

Effect of dietary supplementation of sodium butyrate on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*) fries

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Present study was to evaluate the effect of different levels of sodium butyrate as a feed additive on growth performance, feed utilization and body biochemical composition of *Oreochromis niloticus* fries. Five diets containing supplementation at levels of 0, 0.5, 1, 2 and 3% were fed to fries of Nile tilapia (1 g) at a daily rate of 20 % of their body weight during the first month, reduced to 15% in the last two weeks. Growth performance parameters increased significantly ($P < 0.05$) with applying dietary sodium butyrate. The highest growth was obtained at 2% sodium butyrate level and the lowest feed conversion ratio was obtained at 2% and 3% levels. No effect was detected on the body composition by supplementing of sodium butyrate.

[Key words:-feed additive, sodium butyrate, feed utilization, growth performance, *Oreochromis niloticus*]

Introduction

Feed additives are substances that are generally added in trace amounts to benefit growth rate and health of the animal¹. Some of the feed additives that are used in improving fish growth performance are chemical products particularly hormones and antibiotics that may result in unfavorable side effects². Organic acids are constituents of animal and plant tissue, thus they are widely distributed in nature³. Organic acids are plentiful in extracts of fish tissues and other aquatic organisms⁴. More than 30 years ago, several studies had shown that organic acids or their salts (Na, K or Ca) are capable of influencing animal performance positively when added to diets⁵. They provide energy to aquatic organisms, improve the digestibility of the dietary nutrients such as phosphorus and nitrogen, and inhibit pathogens in the intestinal tract⁶. Some organic acids are added to pellets for the improvement of feed utilization and/or for storage effects^{7, 8}. Several studies demonstrated that some organic acids retard spoilage when added to aquafeed⁹. A number of studies on cold-water and tropical fish species showed that several organic acids and their salts can improve disease resistance, growth and feed utilization in fish¹⁰. Also, It was documented that organic acids and their salts can reduce fish stress and improve immune response and consequently the mortality rate¹¹. Some authors indicated that adding

sodium diformate or calcium formate to the diet increased the weight gain, protein retention and improved the digestibility in the Nile tilapia fingerlings^{12, 13, 14}. On the contrary, some other studies indicated that organic acids may not significantly affect fish performance. A study proved that sodium formate gave a non-significant improvement in the performance of Arctic charr¹⁵. Moreover, other study found that the survival rates of Arctic charr juveniles were not significantly affected by the addition of dietary lactate¹⁶. Meanwhile, the addition of sodium lactate to the diet of Atlantic salmon juveniles did not show prolonged effect¹⁷. Additionally, a blend of sodium formate and butyrate did not improve the feed utilization or growth rate of rainbow trout and reported that an increase in the weight of the intestine and a reduction in the digestibility of amino acids and macro nutrient took place when this blend was added to plant protein-based diets before extrusion¹⁸. Butyric acid and butyrates provide the energy needed for epithelial growth, since the renewal of the human intestinal epithelium requires high energy¹⁹. In addition, butyric acid and butyrates influenced cellular functions related to human gut health²⁰. Furthermore, sodium butyrate slightly increased the growth and reduced FCR in catfish when added to the fishmeal diet while no improvement occurred in the catfish received the

butyrate together with defatted soya²¹. Tilapia is the most important farmed fish not only in Egypt but also in many regions worldwide as it is considered the second produced species in the world^{22, 23, 24}. However, they may be subjected to stress and disease infection due to high stocking density and artificial feed. Based on the significant effects of organic acids on fish performance, the present study has been carried out to investigate the effects of a commercial organic acid salt (sodium butyrate) on growth and feed utilization of Nile tilapia fries.

Materials and Methods

Nile tilapia fries (0.1 g average weight) were obtained from a private Fish Hatchery (Kafr El-Sheikh), Egypt. Fish were transported to Aquaculture Experimental Station of the National Institute of Oceanography and Fisheries (NIOF), Alexandria Governorate, Egypt. The fish were acclimatized to the experimental conditions for two weeks before the start of the experiment and were fed the control diet. Then, duplicate groups of the fish were stocked in 16 glass aquaria (70 L each) at a density of 10 fish per aquarium. Prior to the initiation of the feeding trial, the diet was gradually replaced with the test diets prepared for the experiment. Aquaria were cleaned daily and faeces were siphoned before the daily first feeding. Siphoned water was replaced by aerated de-chlorinated water from the storage tank. Each aquarium was also provided with aeration through air pump. Temperature was adjusted by electric heaters. Five isonitrogenous (40% crude protein) and isocaloric (480 Kcal/100 g) diets were formulated and prepared as shown in Table 1.

