the diet, they act as preserving agents, inhibiting microbial growth and diminishing a possible intake of pathogenic organisms (LÜCKSTÄDT, 2007). In the intestinal tract, they inhibit the growth of pathogenic bacteria; they may modify intestinal microbiota (DEFOIRDT *et al.*, 2009; SCHRYVER *et al.*, 2010; MINE and BOOPATHY, 2011) and thereby increase survival (DEFOIRDT *et al.*, 2006; DEFOIRDT *et al.*, 2007; LIU *et al.*, 2010; NHAN *et al.*, 2010). In animal metabolism, organic acids can also affect the action of digestive enzymes; organic acids can serve as a source of energy through the citric acid cycle or the carboxylic acid cycle (LÜCKSTÄDT, 2008). In addition, organic acids might alter the availability and absorption of certain nutrients, such as phosphorus, lipids, and amino acids (BARUAH *et al.*, 2005, 2007; MORKEN *et al.*, 2011; KHAJEPOUR and HOSSEINI, 2012; SARKER *et al.*, 2012a, 2012b).

The current study aimed to evaluate the dietary inclusion of sodium acetate and sodium citrate for fat snook fingerlings (*C. parallelus*), through their effects on growth performance and intestinal bacteria counts.

MATERIALS AND METHODS

This work was performed at the Laboratório de Piscicultura Marinha (LAPMAR), Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil. The fat snook fingerlings were obtained through natural spawning by hormonal induction (CERQUEIRA, 2010), cultured for 69 days after hatching (DAH), to temperature and salinity averages of 23°C and 35‰, respectively.

In the experiment, 3,000 fat snook fingerlings were used, with an initial average weight of 0.57 ± 0.10 g and an average length of 3.65 ± 0.19 cm. After the initial biometry, the fingerlings were randomly distributed into 12 tanks of 50 L useful volume at a density of 5 fingerlings L⁻¹ with three treatments containing four replicates each. The fish were acclimated for one week. The water of the tanks was maintained under controlled temperature by thermostats and heaters (100 W),

constant aeration, and daily renewal of 65% total volume of each tank. The photoperiod during the experiment was 9 h light. Temperature, pH and dissolved oxygen, and weekly total ammonia were measured daily using methods described by APHA (1995).

The treatments were as follows: control group (without supplementation), one group supplemented with sodium acetate, and another group supplemented with sodium citrate. The diet used in the experiment was a commercial diet

containing 57% crude protein (INVE Aquaculture, U.S.A NRD 0.8/1.2 and NRD 1.2/2.0) (Table 1). The diets supplemented with organic salts were sprayed with cod liver oil (10 mL per kg of feed) previously homogenized with 30 g sodium acetate or sodium citrate. The final concentration of organic salts in the diets was 3%. The control diet also received the cod live oil without the addition of organic salts. The growth performance experiment lasted 30 days.

Table 1 - Proximal composition of the commercial diet (INVE) used in the experiment.

Nutrient	(%)
Crude Protein	57
Crude Fiber	1
Ether Extract	14.5
Ash	13
Misture	7
Calcium	1.7
Phosphorus	1.4
Energy (kcal kg ⁻¹)	4551

At the end of the trial performance parameters were calculated using the following formulas:

$$\text{Survival (\%)} = \frac{\text{number of final fish}}{\text{number of stocked fish}}$$

$$\text{Final weight (g)} = \frac{\text{final biomass(g)}}{\text{number of fish}}$$

$$\text{Feed conversion ratio} = \frac{\text{amount of feed(g)}}{\text{final biomass (g)}}$$

$$\text{Yield (kg m}^{-3}\text{)} = \frac{\text{final biomass (kg)}}{\text{tank volum (m}^3\text{)}}$$

$$\text{Condition factor (\%)} = 100 \times \frac{\text{Final weight (g)}}{\text{Final length}^3 \text{ (cm}^3\text{)}}$$

At the end of the experiment 10 fingerlings were randomly collected from each tank for microbiological analysis of gut. Fingerlings were washed with 70% alcohol to remove bacteria attached to the external body surface without affecting the internal bacteria. The fish guts were then excised with the use of forceps and scalpel, and were homogenized in sterile saline solution in 3% of NaCl, in a grail. The samples were then

serially diluted (1/10), seeded in Agar Marine culture medium (for total heterotrophic marine bacteria) and Agar Thiosulfate, Citrate, Bile and Sucrose (TCBS; selective medium for vibrios). After an incubation of 24 h at 26°C, total counts of colony forming units (CFU) were performed.

