

**O butirato de sódio melhora o desempenho de juvenis de tilápia, *Oreochromis niloticus*
(Linneaus, 1758)**

**Sodium butyrate improves the performance of juvenile tilapia, *Oreochromis niloticus*
(Linneaus, 1758)**

**El butirato de sodio mejora el rendimiento de la tilapia juvenil, *Oreochromis niloticus*
(Linneaus, 1758)**

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Resumo

Com o objetivo de avaliar o efeito do butirato de sódio sobre o desempenho de juvenis de tilápia foram utilizados 400 peixes ($28,55 \pm 1,55$ g), distribuídos casualmente em 20 tanques (250 L) interligados num sistema de circulação fechada de água. Durante 55 dias foram testados cinco níveis de butirato de sódio (0, 0,5, 1,0, 1,5 e 2,0 g kg⁻¹) sendo cada unidade experimental representada por 20 peixes. Para avaliação dos tratamentos foram analisados o desempenho zootécnico, o índice hepatossomático, a relação viscerossomático, a composição centesimal dos peixes, a hematologia e a histologia do intestino. Foram observadas diferenças significativas com comportamento quadrático para o ganho de peso dos peixes e o fator de crescimento específico, sendo 1,21 g kg⁻¹ o melhor resultado encontrado para o ganho de peso, enquanto para o fator de crescimento foi encontrado 1,13 g kg⁻¹ do ácido orgânico. A conversão alimentar e a relação viscerossomático apresentaram comportamento linear, reduzindo os valores em relação ao aumento do nível de butirato de sódio. Na análise hematológica os níveis de triglicérides apresentaram diferença significativa para o tratamento com 0,5 g kg⁻¹, que foi 190,75 mg dl⁻¹. Na análise histológica dos intestinos e composição centesimal dos peixes não houve diferença significativa entre os tratamentos. Os dados avaliados demonstram ação efetiva do butirato de sódio com o melhor resultado para o desempenho dos peixes no nível de 1,21 g kg⁻¹.

Palavras-chave: Ácido orgânico; Hematologia; Histologia intestinal; Promotor de crescimento.

Abstract

In order to evaluate the effect of sodium butyrate on the performance of juvenile tilapia 400 fish (28.55 ± 1.55 g) were used, distributed randomly in 20 tanks (250 L) connected in a closed water circulation system. For 55 days, five levels of sodium butyrate (0, 0.5, 1.0, 1.5 and 2.0 g kg⁻¹) were tested, with each experimental unit represented by 20 fish. To evaluate the treatments, the zootechnical performance, the hepatosomatic index, the viscerosomatic relationship, the centesimal composition of the fish, the hematology and the histology of the intestine were analyzed. Significant differences were observed with quadratic behavior for fish weight gain and specific growth factor, with 1.21 g kg⁻¹ being the best result found for weight gain, while for growth factor 1.13 g kg⁻¹ of organic acid. The feed conversion and the viscerosomatic relationship showed linear behavior, reducing the values in relation to the increase in the level of sodium butyrate. In the hematological analysis, triglyceride levels showed a significant difference for the treatment with 0.5 g kg⁻¹, which was 190.75 mg dl⁻¹.

In the histological analysis of the intestines and fish composition, there was no significant difference between treatments. The evaluated data demonstrate an effective action of sodium butyrate with the best result for fish performance at the level of 1.21 g kg⁻¹.

Keywords: Growth promoter; Hematology; Intestinal histology; Organic acid.

Resumen

Para evaluar el efecto del butirato de sodio en el rendimiento de tilapia juvenil se utilizaron 400 peces (28.55 ± 1.55 g), distribuidos aleatoriamente en 20 tanques (250 L) conectados en un sistema cerrado de circulación de agua. Durante 55 días se probaron cinco niveles de butirato de sodio (0, 0.5, 1.0, 1.5 y 2.0 g kg⁻¹), representando cada unidad experimental 20 peces. Para evaluar los tratamientos se analizó el desempeño zootécnico, el índice hepatosomático, la relación viscerosomática, la composición centesimal de los peces, la hematología e histología del intestino. Se observaron diferencias significativas con el comportamiento cuadrático para la ganancia de peso de los peces y el factor de crecimiento específico, siendo 1.21 g kg⁻¹ el mejor resultado encontrado para la ganancia de peso, mientras que para el factor de crecimiento 1.13 g kg⁻¹ de ácido orgánico. La conversión alimenticia y la relación viscerosomática mostraron un comportamiento lineal, reduciendo los valores en relación al aumento del nivel de butirato de sodio. En el análisis hematológico, los niveles de triglicéridos mostraron una diferencia significativa para el tratamiento con 0.5 g kg⁻¹, que fue 190.75 mg dl⁻¹. En el análisis histológico de los intestinos y la composición del pescado, no hubo diferencia significativa entre tratamientos. Los datos evaluados demuestran una acción eficaz del butirato de sodio con el mejor resultado para el rendimiento de los peces al nivel de 1.21 g kg⁻¹.

