Effects of Nano-sustained Release Sodium Butyrate on Growth and Intestinal Cell Proliferation of Grass Carps

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Abstract Taking grass carps with the initial weight of about 20 g as the research object, the basic feeds of grass carps were added with 0.0%, 0.1%, 0.3%, 0.6%, 0.8%, and 1% of nano-sustained release sodium butyrate to prepare 6 types of experimental feeds with equal nitrogen and energy. The effects of different concentrations of nano-sustained release sodium butyrate were surveyed on growth and intestinal cell proliferation of grass carps. The experiment was carried out in cages with 50 carps per cage, and each treatment was repeated 3 times for 60 days. Experimental results indicated that the addition of nano-sustained release sodium butyrate significantly promoted the growth of grass carps and significantly increased the ratio of intestinal villus to crypt depth. When the addition of nano-sustained release sodium butyrate was 0.6%, the weight increase rate, specific growth rate, fullness and intestinal villus height of grass carps were the highest, which was significantly higher than that of the control group ($P < 0.05$). The study results indicated that addition of appropriate amount of nano-sustained release sodium butyrate can promote the growth of grass carps through increasing the intestinal villus height, and the suitable addition dosage was 0.6%.

Key words Nano-sustained release sodium butyrate, Grass carps, Intestinal cell proliferation, Growth

1 Introduction

Fish meal is an indispensable high quality protein source in aquatic feed. With the development of animal husbandry, the demand is increasing. At the same time, the production of fish meal declines year by year due to a variety of factors, and prices have gone up, which inevitably increases the raw material cost of feeds. In order to solve the problem of feed costs and ensure balanced nutrition, feed factories often use cheap vegetable protein sources as alternative protein for fish meal, such as soybean meal, dregs of rapeseeds after being extracted, and cottonseed meal and so on. The replacement of fish meal by plant protein source not only can save feed cost and reduce the cost of aquaculture, but also can protect fishery resources and alleviate the shortage of fish meal supply. Therefore, the plant protein source is a better substitute for fish meal. However, after feeding the aquatic animals with feeds containing plant protein, their physiological state and physiological indicators will change, and excessive addition may even lead to death, mainly because the source of plant protein damages intestinal villus and leads to intestinal injury of aquatic animals. If not cured in time, the intestinal injury will bring about a series of intestinal diseases. In serious case, it may lead to death of animals. In addition, the plant protein source of feed is low in digestion and absorption rate, resulting in a serious waste of feed and low animal growth rate. From the perspective of water nutrition and pollution, large amount of unabsorbed protein is excreted from the body, resulting in high ammonia in water, water eutrophication, and degradation of water quality, which are unfavorable for growth of aquatic animals. Studies have shown that sodium butyrate can improve small intestinal shape, promote intestinal cell proliferation and maturation, and keep normal form of intestinal epithelial cells, so as to promote the digestion and absorption of the small intestine[1-4]. The active composition of sodium butyrate is butyric acid (short chain volatile fatty acid). In order to avoid its free, volatile and special smell shortcomings in the feed processing and storage, usually the sodium butyrate is treated to become coated product. According to extensive studies, the addition of sodium butyrate in piglets and chickens feed can increase food intake and weight gain, reduce feed coefficient, regulate the intestinal micro-ecosystem and improve the immune function[5-9]. However, there are few studies about application of sodium butyrate in aquatic animal nutrition. Only Zhang Songlin *et al.*[10] reported that the addition of 1.0‰ sodium butyrate in the feed can significantly promote the growth of American eel (*nguilla rostrata*), 37% higher than the control group, and Zheng Ruigeng *et al.*[11] found that sodium butyrate increased the weight of tilapia (*Oreochromis niloticus*) and carp (*Cyprinuscarpio*), but its action mechanism of nutrition has not been reported. In this study, adding the nano-sustained release sodium butyrate in feeds of grass carps, we discussed the effects on growth performance and intestinal growth of grass carp, in the hope of providing theoretical reference for application of sodium butyrate in feeds of grass carps and the action mechanism.

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2 Materials and methods

2.1 Experimental feed With the reference to the nutritional parameters of grass carps and commercial feed formulations, we added 6.0 g nano-sustained release sodium butyrate per kilogram

feed. We crushed raw materials of feed using a 40 mesh sieve, and screened through the SLX-80 soft particle feeder to screen 3.0 mm diameter hard particles, naturally air-dried, we obtained the nano-sustained release sodium butyrate feed for grass carps.

