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Effects of dietary sodium acetate on food intake, weight gain, intestinal digestive enzyme activities, energy metabolism and gut microbiota in cultured fish: zebrafish as a model

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Abstract

Acetate is the most abundant short-chain fatty acids (SCFAs) in the gastrointestinal tract of fish. The aim of this study was to investigate the effects of dietary sodium acetate (NaAc) on food intake, weight gain, intestinal digestive enzyme activities, energy metabolism and gut microbiota of zebrafish (*Danio rerio*). In this study, 1-month-old zebrafish were fed with 0.15% NaAc diet for 4 weeks under satiation feeding. At the end of feeding trial, the level of intestinal acetate in the fish fed with 0.15% NaAc containing diet were significantly increased ($p < 0.05$), which was accompanied by a higher weight gain and daily feeding rate compared with the control group ($p < 0.05$). Dietary supplementation of 0.15% NaAc significantly promoted the body fat mass, energy gain and energy conversion efficiency, as well as intestinal digestive enzyme (amylase) activity in fish ($p < 0.05$). The expression of food intake-related genes (*ghre*, *npy* and *npy7R*) in the brain of fish fed on the diet contacting 0.15% NaAc was higher than that of the control group. Consistently, intracerebroventricular injection of NaAc in the brain of zebrafish also increased the expression of genes related to food intake. The effects of NaAc on orexigenic genes expression in the brain of zebrafish could be prevented by co-injection of atropine, suggesting that acetate played a direct role in controlling appetite through the parasympathetic nerve system. Moreover, there was significantly decrease ($p < 0.05$) in the relative abundance of *Plesiomonas* genus in the fish gut fed on the diet supplemented with NaAc. These data suggest that NaAc has a potential value as fish feed additive in aquaculture.

Key words: Acetate; food intake; metabolism; gut microbiota; zebrafish

1. Introduction

Short chain fatty acids (SCFAs) is one of the major metabolite products of the gut microbiota which plays significant role in several biological processes including regulation of growth, feed conversion, gut microbiota function, intestinal mucosal barrier, immune function as well as energy metabolism (Canfora and Blaak 2017; Canfora et al., 2015; den Besten et al., 2013). Acetate is one of the major SCFAs produced by gut microbiota in fish species (Liu et al., 2019), including, Nile tilapia (*Oreochromis niloticus*) (Minoru and Takashi, 1997), *Odax pullus*, *Aplodactylus arctidens*, *Kyphosus sydneyanus* and *Hermosilla azurea* (Morin et al., 2005; Pat et al., 2006) as well as the classic vertebrate model zebrafish (*Danio rerio*) (Zhang et al., 2019).

Several studies demonstrated the potential benefits of sodium acetate in many species of aquatic animals. Recent study has indicated that addition of certain concentration (100 mmol/L or 200 mmol/L) of sodium acetate alleviated the intestinal inflammation induced by high-carbohydrate mainly though inhibiting the MAPK and NF-κB signaling pathways in Nile tilapia (Li et al., 2019). Nile tilapia fed on diets supplemented with 0.5% of calcium lactate + sodium acetate blend (OSB), increased in protein and ash content of fish body composition; moreover, diet supplemented with 1% of OSB enhanced their growth, feed utilization, and health status in Nile tilapia (Magdy et al., 2017). However, Ebrahimi et al. (2017) showed that dietary supplementation of sodium acetate showed no effect on growth, feeding efficiencies, or muscle fatty acid composition, and significantly decreased intestinal SCFAs in red hybrid tilapia (*Oreochromis* sp.). The organic acid salt of SCFAs have been considered to be potential feed additives. Recent studies have shown that use of sodium butyrate as a feed additive contributes to the restoration of intestinal homeostasis and disease resilience in a marine teleost gilthead sea bream (*Sparus aurata*) (Estensoro et al., 2016; Piazzon et al., 2017). Thus far, however, there is no unified opinion on the potential impact of NaAc on fish.

The neuroendocrine regulation of food intake has been well known in multiple fish families (e.g., cyprinidae, gadidae and salmonidae) (Helene, 2016; Soengas et al., 2018; Volkoff, 2016). Major appetite regulators, known as putative orexigenic and anorexigenic factors, are widely expressed both in the brain and gastrointestinal tract of fish (Rønnestad et al., 2017; Volkoff, 2014). The genes differentially expressed in fish brain have been involved in feeding behavior, lipid metabolism and appetite regulation (Schartl et al., 2019). It has been demonstrated that acetate can act as signaling molecules via gut-brain neural circuits in mammals (Perry et al., 2016). However, the above studies in farmed fish species have not explored this function mechanism of acetate. So we want to know if acetate plays a role in the neural regulatory system in fish.

Moreover, gut microbiota play an important role in the regulation of nutrition and disease resistance in fish (Butt and Volkoff, 2019). Production of acetate by gut microbiota through fermentation of indigestible carbohydrates have been reported in several fish species including zebrafish (Zhang et al., 2019), carp (*Cyprinus carpio* L.) (Kihara and Sakata, 2002), tilapia (Minoru and Takashi, 1997) and *Kyphosus sydneyanus* (Moran et al., 2005). However, addition of exogenous acetate (in the form of sodium acetate) on the effect of gut microbiota in cultured fish is unknown.

Since evaluation of diets in aquaculture species is costly and requires time consuming trials (Ulloa et al., 2014). Currently the use of zebrafish as nutritional model for aquaculture has been increasing. Moreover, due to the whole genome sequenced which permit to use new technologies to study the molecular mechanisms of the host's response to the diets (Ribas and Piferrer, 2014; Ulloa et al., 2014). In this study, zebrafish was used as the model to investigate the effects of dietary NaAc on food intake, weight gain, intestinal digestive enzyme activities, energy metabolism and gut microbiota in cultured fish.

2. Materials and Methods

2.1 Animals and treatments

All experimental and animal care procedures were conducted in agreement with protocols approved by the Feed Research Institute of the Chinese Academy of Agricultural Sciences Animal Care Committee, under the auspices of the China Council for Animal Care (assurance No. 2017-AF-FRI-CAAS-001). Adult zebrafish (1-month-old) were purchased from China Zebrafish Resource Center (CZRC), and housed in a recirculating system with controlled temperature (28℃) and light conditions (12 h light–dark cycle). The fish were randomly assigned to 2-L tanks in a recirculating system with stocking density of 18 fish per tank. During the feeding period, the rearing temperature was 25–28℃, the dissolved oxygen was > 6.0 mg oxygen L^{-1}, the pH was 7.0–7.2, the nitrogen content in water may include ammonium (as NH_4^+) was < 0.50 mg nitrogen L^{-1}, and nitrogen-containing anion (as NO_2^-) was < 0.02 mg nitrogen L^{-1}.