The bacteriological count data were transformed into log₁₀ (x+1). Data homoscedasticity was assessed by the test of Bartlett. The data were then subjected to unifactorial variance analysis. Where necessary, performance of medium separation was carried out by the test of Tukey. All the statistical tests were evaluated with a significance level of 5% (ZAR, 2010).

RESULTS

During the experimental period, the water of all tanks showed average values of pH 7.6 ± 0.1, dissolved oxygen 4.1 ± 0.6 mg L⁻¹, temperature 25.85 ± 0.35 °C, and total ammonia 0.07 ± 0.01 mg L⁻¹. These data did not differ among the different treatments.

Table 2 shows the observed results of zootechnical parameters. Fish supplemented with sodium acetate showed higher final weight, length, and yield, compared with the control group fish. The fish supplemented with sodium

citrate did not differ from the fish under other treatments. Survival, feed conversion ratio, and condition factor showed no significant differences between the fish of different groups.

Table 2 - Growth performance of fat snook (*Centropomus parallelus*) fed diets supplemented with 3% sodium acetate and 3% sodium citrate, and without supplementation.

Growth performance	Control	Acetate	Citrate
Survival (%)	96.4 ± 3.2	99.6 ± 0.5	98.8 ± 0.5
Final weight (g)	1.27 ± 0.24 ^b	1.72 ± 0.08 ^a	1.47 ± 0.20 ^{ab}
Final length (cm)	4.98 ± 0.29 ^b	5.62 ± 0.16 ^a	5.26 ± 0.25 ^{ab}
Feed conversion ratio	1.17 ± 0.46	0.72 ± 0.09	0.89 ± 0.15
Yield (kg m ⁻³)	6101 ± 983 ^b	8572 ± 393 ^a	7277 ± 966 ^{ab}
Condition factor (%)	1.02 ± 0.02	0.99 ± 0.03	1.00 ± 0.02

Different letters represent statistically significant differences ($p < 0.05$) between treatments on the Tukey test.

The intestine of fat snook fingerling showed lower counts of total heterotrophic marine bacteria (Marine Agar) in fish supplemented with acetate and citrate in relation to the fish of the control group. A lower count of green colonies on

TCBS agar, sucrose non-fermenting bacteria, was also observed in fish supplemented with sodium acetate compared with the fish supplemented with sodium citrate or the fish without supplementation (Table 3).

Table 3 - Bacteriological counts intestines of fingerling fat snook (*Centropomus parallelus*) supplemented with 3% sodium acetate and 3% sodium citrate, and without supplementation.

	Control	Acetate	Citrate
Agar Marine (log ₁₀ UFC g ⁻¹)	7.73 ± 0.88 ^a	5.49 ± 0.09 ^b	5.84 ± 0.29 ^b
Agar TCBS-YC (log ₁₀ UFC g ⁻¹)	4.83 ± 0.62	4.41 ± 0.49	3.46 ± 0.31
Agar TCBS-GC (log ₁₀ UFC g ⁻¹)	5.72 ± 0.27 ^a	2.61 ± 0.08 ^b	4.23 ± 0.49 ^a

YC - yellow colonies on TCBS Agar, sucrose-fermenting; GC - green colonies on TCBS Agar, sucrose non-fermenting. Different letters represent statistically significant differences ($p < 0.05$) between treatments on the Tukey test.

DISCUSSION

Following CERQUEIRA (2010), the water quality remained adequate throughout the experiment. All parameters were within those acceptable for fat snook cultivation.