Palabras clave: Ácido orgânico; Hematología; Histología intestinal; Promotor de crecimiento.

1. Introduction

High density and feeding rates in aquaculture production systems, result in a decrease in the water quality of the crop, stress, and greater susceptibility to infectious diseases (Longhi, et al., 2012). Sanitary control has the purpose of improving production performance, getting reductions in mortality and in the decrease in zootechnical performance, besides bringing less risk to consumer health (Figueiredo & Leal, 2008).

According to Kowalska, et al. (2010), lesions in the intestines of fish can cause inflammation, and some changes in the intestinal villi. In this sense, Portz (2006) describes that to improve the health status of fish, the need of balanced diets improves productivity, better response to stress, and resistance to pathogens. According to Mountzouris, et al. (2006), strategic nutritional alternatives are needed to improve productivity.

Antibiotics treat bacterial incidence, and if not used correctly, are harmful to aquaculture production and to the increased risk of problems in humans (Hernadéz-Serrano, 2005). Considering that organic acids influence gram-negative bacteria (Lückstädt, 2008), the commercial use of these compounds in fish diets is advisable, as are medium-chain acids, in disease control and in improving growth performance, acting as growth promoters (Wing-Keong, et al., 2009).

Knowledge of sanity in the production of aquatic organisms with the development of technologies may help in the greater success of the activity (Tavares-Dias, et al., 2006). Butyric acid has a beneficial effect on the intestine (Galfi & Bokori, 1990), and influencing body weight gain (Kotunia, et al., 2004), thus the use of sodium butyrate in the diet of juveniles of tilapia as a growth promoter.

2. Methodology

The study was conducted at Laboratory of Fish Nutrition (AQUANUT) of the State University of Santa Cruz, Ilhéus-BA. Twenty tanks (250 L), were used, mounted in a closed water recirculation system with biological filter, and aeration using a blower. Four hundred juveniles of tilapia GIFT, *Oreochromis niloticus*, (28.55 ± 1.55 g), were stored in tanks, at density of 25 fish per tank, for 55 days. The experimental design was completely randomized, performed with four treatments and a control group, and four repetitions. The diets were isoenergetic and isoproteic, differing only in the concentration of sodium butyrate, which was exempt from the control group, and of 0.5, 1.0, 1.5 and 2.0 g kg⁻¹ for the other treatments, these dosages being based on the authors Wing-Keong Ng, et al., 2009 and Lim, , et al. 2010. The sodium butyrate used was Adimix Pure, from Nutriad Nutrição Animal Ltda., and 98% - concentration.

Every day the water quality parameters, as dissolved oxygen, temperature and pH were monitored with a digital multi-parameter model YSI Pro Plus. Every week the ammonia (NH₃) was measured, using a bench-top photo colorimeter (HANNA, model HI83203). To

maintain the water quality, the tanks were siphoned twice a week, to remove feed and feces waste. The filtration system also was daily drained to remove solid particles.

The fish were adapted for 15 days, fed with extruded commercial feed, with 2 mm diameter, containing 400 g kg⁻¹ of crude protein, 80 g kg⁻¹ of ether extract, 60 g kg⁻¹ of crude fiber, and 100 g kg⁻¹ of moisture. Fish were fed three times a day (8:00 am, 12:00 pm and 4:00 pm) until apparently satiety.

The experiment diets (Table 1) were formulated with the SUPER CRAC® program, and with the nutritional recommendations of Furuya, et al. (2012), being isoproteic and isoenergetic, with 368.10 g kg⁻¹ of crude protein and 4266.40 kcal kg⁻¹ of crude energy. The diets had average values of ether extract of 87.01 g kg⁻¹, humidity of 60.80 g kg⁻¹ and ash of 91.62 g kg⁻¹.