Zebrafish were fed on different diets containing different doses of NaAc (0, 0.05%, 0.1%, 0.15% and 0.2%) for 4 weeks. Three replicate tanks were randomly assigned per treatment group. Zebrafish (66.0 ± 0.7 mg) were fed diets to apparent satiation each time, twice a day (9:00, 16:00). The formulation of basal diet and the experimental diets as well as their chemical compositions were shown in Supplemental Table 1.

2.2 Growth measurements

To evaluate the effects of NaAc on survival rate, food intake, weight gain and feed efficiency, zebrafish from each group were weighed and calculated according to previous reports (Guo et al., 2017). Survival rate (%) =100 × final survival individuals/initial individuals, Daily feeding rate (%/d) = 100 × total feed consumed/[days × (IBW + FBW)/2], Weight gain (WG) = [100 × (final body weight − initial body weight)/initial body weight], and Feed efficiency = [(final body weight − initial body weight)/feed intake].

2.3 Body proximate composition analysis

After 4 weeks feeding trial, zebrafish undergone starvation for 24 h, and then were sacrificed to analysis body proximate composition as previously described (Thiex et al., 2003; Guo et al., 2017). Total protein of whole fish body was determined by a semi-automatic Kjeldahl System. The ash content was determined by high temperature burning method. The moisture content was determined by oven drying method. And the crude fat content was determined using the Soxtec method.

2.4 Intestinal digestive enzyme activities

The activities of intestinal digestive enzymes were detected according to the instructions of ELISA assay kits for testing protease, lipase and amylase of zebrafish (Shanghai Enzyme-linked Biotechnology Co., Ltd). The whole intestine was collected 4 h after the last feeding from the adult zebrafish fed with 0.15% NaAc diets or the control group (n = 8). The whole intestinal samples were homogenized and mixed with sodium phosphate buffer (0.05 M, pH 7.0; wt:vol = 1:9). After centrifugation at 1500×3 g at 4℃ for 10 min, the supernatant was collected for testing the activity of intestinal digestive enzymes.

2.5 Respirometry

The standard and feeding metabolic energy in zebrafish was evaluated through measurement of oxygen consumption using the 8-chamber (2.3 L) respirometer located in a temperature-controlled room, as previously described (Guo et al., 2017). Firstly, zebrafish were feed-deprived for 24 h prior to being placed into the chambers, the chamber without fish served as a control group. The fish were then fed with 0.15% NaAc diets or control diets at equal amount (close to apparent satiation) for 3 days (1-3 d). On 4th day, the water in chambers was changed and the initial oxygen content in water was measured. The fish were continued to feed for another day as usual. The final oxygen content in water was measured after 24 h (5 d). Oxygen consumption was measured over this 24 h period to provide total metabolism values. Then, zebrafish were feed-deprived for 3days (5-7 d). On 8th day, the water in chambers

was changed and the initial oxygen content in water was measured. The fish continued to feed-deprived for another day as usual. The final oxygen content in water was measured after 24 h (9 d). Oxygen consumption was measured over this 24 h period to provide standard metabolism values. The concentration of oxygen was determined by using a polarographic oxygen electrode with a temperature sensor (lower limit: 4.0 mg O_2/L, Yellow Springs Instruments, Inc.). Oxygen consumption was converted to energy by using a conversion factor of 13.84 J/mg O_2. Total metabolic energy minus standard metabolic energy equals feeding metabolic energy. Notably, the fish used for this measurement were not applicable for further testing.

2.6 Automated measurement of larvae swimming activity

The swimming activity of zebrafish larvae that fed with control diet or diet containing 0.15% of NaAc over 7 d were measured by an infrared tracking device (WMicroTracker) (Goya et al., 2016). Zebrafish larvae were distributed into a 24-well microplate at 1 larva/2 ml GZM/well and 4 replicate wells were used for each treatment. Swimming activity of larvae was tracked for three random days, 22 h each day, with the remaining 2 h for feeding. Zebrafish larvae were fed twice a day (9:00, 17:00) at equal amount (close to apparent satiation), with each lasting about an hour. Swimming activity was calculated by summing up the number of activity events for 30 min.

2.7 Gas chromatography-mass spectrometry (GC-MS)

The concentration of SCFAs in gut contents was measured by gas chromatography-mass spectrometer (GC-MS). Zebrafish fed on diet supplemented with 0.15% NaAc for 4 weeks, gut content samples were collected at 4 h post the last feeding. Briefly, gut contents sample were weighed (20 mg), lyophilized and resuspended with 200 µl MeOH. Samples were mixed with sonication for three times with 10 min for each, followed by centrifugation at 12000 rpm for 10 min, the supernatant was collected for GC-MS analysis. GC-MS was performed on a

GCMS-QP2010 Ultra with an autosampler (SHIMADZU) and the Rtx-wax capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness, SHIMADZU). Oven temperature was programmed from 60 to 100℃ at 5 ℃/min, with a 1 min hold, to 150℃ at 5 ℃/min, with a 5 min hold, to 225℃ at 30 ℃/min, with a 20 min hold. Injection of a 2 μl sample was performed at 230℃. Helium, at a flow rate of 1.2 $ml \cdot min^{-1}$, was the carrier gas. Electronic impact was recorded at 70 eV. Peaks were integrated using Agilent ChemStation software (Agilent Technologies, Oxford, UK). SCFA contents were quantified by single-point internal standard method. Peak identity and internal response factors were determined using a 1-mM calibration cocktail including acetate, propionate and butyrate. Data were normalized according to the weight of original sample used.

2.8 Bacterial 16S ribosomal RNA gene sequencing

Gut microbiota from the experimental zebrafish were analyzed using bacterial 16S ribosomal RNA gene sequencing. Zebrafish were fed with 0.15% NaAc diet for 4 weeks, then at 4 h post the last feeding, the digesta samples were collected from 6 fish in each group and pooled as a replicate. DNA was extracted from each pooled sample using a Fast DNA SPIN Kit for Soil (MP Biomedicals), according to the manufacturer's instructions. The 16S V3–V4 region was amplified using the primers U341F (5′-CGGCAACGAGCGCAACCC-3′) and U806 (5′-CCATTGTAGCACGTGTGTAGCC-3′). 16S rRNA gene sequencing was performed at the Realbio Genomics Institute (Shanghai, China) using the Illumina HiSeq platform. The raw pair-end readings were then subjected to a quality-control procedure with the use of UPARSE. The qualified reads were clustered to generate operational taxonomic units (OTUs) at the 97% similarity level by using USEARCH. A representative sequence of each OTU was assigned to a taxonomic level in the RDP (Ribosomal Database Project) database by using the RDP classifier. Principal components analysis and heat-map analysis were performed using R software version 3.1.0.