The results of this study showed better growth performance of fish supplemented with organic salts, mainly sodium acetate. RINGO (1991) and RINGO *et al.* (1994) observed that dietary supplementation with 1% lactic acid improved the growth and feed conversion ratio of

salmon (*Salvelinu salpinus*). European sea bass (*Dicentrarchus labrax*) supplemented with 2% to 5% polyhydroxy-butyrate (PHB) also showed greater weight gain and conversion compared with fish without supplementation (SCHRYVER *et al.*, 2010).

Some studies with marine and diadromous fish report improvement in nutrient use after supplementation with organic acids or their salts. Rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with 1% sodium formate showed

improvements in the digestibility of lipid, protein, and ash (MORKEN *et al.*, 2011). Those supplemented with 1% formic acid showed improvement in the digestibility of phosphorus, magnesium, and calcium (VIELMA and LALL, 1997). Citric acid supplementation (1% to 3%) increased weight gain and retention of nitrogen and phosphorus, improving feed conversion ratio of red sea bream (*Pagrus major*) (SARKER *et al.*, 2005). Supplementation with 3% citric acid also improved mineral availability and retention of calcium and phosphorus in rohu (*Labeo rohita*) and European sturgeon (*Huso huso*) (BARUAH *et al.*, 2005, 2007; KHAJEPOUR and HOSSEINI, 2012).

Changes in the gut microbiota community, aimed at reducing the concentration of potentially pathogenic bacteria, constitute a major benefit from the addition of feed additive growth promoters used in aquaculture. Like in this study, dietary supplementation of a probiotic (*Lactobacillus plantarum*) decreased the concentration of total heterotrophic bacteria and *Vibrio* spp. in the fat snook gut (BARBOSA *et al.*, 2011). Studies with lactic acid bacteria have demonstrated their potential inhibition, especially against Gram-negative bacteria, due to the production of organic acids such as acetic acid and lactic acid (VAZQUEZ *et al.*, 2005).

Other studies on supplementation with organic acids or their salts in fish observed changes in intestinal microbiota. European sea bass (*D. labrax*) supplemented with 2% to 5% polyhydroxy-butyrate (PHB) showed changes in the gut bacterial community compared with non-supplemented fish, with the fish receiving supplementation obtaining better weight gain and feed conversion ratio (SCHRYVER *et al.*, 2010). Hybrid tilapia (*Oreochromis sp.*) fed a diet supplemented with 0.1% to 0.3% of a mixture of organic acids (not identified by the authors) showed a lower total count of bacteria in feces and intestinal tract (NG *et al.*, 2009). *Macrobrachium rosenbergii* larvae fed brine shrimp enriched with PHB decreased total bacteria and *Vibriosis*, besides presenting better development (NHAN *et al.*, 2010). Juvenile *Litopenaeus vannamei* supplemented with 0.4% to 2% of a commercial inorganic acid, based on calcium sulfate, also showed changes in the intestinal microbiota (ANUTA *et al.*, 2010).

In the present study, besides the number of

total bacteria, sodium acetate reduced sucrose non-fermenting *Vibriosis*. Species that are part of this group are *V. metschnikovii*, *V. parahaemolyticus*, *V. mimicus*, *V. hollisae*, *V. vulnificus*, and *V. damsela* (MAUGERI *et al.*, 2000), this last subsequently identified as *Photobacterium damsela* (OSORIO *et al.*, 2000). Many of these are potential pathogens for marine fish (LABELLA *et al.*, 2011; LOPEZ *et al.*, 2012; THUY *et al.*, 2013).

CONCLUSION

Through the results of this study, it was possible to evaluate for the first time dietary supplementation of organic salts for Brazilian native marine fish, where the inclusion of 3% sodium acetate to fat snook fingerlings (*Centropomus parallelus*) increased the final weight, final length, and yield, and decreased the total heterotrophic bacteria and sucrose non-fermenting *vibrionaceas* in the gut.

ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Ministério da Pesca e Aquicultura (MPA), and Financiadora de Estudos e Projetos (FINEP/RECARCINA), for their financial support.

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