Table 1. Composition of experimental diets for juvenile tilapia, with addition of different levels of sodium butyrate.

| Ingredients (g kg ⁻¹) | Inclusion levels of sodium butyrate (g kg ⁻¹) | | | | |
|---------------------------------------|---|-----------------|-----------------|-----------------|-----------------|
| | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
| Soybean meal 45% | 310.00 | 310.00 | 310.00 | 310.00 | 310.00 |
| Corn meal | 200.00 | 200.00 | 200.00 | 200.00 | 200.00 |
| Fish meal (55%) ¹ | 145.00 | 145.00 | 145.00 | 145.00 | 145.00 |
| Wheat bran | 124.00 | 124.00 | 124.00 | 124.00 | 124.00 |
| Maize starch | 83.00 | 83.00 | 83.00 | 83.00 | 83.00 |
| Meat and bone meal (45%) ¹ | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 |
| Corn gluten (60%) | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| Soy oil | 19.00 | 19.00 | 19.00 | 19.00 | 19.00 |
| Premix ² | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| Antioxidant ³ (BHT) | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Common salt | 3.00 | 2.50 | 2.00 | 1.50 | 1.00 |
| Sodium butyrate | 0.00 | 0.50 | 1.00 | 1.50 | 2.00 |
| Total | 1.000.00 | 1.000.00 | 1.000.00 | 1.000.00 | 1.000.00 |

¹Percentage of crude protein. ²Mineral vitamin premix (Composition/ kg of product): vit. A = 6,000,000 IU; vit. D3 = 2,250,000 IU; vit. E = 75,000mg; vit. K3 = 3,000mg; vit. Thiamine = 5,000mg; riboflavin = 10,000mg; vit. pyrodoxin = 8,000mg; biotin = 2,000mg; vit. C = 192,500 mg; niacin = 30,000mg; folic acid = 3,000mg; Fe = 100,000mg; Cu = 600mg; Mn = 60,000mg; Zn = 150,000mg; I = 4,500mg; Cu = 15,000mg; Co = 2,000mg; If = 400mg. ³BHT = Butylhydroxy-toluene. Source: Authors.

In the composition of the diet it is possible to observe the inclusion of sodium butyrate according to its treatment, and after the formulation the diets were processed by extrusion. The diets were processed in the Laboratory of Fish Nutrition - AQUANUT, in a pelleted form with a diameter of 2 mm. The ingredients were ground in a knife mill, with a maximum granulometry of 1 mm and subsequently mixed with the addition of soy oil and humidification with water at 35° C, and pelleted. After the production process, the feed pellets

were dried in a forced ventilation oven at 50° C. Then the diets were kept at a temperature of approximately -10 °C.

During the experiment, fish were fed three times a day until apparently satiety at 8:00, 12:00 and 16:00 hours. At the end of the experiment, the fish fasted for 24-hour, and subsequently weighed and counted for biometrics. After obtaining the biometrics, the feed consumption and the calculations quantify the performance parameters, which were the weight gain, apparent feed conversion and specific growth rate.

The blood, liver and intestine collection were from one fish of each experimental unit. The fish, liver and total viscera were weighted to the hepatosomatic and viscerosomatic index register. Fish were weight in a BL3200H (Shimadzu) scale. Hematological analyzes were performed at the Hematology Laboratory of the Veterinary Department of UESC. Blood samples were collected by puncturing the fish's caudal vein. Reflotron Plus equipment (Roche) was used to read the collected samples.

For histological analysis, to remove fragments from the anterior and middle portions of the intestine, the specimens were dissected, and fragments fixed in Bouin's solution (TIMM, 2005) for 24 hours and after that period transferred to 70% alcohol, followed by all routine techniques up to paraffin embedding. Sequentially, the samples were cut, using a microtome, into 6 µm thick sections and stained with Hematoxylin and Eosin (H-E). The slides were photographed with the aid of a digital camera coupled to an optical microscope and then analyzed and described. A comparison of the height of the intestinal villi of fish submitted to experimental diets containing different levels of organic acid, and measurements were made using the Image-Pro Plus program (Media Cybernetics - Version 6.2). The slide preparation and reading procedures occurred at the Histopathology Laboratory, Department of Veterinary Medicine, UESC.

A second group of fish (one per experimental unit) was lyophilized for later quantification of the proximate composition. The bromatological analyzes of the rations and samples of the fish in the experiment were carried out at the Animal Nutrition Laboratory of the Department of Agricultural and Environmental Sciences at UESC, all according to the procedures described by AOAC (2000), being they crude protein, crude energy, ether extract, dry matter and ash (942.05).

The results went through to analysis of variance and for performance evaluation, and regression analysis, and for the metabolic parameters, Tukey's test was applied, considering the 5% probability among the results of the sample means (R Development Core Team, 2008).

3. Results and Discussion

The physical-chemical parameters of water quality, dissolved oxygen saturation of $41.30 \pm 11.31\%$, temperature of 27.98 ± 0.69 °C, pH of 5.97 ± 0.29 , showed little variation, and the average level of ammonia (NH₃) was 2.80 ± 1.15 mg L⁻¹.