2.9 Intracerebroventricular (ICV) injection of sodium acetate and atropine

Zebrafish were fed control diet at 10:00 am of the day before intracerebroventricular (ICV) injection. Zebrafish were handled with hypothermic anesthesia, the operation of ICV injection was performed under Axio Zoom.V16 (ZEISS, Oberkochen, Germany). After 24 h fasting, zebrafish in the experimental group were injected with 75 mg/kg NaAc, 75 mg/kg NaAc + 0.1 mg/kg atropine, respectively. In the injection of NaAc combined with atropine, 75 mg/kg NaAc were infused with 0.1 mg/kg atropine, and then injected into zebrafish synchronously. The control group was injected with an equivalent osmotic sodium chloride. The ICV injection dose of NaAc was calculated according to the addition rate of NaAc in feed (0.15%) and the daily feeding rate (nearly 5%) based on the above feeding experiment. Dose of atropine is determined relative to previous study (Yamazaki et al., 2006). The injection accuracy was determined by the position of evans blue dye (0.5 μl) in the ventricle. At indicated time 2 h, 4 h and 6 h post injection, multiple parameters are measured, including food intake, and the expression levels of orexigenic and anorexigenic gene in zebrafish brain tissue.

2.10 Quantitative PCR analysis

Total RNA was isolated from the brain tissue of zebrafish and extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). First-strand complementary DNA synthesis (cDNA) was performed using the Superscript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR reaction was performed using the SYBR Green Supermix (TianGen, Beijing, China) on the LightCycler 480 (Roche). The primer sequences were listed in Supplementary Table 2.

2.11 Statistical analysis

The data in this study are presented as values with standard error of measurement (mean ± SEM). All statistical analyses were performed in GraphPad Prism Version 6

(GraphPad Software). The significance of differences in mean values between two groups was analyzed using Student's t-test. Comparisons between multiple groups were analyzed using one-way ANOVA followed by a Duncan's test. Differences were considered significant when the *p*-value was less than 0.05, 0.01 or 0.001, $p < 0.05$(*), $p < 0.01$(**) and $p < 0.001$ (***), A *p*-value greater than 0.05 was considered as no significance difference (NS).

3. Results

3.1 Dietary sodium acetate promoted food intake, energy gain, and intestinal digestive enzyme activity in zebrafish

Under apparent satiation feeding, zebrafish were fed on diets supplemented with NaAc at different concentrations (0.00%, 0.05%, 0.1%, 0.15% and 0.2%) for 4 weeks. The results showed that survival rates were similar in all groups (Supplemental Figure. 1A). Zebrafish fed with 0.15% NaAc diet showed a higher weight gain and daily feeding rate than those fed diets containing other doses of NaAc (Supplemental Figure. 1B, C). All the added concentrations of NaAc had no effect on the feed efficiency (FE) of zebrafish (Supplemental Figure. 1D). Therefore, supplementation of 0.15% NaAc in the diets was identified as the optimum treatment dose for further study.

Subsequently, another feeding experiments including control and 0.15% NaAc diet group were conducted. Zebrafish were fed on control diet or diet containing 0.15% of NaAc under apparent satiation feeding for 4 weeks. The concentration of acetate acid in the gut contents of zebrafish fed with 0.15% NaAc containing diets was significantly increased compared with the control group (Fig. 1A). There was no significant difference in the survival rate between the 0.15% NaAc-fed zebrafish and the control group (Fig. 1B). The body weight gain (Fig. 1C) and daily feeding rate (Fig. 1D) were significantly increased in 0.15% NaAc fed zebrafish. Feed efficiency (FE) in zebrafish fed with 0.15% NaAc diets was similar to that of the control group (Fig. 1E).

To analyze the effects of dietary NaAc on the metabolic system, we examined the body composition in zebrafish. There was no significant change in moisture (Fig. 2A), crude protein (Fig. 2B) and crude ash content (Fig. 2C) between the treatment group and the control group, but the level of crude lipid was significantly elevated in zebrafish fed with 0.15% NaAc diets versus the control group ($p < 0.01$; Fig. 2D). Accordingly, both the energy gain and energy conversion efficiency were increased significantly in zebrafish fed with 0.15% NaAc diets ($p < 0.01$; Fig. 2E, F), indicating that sodium acetate promoted zebrafish energy accumulation process. Diet supplemented with 0.15% of NaAc also affected the activities of intestinal enzymes. The activity of intestinal amylase in zebrafish fed with 0.15% NaAc supplemented diet was significantly higher than that of the control group ($p < 0.05$; Fig. 2G).

3.2 Dietary sodium acetate increased orexigenic expression profile.

We assessed the expression of food intake-related genes in zebrafish fed on the diet containing 0.15% NaAc under apparent satiation feeding. The relative expressions of orexigenic (feeding-promoters) genes (*ghre*, *npy* and *npy7R*) in the brain tissue were significantly up-regulated after 0.15% NaAc administration compared with the control group ($p < 0.05$; Fig. 3A). The relative expression of anorexigenic (feeding-inhibitors) genes including *mc4R*, *grp* and *leptina* showed no significant difference between 0.15% NaAc fed zebrafish and control group (Fig. 3A). We hypothesized that the orexigenic effects of acetate may be associated with the regulation of the central nervous system. To test this hypothesis, NaAc was directly applied to the brain tissue of zebrafish via ICV injection. The results showed that ICV injection of NaAc (75 mg/kg) also resulted in significant increase in the cumulative food intake of the fish compared with the fish of control group 48 h after injection (Fig. 3B). The relative expression of both orexigenic and anorexigenic genes were significantly up-regulated by the administration of NaAc. The *ghre* gene was significantly up-regulated at 2 h ($p < 0.05$) while the expression of *npy7R* gene was significantly higher ($p < 0.05$) throughout the time course (2 h, 4 h and 6 h) of NaAc stimulation (Fig. 3C-E). At 6 h, the relative expression of anorexigenic genes *mc4R*

and *cart* was significantly up-regulated by NaAc compared with the control group ($p < 0.01$; Fig. 3F-H).

3.3 Sodium acetate administration affected the parasympathetic activation

Administration of NaAc also affected the parasympathetic system. When we inject NaAc combined with atropine (the parasympathetic blocker), the expression of *ghre* and *npy7R* gene was decreased compared with that of NaAc group (Fig. 4A-C), and the relative expression of anorexigenic genes *mc4R* and *cart* were also significantly down-regulated by atropine (Fig. 4D-F), indicating that the effects of NaAc on food intake-related genes can be invalidated by atropine compound. These results demonstrated that acetate played a great role in the regulation of food intake in zebrafish via parasympathetic activation, and it may act as a regulatory signal.