2.2 Animal feeding and management We put 50 grass carps in each cage, and each carp had initial weight of about 20 g and uniform size. We fed them for 60 days, repeated three times per treatment. We took the group without addition of sodium butyrate as the control group. We bought coated sodium butyrate and took the addition of 0.6% coated sodium butyrate as treatment 1 group, respectively, and the nano-sustained release sodium butyrate with addition of 0.6% as treatment 3 group. We fed them at 8:30, 12:30, and 16:30 for three times, increased the feeding amount with the increase in carp weight. In the first week, the feeding amount was 5% of the carp weight. Later, it increased by 1% per week. We fed them under natural conditions. Every day, we made a record of weather, water temperature, feeding amount and feeding conditions.

2.3 Sample collection, analysis, and calculation After completion of the culture, we measured the performance indicators of the grass carps, and mainly calculated the average weight, weight gain, survival rate and specific growth rate of the grass carps. Later, we randomly selected six grass carps in each cage and calculated the fullness.

We randomly selected 6 fishes and separated the intestine and washed with physiological saline. After weighing, we fixed the midintestine in 4% paraformaldehyde solution and then dehydrated according to the routine tissue sectioning procedure, embedded with paraffin, sectioned and HE stained. We observed with the aid of microscope, put them in 40 times magnified section, randomly selected 10 pieces of intestinal villus, measuring villi height and adjacent crypt depth, and calculate the villi height / crypt depth.

2.4 Data statistics and analysis With the aid of Imagepro plus software, we analyzed intestinal section images. All experimental data were expressed as mean value ± standard deviation ($\bar{x} \pm SD$). Using Excel and SPSS 17.0 software, we analyzed and made statistics of the data. On the basis of single factor variance analysis (ANOVA), we compared the difference between groups using Duncan multiple comparison method ($P < 0.05$).

3 Results and analyses

3.1 Effects of nano-sustained release sodium butyrate on growth performance of grass carps From Table 1, we can see that there are significant changes in the weight gain, specific growth rate and fullness of the control group and the experimental group ($P < 0.05$), and 0.6% nano-sustained release sodium butyrate had the best effect. According to the preliminary analysis, we concluded that 0.6% nano-sustained release sodium butyrate has optimum effect on the growth performance of grass carps.

Table 1 Growth performance of grass carps

Item \ Group	Control group	Treatment 2 (market available coated sodium butyrate)	Treatment 3 Nano-sustained release sodium butyrate (0.6%)
Initial weight // g	18.85 ± 1.11	18.00 ± 0.52	19.35 ± 0.10
Final weight // g	39.67 ± 3.66^a	45.40 ± 7.55^b	58.96 ± 19.08^c
Weight gain // %	110.84 ± 20.74^a	153.07 ± 46.78^b	204.52 ± 97.79^c
Survival rate // %	95 ± 2.30^a	96 ± 1.89^a	98 ± 2.13^b
Specific growth rate // % / d	1.24 ± 0.05^a	1.54 ± 0.03^b	1.86 ± 0.06^c
Fullness // g / cm %	0.16 ± 0.07^a	0.21 ± 0.12^b	0.22 ± 0.07^b

Table 2 Intestinal mucosal shape of grass carps

Item \ Group	1 (Control group)	2 (market available coated sodium butyrate)	3 (Nano-sustained release sodium butyrate 0.6%)
Intestinal villi height // μm	1150.95 ± 317.19^a	1220.93 ± 160.36^b	1857.15 ± 175.05^c
Crypt depth // μm	128.66 ± 45.78^a	109.78 ± 26.42^b	96.97 ± 50.88^c
Intestinal villi height / crypt depth	8.95 ± 1.38^a	11.12 ± 2.63^b	19.16 ± 1.16^c