Diets contained five levels of sodium butyrate (SB) (0, 0.5, 1, 2, or 3%; designated as: control (SB0), SB0.5, SB1, SB2 and SB3) as a feed additive. Each diet was processed by blending dry ingredients, mechanically mixed, pressure pelleted by using meat mincer, air dried at room temperature, sieved to obtain appropriate size and stored in the deep freezer at -20 °C. The fish were fed manually three times a day, 7 days a week, for 45 days at a daily rate of 20 % of their body weight during the first month, reduced to 15% in the last two weeks. Fish in each aquarium were counted and collectively weighed every 2 weeks throughout the feeding trials and the amounts of feed consumed readjusted accordingly.

Total amount of feed consumed by the fish in each aquarium, during the study period was determined, and average amount for each individual fish was

calculated. At the end of the experiment, fish in each aquarium were collected, counted and weighed collectively. Then, fish were oven-dried at 60 °C for 24 hours, grinded by electric grinder, and stored in labelled plastic bags and frozen at -20°C for final body composition analysis. Experimental diets and fish samples were analysed for dry matter and ash content according to²⁵, crude protein (%N x 6.25) by the Kjeldahl method and total lipid was determined using the Soxhlet apparatus. Crude fiber content was determined in the defatted sample and the gross energy (GE) content of the diets was estimated according to²⁶. Nitrogen free extract (NFE) was calculated based on the following equation:

$$\text{NFE} = 100 - [\% \text{Ash} + \% \text{Ether Extract} + \% \text{Protein} + \% \text{Fiber}]$$

Growth rates and feed utilization efficiency were calculated using the following equations:

- Percent weight gain (%WG) = $100 [(W2-W1)/W1]$.
- Specific growth rate (%SGR) = $100 [(\ln W2 - \ln W1)/T]$.

Where, W1 = initial weight (g), W2 = final weight (g) and T = time of experiment (days).

Table 1—The formulation (percentage dry weight) of the test diets used in the experiment.

Ingredients (%)	Control	SB0.5	SB1	SB2	SB3
Fishmeal	52.0	52.0	52.0	52.0	52.0
Corn flour	39.0	38.5	38.0	37.0	36.0
Plant oil (sunflower)	4.0	4.0	4.0	4.0	4.0
Vitamin & mineral premix ¹	3.0	3.0	3.0	3.0	3.0
CMC	2.0	2.0	2.0	2.0	2.0
Sodium butyrate	0.0	0.5	1.0	2.0	3.0
Total	100.0	100.0	100.0	100.0	100.0
Chemical composition (%)					
Dry matter	89.00	97.46	89.87	89.50	92.80
Crude protein	41.27	42.14	41.72	41.34	40.87
Ether extract	9.20	9.23	9.67	9.85	10.25
Crude fiber	2.92	3.09	3.22	3.46	2.95
Ash	8.73	8.35	8.26	8.37	8.18
NFE	37.88	37.19	37.58	36.98	37.75
GE	479.70	482.00	485.42	482.50	487.00

¹Vitamin and mineral premix (per kg of mixture) contains: 15000 IU vitamin A, 01500 IU vitamin D3, 2.0 mg vitamin E, 2 mg vitamin K3, 2.5 mg vitamin B2, 10 mg vitamin B3, 3 mg vitamin B6, 2 mg vitamin B1, 5 mg vitamin B12, 5.5 mg pantothenic acid, 1 mg niacin, 2 mg folic acid, 100 mg choline, 4 g copper, 300 mg iodine, 30 g iron, 60 g manganese, 50 g zinc, 100 mg selenium, 855.5 g calcium carbonate.

Table 2—Growth rates and Feed utilization efficiency (mean $\pm$ SE) of Nile tilapia *O. niloticus* fry fed different concentrations of sodium butyrate