3.4 Dietary sodium acetate attenuated energy metabolism

The metabolic energy in zebrafish was evaluated via the measurements of standard metabolic energy (Rs), feeding metabolic energy (Rf), and swimming activities. The standard metabolic energy in the fish fed with 0.15% NaAc supplemented diets was significantly lower than that of control group ($p < 0.01$; Fig. 5A). There was no significant difference on feeding metabolic energy between the 0.15% NaAc-fed group and the control group (Fig. 5B). In zebrafish larvae fed with 0.15% NaAc supplemented diet, swimming activities were significantly lower than that of control group ($p < 0.05$; Fig. 5C, D).

3.5 Dietary sodium acetate improved the composition of gut microbiota

To evaluate the response of intestinal microbiota to the intake of sodium acetate, the composition of intestinal microbiota of zebrafish was analyzed by sequencing the 16s rRNA gene amplicons. At the phylum level, we observed that the dominant bacterial communities were mainly Fusobacteria, Proteobacteria, and Firmicutes, and exhibited no significant difference between two groups (Table 1, Fig. 6A). However,

at the genus level, the relative abundance of *Plesiomonas* was significantly lower in the 0.15% NaAc group than control (Table 2, Fig. 6B). The diversity indexes of the gut microbial community including Chao1, and observed species were higher in the 0.15% NaAc group than in the control group (p <0.05) (Table 3). Partial least squares discrimination analysis (PLA-DA) showed separated clustering of the control group and NaAc group, indicating a marked alteration of the microbiota by dietary NaAc (Fig. 6C).

4. Discussion

Acetate is the most abundant SCFA produced by gut microbiota in vertebrate, which exerts various effects to host physiology (Canfora and Blaak, 2017; den Besten et al., 2013; Koh et al., 2016). Administration of acetate or sodium acetate in aquatic animal showed some positive effects on digestibility (Ebrahimi et al., 2017; Sugiura et al., 2006). The application of acetate in aquafeed is still hampered by the lack of data related to its physiological effects on fish. Thus we evaluated the physiological effect of acetate on zebrafish, including food intake, parasympathetic activation, energy homeostasis and gut microbiota alteration.

Like in mammals, feeding behavior in fish is regulated by a feeding centra in the brain (Helene, 2016; Sebaihi et al., 2019). In this study, acetate supplemented diet and intraventricular infusion of acetate promoted appetite and food intake of zebrafish, which was eliminated by administering a parasympathetic blocker molecule atropine. Parasympathetic nerve system comprising vagal and pelvic nerves is part of autonomic nerve system and provides excitatory innervation from central nerve system (CNS) to intestine (Brinkman et al., 2019). Thus, CNS and parasympathetic nerve system take important part in the effect of appetite promotion of acetate in zebrafish. On the one hand, acetate cross blood-brain barrier via monocarboxylic acid carrier, thus to stimulate feeding centra and CNS. Then hormone or neuropeptide signal produced by feeding centra or CNS is transmitted to gut via endocrine pathway or parasympathetic efferent fibers. In this study, dietary acetate regulated the

expression of orexigenic factors, including *ghrelin*, neuropeptide Y (*NPY*) and one of neuropeptide Y receptors (*NPY7r*). *NPY* and *ghrelin* have been identified as orexigenic hormones in various fish species, such as goldfish, zebrafish and Nile tilapia (Helene, 2016). Central injection of ghrelin and NPY stimulated food intake in fish (Unniappan et al., 2002). Thus, dietary acetate promotes zebrafish appetite and food intake via neuroendocrine regulation involving with the production of ghrelin and NPY in brain. Meanwhile, higher NPY7r expression enhances the sensitivity of NPY in brain. In the context of restricted feeding, appetite- and food intake-controlling endocrine systems in teleost fish are of particular interest, and require further study.

Energy homeostasis depends on the balance between energy intake and energy expenditure. Energy gain is the comprehensive result of energy intake and energy expenditure. Intestinal digestive enzyme activity adapt to alteration in dietary composition (Corring, 1980). Intestinal digestive enzymes is important to enzyme breakdown of dietary components. In this study dietary acetate increased amylase activity, which indicated acetate can promote the utility of starch to fish. Thus, in this study, increased food intake and intestinal digestive enzyme activity led to elevated energy intake. Moreover, the standard metabolic energy (Rs) and swimming activities were significantly decreased, indicating that the reduction of energy consumption in zebrafish. Thus, improvement in energy gain by acetate supplemented diet was attributable to increased energy intake and deceased energy expenditure. The application of acetate derived from gut microbiota or other sources may have an impact on the control of the weight via affecting on the energy intake and energy expenditure. Mice fed on diet supplemented with sodium acetate resulted reduction in weight gain caused due to high fat diet (den Besten et al., 2015). Similarity, oral administration of sodium acetate supplemented diet (5% wt./wt.) to mice suppressed high fat diet induced weight gain by 72% compared with the mice fed on HFD (Lu et al., 2016). Furthermore, Kondo et al. (2009) demonstrated that, intragstric administration of acetic acid to high fat diet fed mice reduced weight gain

and fat accumulation compared with the control fed only in high fat diet. Moreover, evidences show that both ghrelin and NPY decreases energy expenditure and stimulates lipogenesis (Porteiro et al., 2013; Lutz, 2015). Thus, the body fat accumulation in zebrafish induced by acetate supplemented diet might be resulted from increased energy gain, which is mediated by *ghrelin*- and *NPY*-mediated signalling pathways. Likewise, the accumulation of acetate in the hypothalamus resulted to affect appetite regulation via glutamate-glutamine transcellular cycle, which resulted in increments in lactate and gamma aminobutyric acid (GABA) production after both intraperitoneal acetate injections and after colonic fermentation of 13C-labelled carbohydrate (Frost et al., 2014). In addition to this, the mice showed a peak in serum acetate levels after intraperitoneal acetate injections, which was associated with changes in the expression of neuropeptides (AMPK and acetyl CoA carboxylase (ACC) that regulate appetite reduction (Frost et al., 2014).

Furthermore, we also analyzed the gut microbiota of zebrafish. The results showed that intestinal flora of zebrafish mainly consisted of Fusobacteria, Proteobacteria, Firmicutes, which is consistent with the report of Roeselers et al. 2011. Similar with this study, rats fed with high fat diet showed an increased colonic and whole body acetate turnover together with a shift on the bacterial at phylum level (Perry et al., 2016). Proteobacteria was primarily represented by the genus *Plesiononas* (Roeselers et al., 2011). *Plesiomonas* were recognized as potential aquatic animal pathogen (Pękala-Safińska, 2018). Our research observed that addition of 0.15% NaAc reduced the relative abundance of *Plesiomonas*. This results similar to the research in Siberian sturgeon, which reported that the amount of intestinal content acetate had negative correlation with *Plesiomonas* (Geraylou et al., 2013). Therefore, we supposed that NaAc may have positive impact on zebrafish intestinal health.

