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Research Article

Effects of Dietary Sodium Acetate on Growth Performance, Fillet Quality, Plasma Biochemistry, and Immune Function of Juvenile Golden Pompano (*Trachinotus ovatus*)

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Six isonitrongenous and isolipidic diets were formulated to feed 360 fish (mean initial body weight: 6.06 ± 0.02 g) with graded levels of sodium acetate (SA1 (0), SA2 (250 mg/kg), SA3 (500 mg/kg), SA4 (1000 mg/kg), SA5 (2000 mg/kg), and SA6 (4000 mg/kg)) for 8 weeks. The results revealed that weight gain rate, specific growth rate, and feed intake of golden pompano were significantly increased in SA3 and SA4 treatments ($p < 0.05$). Dietary SA level significantly improved lipid contents and gumminess of muscle ($p < 0.05$). Supplement levels of dietary SA significantly influenced plasma biochemical parameters containing triglyceride, glucose, high-density lipoprotein cholesterol, complements, and immunoglobulin M ($p < 0.05$). Appropriate supplement levels of dietary SA significantly increased enzyme activities of hepatic superoxide dismutase and total antioxidant capacity and decreased malondialdehyde contents ($p < 0.05$). Furthermore, appropriate SA levels downregulated the expression levels of Keap1, IL-8, TNF-α, and NF-κB genes and upregulated the expression levels of Nrf2 and TGF-β genes of the spleen ($p < 0.05$). Excess SA caused negative effects for *Trachinotus ovatus*. The optimum supplement level of SA for juvenile golden pompano was evaluated to be 1423.67 mg/kg based on WGR.

1. Introduction

The harvest of intensive aquaculture greatly relied on reducing the influence of disease in farmed fish [1]. Antibiotics has been intensively used to control disease in an aquatic organism [2]. Overused antibiotics used in feed induced hazards in health and environment, which brought about the ban of antibiotics in many countries [2]. Great attentions have arisen in seeking alternatives to antibiotics. Short-chain fatty acids (SCFAs) are thought to safe feed additives and have been applied in aquaculture recently [3]. SCFAs belong to organic acids, such as acetic acid, propionic acid, and butyric acid [4]. Dietary SCFAs exist in several forms, such as calcium, sodium, and potassium salts [5]. SCFAs are generally easily absorbed and promote digestive function of the intestinal epithelia in fish [6]. SCFAs are involved in ameliorating growth, immune responses, and intestinal microorganisms in fish [7, 8].

Acetate is one of the major SCFAs and is produced from pyruvate (through acetyl-CoA or the Wood-Ljungdahl pathway), arabinoxylan (through intestinal bacteria), and several amino acids (through deamination) [3, 9, 10]. In aquatic animals, sodium acetate (SA) has been added in the diets of Arctic charr (*Salvelinus alpinus*) [11], common carp (*Cyprinus carpio*) [4], zebrafish [12], yellowfin seabream (*Acanthopagrus latus*) [6], and Siberian sturgeon (*Acipenser baerii*, Brandt, 1869) [13], which showed effective improvements in growth performance and immune responses. In addition, a recent study of Nile tilapia (*Oreochromis niloticus*) indicated that SA regulated the MAPK and NF-κB signaling

pathways to alleviate high-carbohydrate-triggered intestinal inflammation [14]. The studies of dietary SA on the growth and immune functions in fish are still limited although several researches have been performed.

Golden pompano, *Trachinotus ovatus* is an economically important fish species because of its advantages of growth, flesh quality, and salinity tolerance [15]. Unfortunately, golden pompano often suffered from inflammation and disease due to intensive culture pattern [16]. SA supplementation might provide a new idea to relieve these symptoms of *T. ovatus* after the antibiotics were banned. The aim of the present study was to assess the effects of dietary sodium acetate for juvenile *T. ovatus* according to the growth performance, fillet quality, plasma biochemistry, and immune function.

2. Materials and Methods

2.1. Ethics Statement. All animal processes were approved by the Ethical Committee of Shanghai Ocean University (Shanghai, China).

2.2. Experimental Diets. The feed formulations are presented in Table 1. Six isoproteic (442.2 g/kg) and isolipidic (106.4 g/kg) diets were formulated through supplementing SA (0, 250, 500, 1000, 2000, and 4000 mg/kg), which were named as SA1, SA2, SA3, SA4, SA5, and SA6, respectively. SA (purity ≥ 99.0%) is provided by Sangon Biotech Co., Ltd. (Shanghai, China). Dietary protein was supplied by fish meal, soybean meal, soy protein concentrate, peanut meal, and pork powder. Dietary lipid was supplied by fish oil and soybean lecithin. All the components were pulverized into dust and sieved via a 425 μm mesh sifter. Then, they were thoroughly blended with fish oil and soybean lecithin in Hobart-type mixer (SZ25, Guangzhou Xuzhong Food Machinery Co., Ltd.) until homogenized. Next, water was supplied to shape a stiff dough and 2 and 2.5 mm diameter feed granules were obtained using a granulator. After drying, they were stored at -20°C until use.