Growth Parameters	% Sodium butyrate				
	0.0	0.5	1.0	2.0	3.0
FW ¹	0.77 $\pm$ 0.01 ^b	0.84 $\pm$ 0.01 ^{ab}	0.80 $\pm$ 0.10 ^{ab}	1.10 $\pm$ 0.20 ^a	0.95 $\pm$ 0.02 ^a
WG ²	0.67 $\pm$ 0.01 ^b	0.74 $\pm$ 0.01 ^{ab}	0.70 $\pm$ 0.10 ^{ab}	1.00 $\pm$ 0.20 ^a	0.85 $\pm$ 0.02 ^a
%WG ³	670.00 $\pm$ 10.00 ^b	735.00 $\pm$ 5.00 ^{ab}	700.00 $\pm$ 100.00 ^{ab}	1000 $\pm$ 170 ^a	850 $\pm$ 20 ^a
SGR ⁴	4.54 $\pm$ 0.03 ^b	4.72 $\pm$ 0.02 ^{ab}	4.60 $\pm$ 0.30 ^{ab}	5.31 $\pm$ 0.35 ^a	5.02 $\pm$ 0.10 ^{ab}
SR ⁵	86.65 $\pm$ 6.65 ^b	100.00 $\pm$ 0.00 ^a	100.00 $\pm$ 0.00 ^a	96.65 $\pm$ 3.35 ^a	100.00 $\pm$ 0.00 ^a
FI ⁶	1.90 $\pm$ 0.10 ^a	2.10 $\pm$ 1.10 ^a	1.90 $\pm$ 0.20 ^a	2.40 $\pm$ 0.30 ^a	2.00 $\pm$ 0.00 ^a
FCR ⁷	2.84 $\pm$ 0.09 ^b	2.84 $\pm$ 0.20 ^b	2.71 $\pm$ 0.15 ^{ab}	2.40 $\pm$ 0.15 ^a	2.35 $\pm$ 0.05 ^a
PER ⁸	0.86 $\pm$ 0.03 ^b	0.84 $\pm$ 0.10 ^b	0.91 $\pm$ 0.05 ^{ab}	1.03 $\pm$ 0.10 ^a	1.05 $\pm$ 0.02 ^a
PPV ⁹	12.74 $\pm$ 0.71 ^b	12.80 $\pm$ 0.70 ^b	13.00 $\pm$ 0.50 ^b	15.40 $\pm$ 1.00 ^a	16.12 $\pm$ 0.90 ^a

Values in the same raw with different superscripts are significantly different ($P < 0.06$). ¹FW=final weight (g/fish), ²WG=weight gain (g/fish), ³%WG=percent weight gain, ⁴SGR=specific growth rate (%/day), ⁵SR=survival rate (%), ⁶FI=feed intake (g), ⁷FCR=feed conversion ratio, ⁸PER= protein efficiency ratio and ⁹PPV= protein production value (%).

- Feed conversion ratio (FCR) = Dry feed intake (g) / Fish live weight gain (g).
- Protein efficiency ratio (PER) = Fish weight gain (g) / Protein intake (g).
- Protein productive value (PPV) = 100 [Protein gain (g) / Protein intake (g)].

Results of fish growth rates, feed utilization efficiency and body composition of fish in both experiments were subjected to one-way analysis of variance (ANOVA) to test the effect of sodium butyrate on fish performance. Data were analyzed using SPSS, version 15.0. Differences between means were compared using Duncan multiple range test at $P < 0.05$ ²⁷.

Results

The results of growth rates of Nile tilapia fry fed the test diets are summarized in Table 2. Statistical analyses using one-way analyses of variance (ANOVA) and Duncan demonstrated that there were significant differences ($P < 0.06$) between growth rates indices of the fish in the different treatments. The best FW, WG, % WG, SGR, FCR, PER and PPV were shown in fish fed 2 or 3% sodium butyrate in their diet, followed by the fish fed 1 or 0.5% sodium butyrate. Fish fed the control diet had the lowest SGR and the lowest survival rate (86.65%). The best FCR was shown in fish fed 2% and 3% sodium butyrate then increased in fish fed 1% sodium butyrate to be 2.71 followed by 2.84 in tilapia received 0.5 and 0% sodium butyrate in the diet. FCR became the worst in tilapia fed the control sodium butyrate-free diet and 0.5% sodium butyrate. The data in Table 3 show that Nile tilapia treated with different levels of sodium butyrate presented no significant differences in the body composition compared to those of the control group.

Table 3—Body composition (mean $\pm$ SE) in dry weight basis of Nile tilapia *O. niloticus* fry fed different concentrations of sodium butyrate

% Sodium butyrate	Dry matter (%)	Ether extract (%)	Protein (%)	Ash (%)
0 (control)	24.90 $\pm$ 0.50 ^a	30.10 $\pm$ 0.60 ^a	61.60 $\pm$ 0.20 ^a	11.60 $\pm$ 0.20 ^a
0.5	24.60 $\pm$ 0.10 ^a	30.80 $\pm$ 0.80 ^a	62.70 $\pm$ 0.31 ^a	10.90 $\pm$ 0.60 ^a
1.0	25.00 $\pm$ 0.20 ^a	30.70 $\pm$ 1.70 ^a	57.50 $\pm$ 1.50 ^a	11.00 $\pm$ 0.90 ^a
2.0	25.00 $\pm$ 1.20 ^a	31.60 $\pm$ 0.20 ^a	59.80 $\pm$ 2.60 ^a	10.90 $\pm$ 0.20 ^a
3.0	26.00 $\pm$ 0.20 ^a	32.90 $\pm$ 0.60 ^a	58.40 $\pm$ 2.90 ^a	9.80 $\pm$ 0.40 ^a
Initial	28.64	23.00	58.67	17.83

Values in the same column with different superscripts are significantly different ($P > 0.05$).