5. Conclusion

The results of this study showed that dietary NaAc promoted energy gain in zebrafish

through increasing food intake and a reduction of energy consumption. The increased appetite behavior was associated with the parasympathetic nerve system. Sodium acetate is beneficial for the growth performance and health status of zebrafish, suggesting it might to be a potential feed additive in cultured fish.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Supplementary data

Supplementary material

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Figure. 1 Effects of dietary sodium acetate on body weight gain and food intake of zebrafish

Under satiation feeding, zebrafish were fed with diets containing 0.15% sodium acetate (NaAc) for 4 weeks, and zebrafish fed with basal feed served as the control group. (A) The acetate concentration in the intestinal contents of zebrafish fed diets with or without 0.15% NaAc. The level of survival rate (B), body weight gain (C), daily feeding rate (D) and feed efficiency (E) in zebrafish fed with 0.15% NaAc containing diets and the control group. Values are mean ± SEM, n=6 per group. $p < 0.05$(*) $p < 0.01$(**) and $p < 0.001$ (***).

Figure. 2 Effects of dietary sodium acetate on body fat mass and energy deposition

The level of body composition including moisture (A), crude protein (B), crude ash (C) and crude lipid (C) in the zebrafish fed diet with 0.15% NaAc or control diet. The energy gain rate (E) and energy conversion efficiency (F) in zebrafish fed diet with 0.15% NaAc or control diet. (G) The digestive enzyme activity in the zebrafish fed diet with 0.15% NaAc or control diet.

Figure. 3 The expression of food intake-related genes in zebrafish with NaAc administration

The expression of food intake-related genes in zebrafish fed on the diet containing 0.15% NaAc were detected by real-time PCR. (A) The relative expression of food intake-related genes in the brain of zebrafish. (B) Cumulative food intake in the zebrafish with NaAc stimulation in the brain

via intracerebroventricular injection. The relative expression of orexigenic (feeding-promoters) genes in the brain of zebrafish, including genes of *ghre* (C), *npy* (D) and *npy7R* (E) . The relative expressions of anorexigenic (feeding-inhibitors) genes include *mc4R* (F), *grpr* (G) and *cart* (H).

Figure. 4 Effects of sodium acetate on parasympathetic activation in zebrafish

The relative expression of food intake-related genes including *ghre* (A), *npy* (B), *npy7R* (C), *mc4R* (D), *grpr* (E), and *cart* (F) were tested by real-time PCR. Zebrafish were treat with NaAc and the parasympathetic blocker molecules atropine via intracerebroventricular injection.

Figure. 5 The energy metabolism in zebrafish

The changes of energy metabolism were evaluated in the zebrafish fed with 0.15% NaAc containing diet and the control diet. (A) Standard metabolic energy of adult zebrafish (J/d). (B) Feeding metabolism energy (J/d) in adult zebrafish. (C) Swimming activities of zebrafish larvae. (D) Accumulation of swimming activity in zebrafish larvae.

Figure. 6 The composition of gut microbiota in zebrafish

The composition of gut microbiota in zebrafish was analyzed using 16s rRNA sequence analysis. (A) Relative abundance at the phylum level between the two groups (B) The composition of gut microbiota at genus level in zebrafish fed with or without 0.15% NaAc diets. (C) Partial least squares discrimination analysis (PLA-DA) of gut microbiota in the control group and NaAc group zebrafish.

Table 1. The composition of gut microbiota at the phylum level

Phylum	CK	NaAc
Fusobacteria	50.51±16.91	46.34±24.07
Proteobacteria	36.06±8.03	27.74±11.45
Firmicutes	8.58±5.86	15.07±12.16
Actinobacteria	1.82±2.73	2.94±3.29
Chlamydiae	0.85±0.93	0.64±0.45
Bacteroidetes	0.45±0.51	2.90±3.04

Table 2. The composition of gut microbiota at the genus level

Table 3. Diversity index of gut bacteria of zebrafish fed with control or 0.15% NaAc diets

Genus	CK	NaAc
Cetobacterium	50.48±16.94	46.32±24.07
Plesiomonas	19.30±3.86	6.80±7.32*
Aeromonas	10.18±6.58	11.92±7.90
Methylobacterium	2.25±2.22	4.14±3.56
Lactococcus	1.85±1.73	2.89±2.40
Streptococcus	1.78±2.10	3.79±3.19
Lactobacillus	1.50±1.35	3.81±3.59
Hyphomicrobium	0.85±1.39	1.90±2.49
Vibrio	0.70±0.96	0.72±0.92
Nocardioides	0.62±1.01	0.99±0.79
Leuconostoc	0.61±0.58	1.08±0.93
Acinetobacter	0.51±0.90	0.02±0.01
Staphylococcus	0.47±0.54	0.82±0.92

	chao1	Observed species	PD_whole_tree	shannon	simpson
CK	213.2±22.3	171.48±19.16	35.2±7.7	2.7±0.7	0.67±0.06
NaAc	322.4±32.2*	271.75±56.57*	33.2±3.5	3.4±1.5	0.71±0.18

Highlights

- Evaluating the usage of sodium acetate (NaAc) in aquafeed.
- Dietary supplementation of 0.15% NaAc significantly promoted the weight gain and daily feeding rate of zebrafish.
- Acetate played a direct role in controlling appetite of zebrafish through the parasympathetic nerve system.