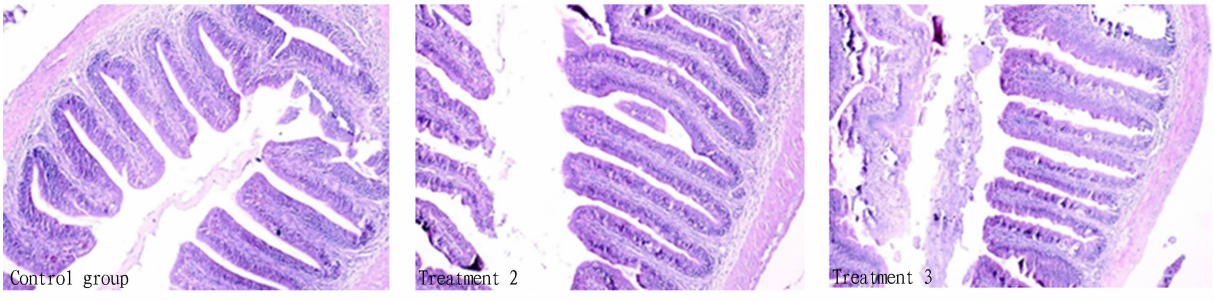

Fig. 1 Intestinal mucosal shape of grass carps

3.2 Effects of nano-sustained release sodium butyrate on intestinal mucosal shape of grass carps The relative length of intestine and relative quality of intestine of grass carps were not significantly different between treatment groups, but the nano-sustained release sodium butyrate significantly affected the intestinal villus height and crypt depth of grass carps. When the addition amount of nano-sustained release sodium butyrate was 0.6% in feeds of grass carps, the intestinal villi height of the grass carp was significantly higher than that of the control group.

From the above, compared with the control group and the market available sodium butyrate, the outdoor cage culture of grass carps fed with 6% nano-sustained release sodium butyrate had better culture effect, higher weight gain rate, and higher villus proliferation.

4 Discussions

There have been extensive studies about the function of sodium butyrate in promoting the growth of livestock and poultry. Piva *et al.* [5] found that the addition of 0.08% sodium butyrate to the feeds of piglets for two weeks could significantly increase the piglet weight by 20% and the daily feed intake by16% ($P<0.05$). Zou Yang *et al.* [6] found that coated sodium butyrate significantly reduced the feed coefficient of 22 – 42 days old broilers. In aquatic animals, Zhang Songlin *et al.* [10] concluded that addition of 1.0% sodium butyrate in feed could promote the growth of eels, which was 37% higher than that of the control group. Zheng Ruigeng *et al.* [11] found that sodium butyrate had excellent effect on increasing the weight of tilapias and carps. In this experiment, the results showed that the addition of a certain amount of nano-sustained release sodium butyrate in feed had function of promoting the growth of grass carps, similar to the studies mentioned above. However, higher concentration of sodium butyrate may not bring better result. In the experimental group added with high concentration of sodium butyrate, the growth effect is not satisfactory, possibly because it inhibits intestinal development of grass carps and reduces digestion and absorption of nutrients. Specific reasons are to be further studied. This also indicated that sodium butyrate has moderate dosage principle in promoting the growth of grass carps. This experiment was carried out in cages, the fat and protein levels of feed conform to the nutritional needs of grass carps. However, the growth rate of grass carps is relatively slow, possibly because of high density in cages, as well as the algae proliferation during the culture process affects the permeability, resulting in slower growth of grass carps. In order to avoid the special smell of sodium butyrate, increase the product mobility, reduce the loss rate, slow down the release rate in the digestive tract of animals, the nano-sustained release sodium butyrate will have higher application value in the production of aquatic feeds.

Through the proliferation and differentiation, intestinal cells promote the growth of intestinal villi and development of the entire intestinal tract. Mucosal epithelial cells and intestinal villi are important functional tissues of the intestine. They participate in intestinal digestion and absorption of nutrients and stress response, and they are closely related with the secretion function of intestine. Studies have shown that the sodium butyrate, as a fast-energy source of intestinal cells[12], can promote regeneration of intestinal cells and stimulate damage recovery of intestinal villi. This experimental indicated that the addition of nano-sustained release sodium butyrate significantly promoted the growth of grass carps and significantly increased the ratio of intestinal villus to crypt depth. When the addition of nano-sustained release sodium butyrate was 0.6%, the weight increase rate, specific growth rate, fullness and intestinal villus height of grass carps were the highest, which was significantly higher than that of the control group ($P<0.05$). The study results indicated that addition of appropriate amount of nano-sustained release sodium butyrate can promote the growth of grass carps through increasing the intestinal villus height, and the suitable addition dosage was 0.6%. The action mechanism of nano-sustained release sodium butyrate is to be further studied.

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