The productive performance of the fish had a quadratic effect for weight gain and specific growth rate (Table 2).

Table 2. Performance of juvenile tilapia fed with different levels of sodium butyrate in the diet.

| | Dietary sodium butyrate levels (g kg ⁻¹) | | | | | CV | p value |
|---------------------------|--|-----------|------------|-----------|-----------|-------|---------|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | | |
| Weight gain (g)** | 81.1±6.02 | 88.8±4.83 | 102.0±5.48 | 90.6±9.08 | 85.1±2.72 | 6.69 | 0.0003 |
| Food conversion* | 1.10±0.02 | 1.05±0.03 | 1.08±0.02 | 1.04±0.02 | 1.01±0.01 | 2.20 | 0.0001 |
| Survival (%) | 98.75 | 96.25 | 100.00 | 100.00 | 98.75 | 3.76 | ns |
| Hepatosomatic index | 1.7±0.75 | 1.9±0.35 | 2.4±1.72 | 2.2±1.79 | 2.0±0.52 | 13.94 | ns |
| Viscerosomatic index (%)* | 11.0±2.41 | 10.2±2.04 | 10.7±2.76 | 10.9±4.18 | 8.8±2.55 | 9.19 | 0.0316 |
| Specific growth rate** | 1.9±0.05 | 2.1±0.05 | 2.2±0.06 | 2.2±0.05 | 2.0±0.05 | 9.52 | 0.0209 |

Efeito Linear*. Food conversion, $y=-0.037x+1.091$; Viscerosomatic index, $y=-0.70x+11.1$, $R^2=0.95$. Efeito Quadrático**. Weight gain, $y=-14.52x^2+31.01x+80.30$, $R^2=0.94$; Specific Growth Rate, $y=-0.28x^2+0.63+1.90$, $R^2=0.81$, $R^2=0.90$. ns – no significance. Source: Authors.

The weight gain and the specific growth rate of fish have an optimal level of 1.075 g kg⁻¹ and 1.13 g kg⁻¹, respectively, estimated through the equation. The food conversion and the viscerosomatic index have a linearly effect and decreased with the increase in including sodium butyrate in the diet.

The weight gain of tilapia treated with 1.21 g kg⁻¹ of sodium butyrate was 20.4% higher than fish not treated with the additive. Robles (2013) recorded a 5% increase in weight gain for *Sparus aurata*, using 3 g kg⁻¹ of sodium butyrate in the diet. According to the author, besides the function of growth promoter, sodium butyrate also improves the intestinal tract. Luz, et al. (2019), feeding pirarucus with sodium butyrate describe the dose of 1.17 g kg⁻¹ as the best results in productive performance. In juvenile grass carp (*Ctenopharyngodon idellus*), the use of 2 g kg⁻¹ of sodium butyrate in the diet improves productive performance (Liu, et al., 2016).

Wing-Keong, et al. (2009) describes an improvement in the productive performance of tilapia after feeding different organic acids at levels between 1.0 and 2.0 g kg⁻¹ in the diet. In

this reference there was no use of sodium butyrate, however the results showed the same trend and dosages evaluated in our study with tilapia. Romano, et al. (2014), report the importance of organic acids, as growth promoters, and also a tool for the control of pathogens. Lim, et al. (2010) evaluated sodium butyrate levels from 0 to 2.0 g kg⁻¹ in diets for *Clarias gariepinus* catfish, improving weight gain. The author reports the gradual increase in gram-positive bacteria for increasing levels of sodium butyrate in these diets.

In the hematological analysis of fish, glucose and cholesterol did not show differences in their levels among treatments (Table 3). Tilapia treated with inclusion of 0.5 g kg⁻¹ had higher (p <0.05) triglyceride levels (190.7±2.06 mg dL⁻¹) when compared to the control group (128.6±2.47 mg dL⁻¹).

Table 3. Mean values ± SE of hematological analyzes of juvenile tilapia fed different levels of sodium butyrate in the diet.

| | Dietary sodium butyrate levels (g kg ⁻¹) | | | | | CV | p value |
|--------------------------------------|--|-------------------------|--------------------------|--------------------------|--------------------------|-------|---------|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | | |
| Glucose (mg dL ⁻¹) | 73.3±0.5 | 88.9±3.61 | 73.7±1.62 | 76.3±0.82 | 118.8±6.53 | 36.72 | ns |
| Cholesterol (mg dL ⁻¹) | 134.5±1.06 | 129.5±1.27 | 135.0±0.51 | 143.7±0.89 | 130.7±1.33 | 8.97 | ns |
| Triglyceride (mg dL ⁻¹)* | 128.6±2.47 ^b | 190.7±2.06 ^a | 179.0±1.69 ^{ab} | 170.2±1.73 ^{ab} | 184.0±2.16 ^{ab} | 16.12 | 0.05 |

CV: coefficient of variation; ns: no significance. * Significant 5% probability (p <0.05). Source: Authors.