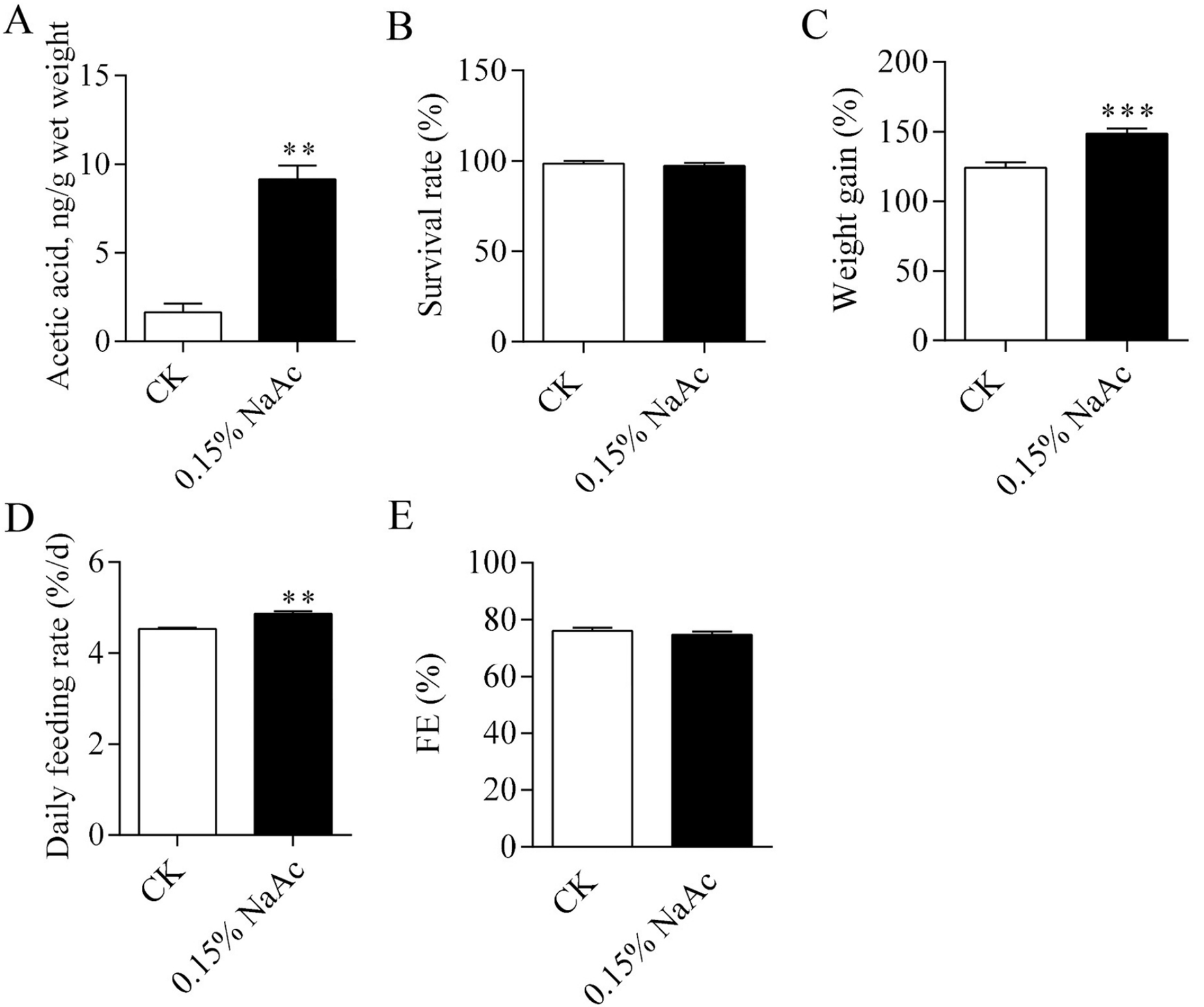
A
Acetic acid, ng/g wet weight
15
10
5
0
**
CK
0.15% NaAc
B
Survival rate (%)
150
100
50
0
CK
0.15% NaAc
C
Weight gain (%)
200
150
100
50
0
***
CK
0.15% NaAc
D
Daily feeding rate (%/d)
6
4
2
0
**
CK
0.15% NaAc
E
FE (%)
100
80
60
40
20
0
CK
0.15% NaAc

Figure 1

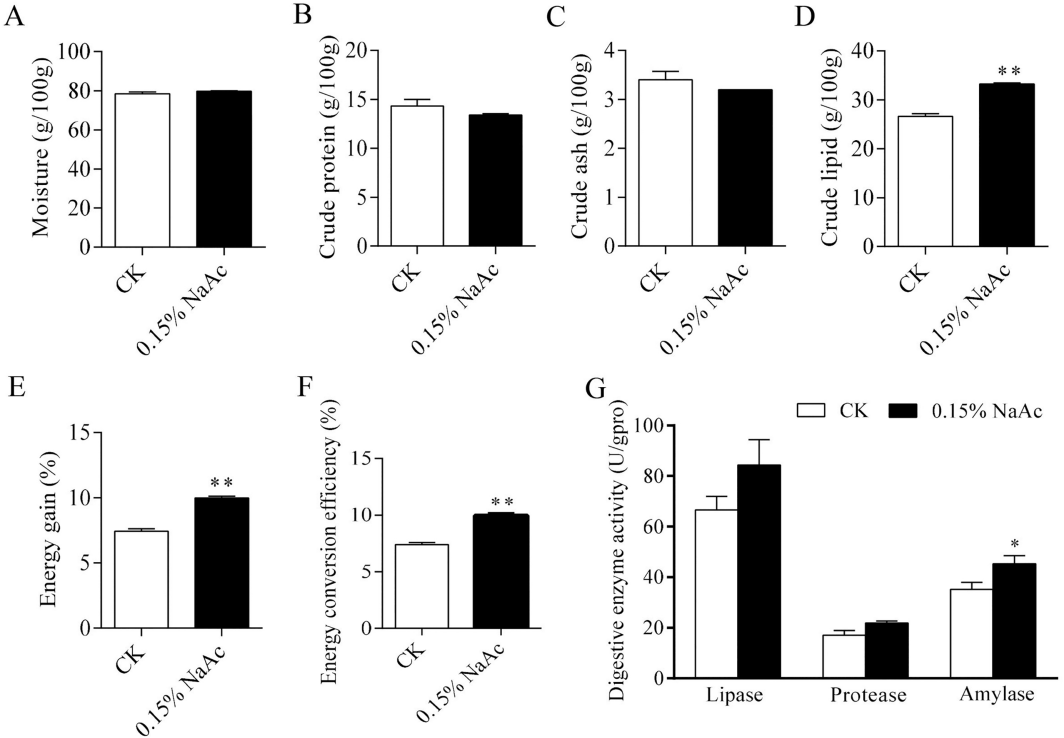
A
Moisture (g/100g)
100
80
60
40
20
0
CK
0.15% NaAc
B
Crude protein (g/100g)
20
15
10
5
0
CK
0.15% NaAc
C
Crude ash (g/100g)
4
3
2
1
0
CK
0.15% NaAc
D
Crude lipid (g/100g)
40
30
20
10
0
**
CK
0.15% NaAc
E
Energy gain (%)
15
10
5
0
**
CK
0.15% NaAc
F
Energy conversion efficiency (%)
15
10
5
0
**
CK
0.15% NaAc
G
CK
0.15% NaAc
Digestive enzyme activity (U/gpro)
100
80
60
40
20
0
*
Lipase
Protease
Amylase