Discussion

There has been increasing interest in the possible use of feed additives in aquaculture as they have demonstrated their ability to increase the growth rate and welfare of cultured aquatic animal^{28, 29, 30, 31, 32}.

The results of the present study indicated that the final weight (FW), weight gain (WG), percent weight gain (% WG) and specific growth rate (SGR) of Nile tilapia fries were improved with increasing dietary sodium butyrate up to 2%. Further increase in sodium butyrate to 3% did not result in any further improvement in growth parameters. These results agreed with previous studies carried out on the use of organic acids as feed additives for Nile tilapia^{33, 34}. On the contrary, A specific study found that dietary organic acids (0.1, 0.2, 0.3% novel organic acid blend and 0.2% potassium diformate) did not significantly improve growth rate of red tilapia³⁵. The authors reported that this result might be due to challenging the fish with *Vibrio anguillarum* on day 10 of the 85-day feeding trial and the growth was measured basing on the surviving, disease-stressed tilapia.

The highest survival rates (96.7 to 100%) were obtained in Nile tilapia fed sodium butyrate diets. Nile tilapia fed the control diet had the lowest survival rate. This effect may be attributed to the ability of organic acids and salts to detoxify the potentially harmful compounds in feeds, improve health, thus mortality decreased. The commercial mixtures of organic acids are used to control pathogenic bacteria in feed ingredients and processed feeds of terrestrial livestock³⁵. Meanwhile, other study reported that the inclusion of potassium diformate (0.3, 0.6, 0.9 and 1.2%) in diets of hybrid tilapia had no significant effect on survival rate³³. These results might be attributed to good water quality and lack of pathogenic infection throughout the experiments.

The effect of dietary organic acids and salts on fish performance is controversial, depending on fish species and size, type and concentration of acid/salt used, and culture conditions. For example, dietary organic acids and their salts have been reported to improve feed utilization efficiency of a number of farmed fish species.

The results of the present study indicated that the feed utilization efficiencies of fish were significantly affected by concentrations of sodium butyrate in the diets ($P < 0.06$), in which feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) were better in fish fed 2 and 3% sodium butyrate in their diet compared to the other concentrations. This may be due to the ability of sodium butyrate to improve digestion and mineral utilization. On the contrary of the above results, other study showed that the inclusion of the organic salt potassium diformate (0.3 and 0.6%) in diets of hybrid tilapia did not improve feed conversion ratio (FCR)³³.

Respecting to another fish species, the inclusion of citric acid in the diet of red sea bream showed significantly better FCR³⁶. Authors attributed that to the increase of phosphorous and nitrogen retention in fish fed these diets which lowered their excretion leading to better FCR. Similar results were reported by³⁷ who found that the inclusion of phosphorous and citric acid in the sea bream diets improved FCR. The authors referred this effect to the presence of citric acid which improved digestibility by lowering pH in the intestines, which resulted in a higher dissociation of mineral compounds, reducing rate of gastric emptying, and formation of easily absorbed chelated mineral complexes.

In support, some researchers found that the combination of 0.015% monosodium phosphate,

0.55% phytase and 0.22% citric acid had improved FCR and PER of common carp compared to the control diet³⁸. The authors attributed that to the ability of citric acid to reduce the pH in intestine and enhance mineral utilization, in addition to, enhancement of phytase activity which improved feed and nutrient utilization. The inclusion of 3% citric acid in diets decreased FCR of the common carp, presumably due to the high feed intake³⁴. Therefore, that study suggested that presence of citric acid in the diet of common carp might have overcome this problem and improved feed digestion³⁴.

The present study indicated that body composition (dry mater, ether extract, protein and ash contents) of Nile tilapia fed different concentrations of sodium butyrate were not significantly affected by dietary treatments. ($P > 0.05$) The results of the present study are in agreement with that of³⁶ who found no significant difference in the body composition of sea bream fed citric acid and those fed a control diet. ³⁵found also that dietary organic acids supplementation did not significantly affect body composition of hybrid tilapia.

Conclusion

In conclusion, this study indicates that the addition of sodium butyrate had a noticeable effect on tilapia growth performance and nutrient utilization. Moreover, it appears from the foregoing discussion that the effects of the sodium butyrate on growth rates and feed parameters are more significant than on body composition or structure. Thus the effect of the organic acids or salts may due to the developmental stages of the fish, the type of organic acids/ blends, feed processing techniques and culture condition may also affect fish performance.

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