The plasma glucose of the tilapia in this experiment had values between 73.3 to 118.8 mg dL⁻¹, values higher than those described by Azevedo, et al. (2006) for tilapia that averaged between 29 ± 10 mg dL⁻¹ to 32 ± 8 mg dL⁻¹. Ogunji, et al. (2007), describe values between 37.07 to 43.73 mg dL⁻¹. In behavioral assessment of fish, Corrêa, et al. (2003) describe the glucose levels of isolated fish (60.02 to 67.85 mg dL⁻¹) and mated fish (110.44 to 136.26 mg dL⁻¹), and the difference in these levels may be stress during the mating of specimens. Regarding triglycerides, the authors observed in isolated animals with levels from 167.87 to 185.68 mg dL⁻¹, and in mating fish values between 210.85 to 221.82 mg dL⁻¹. Ahmed & Sadek (2015) describe a significant increase in glucose values in tilapia after feeding with diets that include sodium butyrate.

Cholesterol levels of tilapia juvenile ranged from 129.5 to 143.75 mg dL⁻¹, with no changes because of the addition of sodium butyrate. Hrubec, et al. (2009) observed similar cholesterol

values, in hybrids of the genus *Oreochromis*, in high culture densities, with average levels of 189 mg dL⁻¹ and in low density average levels of 156 mg dL⁻¹, suggesting a link with the system of cultivation.

In this study, the butyric acid enhanced the triglyceride levels (190.75 mg dL⁻¹) in tilapia fed when compared to animals that did not receive the additive (128.60 mg dL⁻¹). Analyzing the effects of different dietary protein and starch levels on plasma and liver components of Nile tilapia, Wang, et al. (2017) observed triglyceride values between 47.56 to 85.95 mg dL⁻¹. Metwally (2009) observed a difference in triglyceride levels in tilapia after feeding with Cu or Zn, with levels of 142.83 mg dL⁻¹ and 109.31 mg dL⁻¹, respectively.

Histological analyzes of the anterior and middle portions of the tilapia intestine, fed with different levels of sodium butyrate, showed morphometric characterization without significant difference. The intestines of the fish showed no structural differences among treatments, and no intestinal crypt, confirming that the enterocytes originate from the proliferation of undifferentiated cells at the base of the villi, as described by Jobling (1995) and Sena, et al. (2012). Luz, et al. (2019) observed an increase in intestinal villi of pirarucu after feeding with sodium butyrate. Tilapia during the period of sexual reversion and fed with sodium butyrate had a longer length, perimeter and villus area of the anterior region of the intestinal tract (Jesus, et al., 2019). These controversial data may be because of the use of different species, or different doses, or production systems.

The chemical composition of fish (bromatology) for dry matter, crude protein, ethereal extract and ashes did not differ ($p > 0.05$) among treatments (Table 4), which shows that butyrate has no influence on the body composition of tilapia.

Table 4. Mean values of the body chemical composition of tilapia juvenile fed with different levels of sodium butyrate in the diet.

| Variável | Dietary sodium butyrate levels (g kg ⁻¹) | | | | | CV | p value |
|-------------------------------------|--|-------|-------|-------|-------|-------|---------|
| | 0.0 | 0.5 | 1.0 | 1.5 | 2.0 | | |
| Dry matter (g kg ⁻¹) | 275.7 | 278.0 | 287.9 | 291.3 | 275.4 | 3.27 | ns |
| Crude protein (g kg ⁻¹) | 632.6 | 604.4 | 605.8 | 625.6 | 618.8 | 6.72 | ns |
| Crude energy (MJ Kg ⁻¹) | 256.8 | 276.2 | 267.8 | 284.0 | 255.5 | 11.52 | ns |
| Ashes (g kg ⁻¹) | 173.1 | 145.1 | 182.7 | 179.4 | 171.7 | 147.7 | ns |

CV: coefficient of variation; ns: no significance. * Significant 5% probability ($p < 0.05$). Source: Authors.

4. Final Considerations

There were no differences between the intestinal portions and the chemical composition of the tilapia juvenile, however the productive performance was effective by sodium butyrate inclusion in diet. The data showed ideal levels of sodium butyrate for better weight gain and specific growth rate at the level of 1.21 g kg⁻¹. The increased level of sodium butyrate directly influenced the low feed conversion and decrease the viscerosomatic index.

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