Figure 2

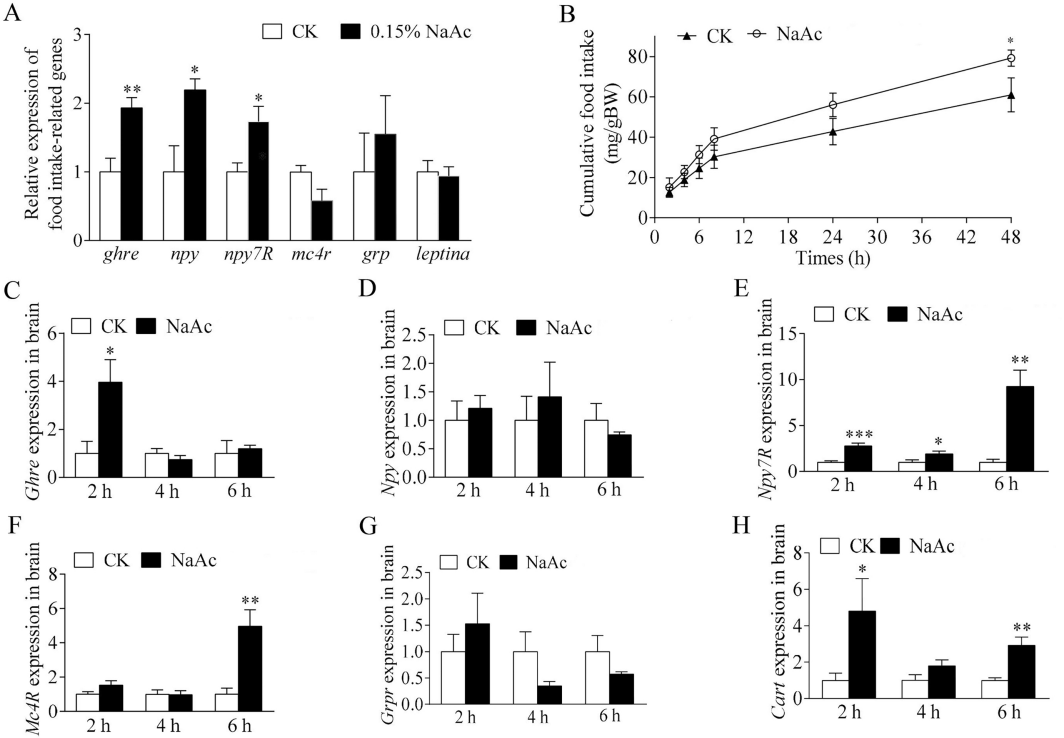
A
CK
0.15% NaAc
Relative expression of food intake-related genes
ghre
npy
npy7R
mc4r
grp
leptina
B
CK
NaAc
Cumulative food intake (mg/gBW)
Times (h)
C
Ghre expression in brain
D
Npy expression in brain
E
Npy7R expression in brain
F
Mc4R expression in brain
G
Grpr expression in brain
H
Cart expression in brain
2 h
4 h
6 h

Figure 3

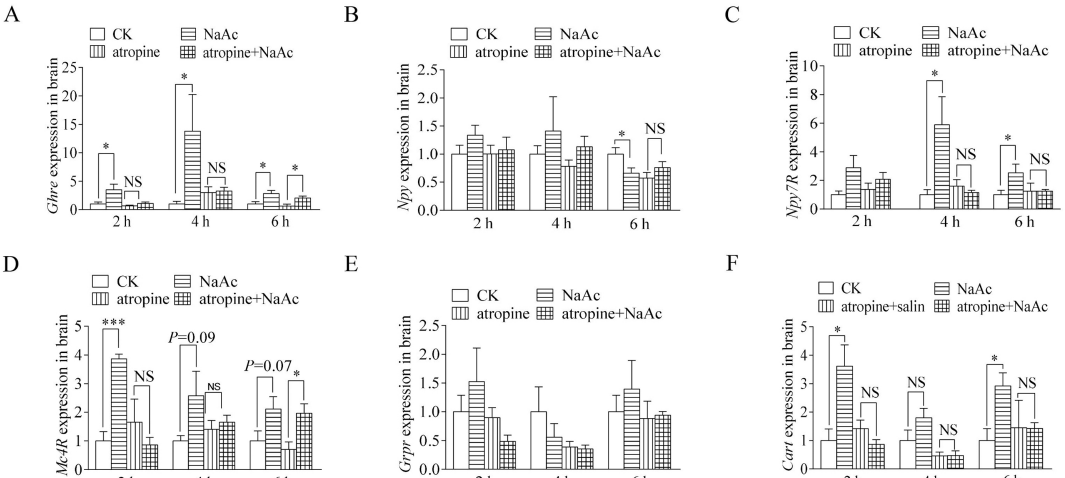
A
CK
atropine
NaAc
atropine+NaAc
Ghre expression in brain
25
20
15
10
5
0
*
NS
*
NS
*
*
2 h
4 h
6 h
B
CK
atropine
NaAc
atropine+NaAc
Npy expression in brain
2.5
2.0
1.5
1.0
0.5
0.0
*
NS
2 h
4 h
6 h
C
CK
atropine
NaAc
atropine+NaAc
Npy7R expression in brain
10
8
6
4
2
0
*
NS
*
NS
2 h
4 h
6 h
D
CK
atropine
NaAc
atropine+NaAc
Mc4R expression in brain
5
4
3
2
1
0
***
NS
P=0.09
NS
P=0.07
*
2 h
4 h
6 h
E
CK
atropine
NaAc
atropine+NaAc
Grpr expression in brain
2.5
2.0
1.5
1.0
0.5
0.0
2 h
4 h
6 h
F
CK
atropine+salin
NaAc
atropine+NaAc
Cart expression in brain
5
4
3
2
1
0
*
NS
NS
*
NS
2 h
4 h
6 h

Figure 4

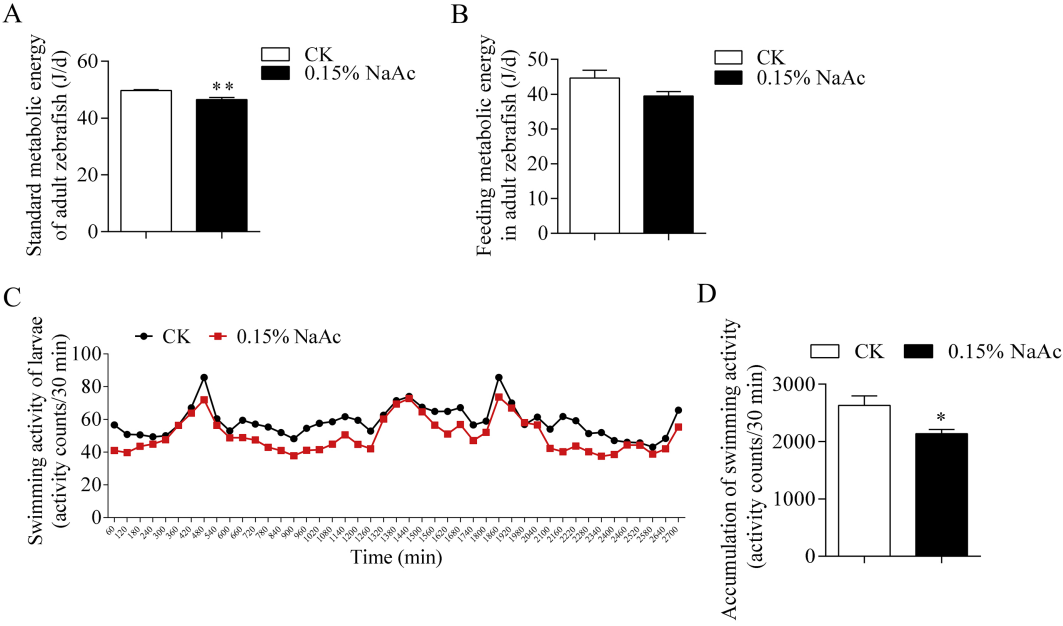
A
Standard metabolic energy of adult zebrafish (J/d)
60
40
20
0
**
CK
0.15% NaAc
B
Feeding metabolic energy in adult zebrafish (J/d)
50
40
30
20
10
0
CK
0.15% NaAc
C
Swimming activity of larvae (activity counts/30 min)
CK
0.15% NaAc
100
80
60
40
20
0
60 120 180 240 300 360 420 480 540 600 660 720 780 840 900 960 1020 1080 1140 1200 1260 1320 1380 1440 1500 1560 1620 1680 1740 1800 1860 1920 1980 2040 2100 2160 2220 2280 2340 2400 2460 2520 2580 2640 2700
Time (min)
D
Accumulation of swimming activity (activity counts/30 min)
CK
0.15% NaAc
3000
2000
1000
0
*

Figure 5

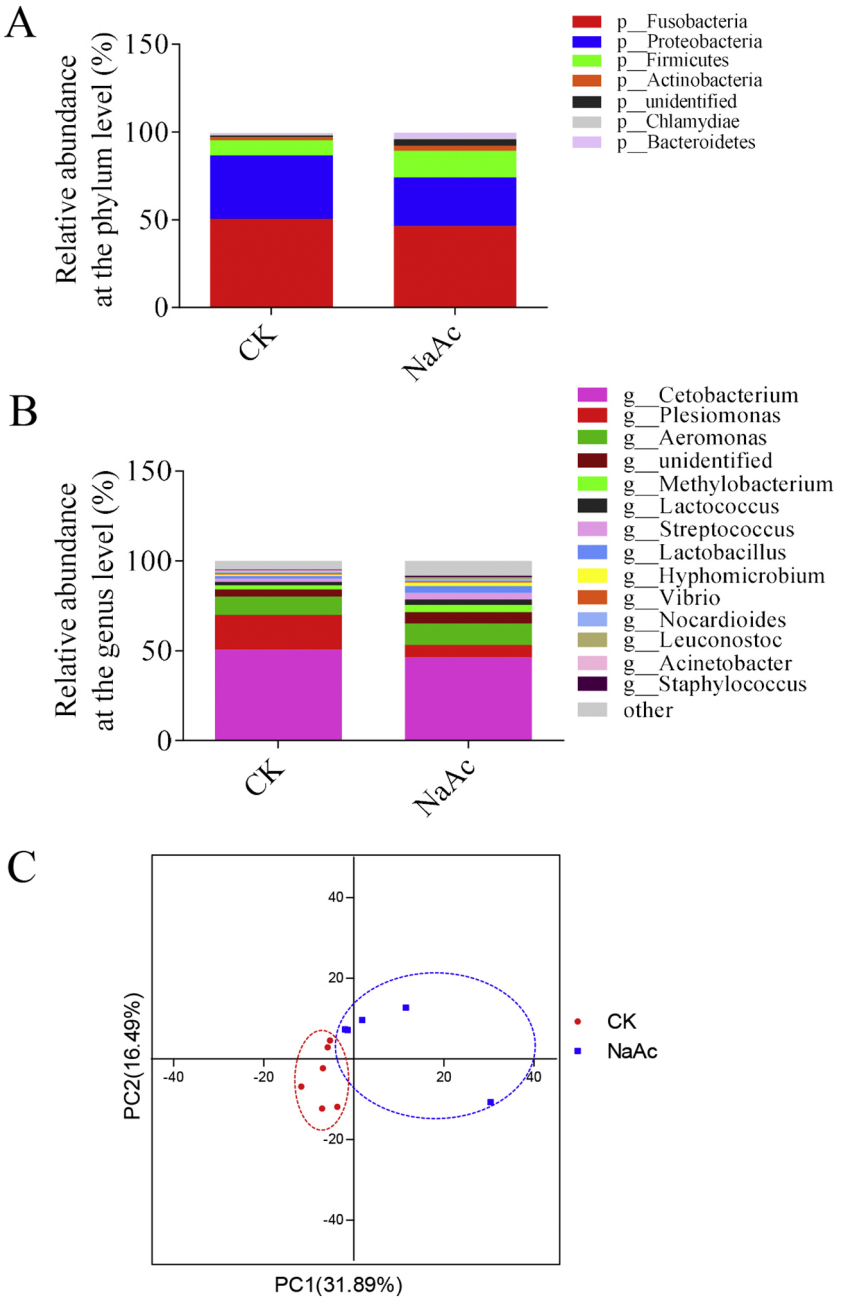
A
Relative abundance at the phylum level (%)
150
100
50
0
CK
NaAc
p__Fusobacteria
p__Proteobacteria
p__Firmicutes
p__Actinobacteria
p__unidentified
p__Chlamydiae
p__Bacteroidetes
B
Relative abundance at the genus level (%)
150
100
50
0
CK
NaAc
g__Cetobacterium
g__Plesiomonas
g__Aeromonas
g__unidentified
g__Methylobacterium
g__Lactococcus
g__Streptococcus
g__Lactobacillus
g__Hyphomicrobium
g__Vibrio
g__Nocardioides
g__Leuconostoc
g__Acinetobacter
g__Staphylococcus
other
C
PC2(16.49%)
40
20
-20
-40
-40
-20
20
40
PC1(31.89%)
CK
NaAc

Figure 6