2.3. Experimental Animals and Design. T. ovatus were purchased from an aquaculture company (Shenzhen, China). Before the beginning of trial, fish were fed SA1 diet for 14 days in polythene cages to acclimate the laboratory conditions and keep equal physiological status, followed by a 24 h of fasting. Subsequently, the experimental fish with homogenous size (average: 6.06 ± 0.02 g) were allotted in 18 pond cages (1 m × 1 m × 1.8 m) with 20 fish per cage. Fish were slowly hand-fed twice a day (7 : 00 and 17 : 00) to visible sense of satiety. The trial lasted 8 weeks and the data (number of dead fish, weight of dead fish and feed intake) in each cage were recorded daily. Water temperature was 31.06 ± 0.30°C, dissolved oxygen 7.78 ± 0.05 mg/L, salinity 15.32 ± 0.20 g/L, and pH 8.24 ± 0.08. Dissolved ammonia was ≤0.05 mg/L, and nitrite was ≤0.01 mg/L.

2.4. Sample Collection. Before sampling, fish were fasted for 24 h. They were anesthetized with eugenol (100 mg/L) for determining the number and weight of fish per cage. Three fish per cage were sacrificed randomly for the measurement of nutrient component of carcass. Five fish per cage were sacrificed and weighed individually. Then, their blood was collected from a caudal venipuncture using 2 mL heparinized syringes. Livers, spleens, and dorsal muscles of aforementioned five fish were aseptically removed and, respectively, collected into tubes and stored at -80°C until further analysis. Another three fish in each cage were caught, and dorsal muscles were collected and immediately performed the texture characteristics analysis.

2.5. Calculations. The metrics of growth performance including the weight gain rate (WGR), specific growth rate (SGR), feed efficiency ratio (FER), feed intake (FI), condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and survival rate were calculated as follows:

$$\begin{aligned}
\mathrm{WGR}\,(\%) &= 100 \times (\mathrm{W_f} - W_i)/\mathrm{W_i},\\
\mathrm{SGR}(\%/\mathrm{day}) &= 100 \times \left(\mathrm{Ln}\,W_f - \mathrm{Ln}\,W_i\right)/\text{number of days},\\
\mathrm{FER} &= \mathrm{W_w}/\text{dry diet feed (g)},\\
\mathrm{FI(g)} &= \text{dry diet feed (g)}/\text{number of fish},\\
K\left(\mathrm{g/cm^3}\right) &= 100 \times W_{bw}/{\mathrm{W_{bl}}}^3,\\
\mathrm{HSI}\,(\%) &= 100 \times W_l/\mathrm{W_{bw}},\\
\mathrm{VSI}\,(\%) &= 100 \times W_v/\mathrm{W_{bw}},\\
\text{Survival rate}\,(\%) &= 100 \times \mathrm{N_f}/\mathrm{N_i}.
\end{aligned} \tag{1}$$

In the above equations, W_f, W_i, W_w, W_{bw}, W_{bl}, W_l, W_v, N_f, and N_i are final average body weight (g), initial average body weight (g), weight gain (g), body weight of fish (g), body length of fish (cm), liver weight of fish (g), viscera weight of fish (g), finial number of fish, and initial number of fish, respectively.

2.6. Chemical Analysis. The proximate composition was measured according to the standard method of Association of Official Analytical Chemists [17]. Moisture was measured on the basis of a constant weight in oven-drying (DHG-9240, Shanghai) at 105°C. Ash was measured by a muffle furnace (FO610C, Yamato Scientific Co., Ltd., Tokyo, Japan) at 550°C for 5 h. Crude protein was determined by the acid digestion furnace (FOSS KT260, Switzerland) and an Auto Kjeldahl apparatus (Kjeldahl™ 2300 System). Crude lipid was mensurated by Soxtec™ 2055 (FOSS, Hoganos, Sweden).

Muscle samples were cut into 10 mm high cylinders before analysis. Texture characteristic measurements were performed using a texture analyzer (TC3, Brookfield, USA) and were estimated based on texture profile analysis (TPA) following a previous method [18]. The model of spherical probe was TA44, and the displacement was 3 mm. The test speed was 0.1 cm/s, and the trigger values were 5 g ($\mathrm{m/s^2}$).

Plasma was separated after centrifuging (3000 r/min, 4°C, 15 min). Total cholesterol (TC), total protein (TP), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), and glucose (GLU) of plasma were quantified using

Table 1: Formulation and nutrition levels of the diets (g/kg dry weight).

Ingredients	SA1	SA2	SA3	SA4	SA5	SA6
Fish meal[a]	250.0	250.0	250.0	250.0	250.0	250.0
Soybean meal[a]	150.0	150.0	150.0	150.0	150.0	150.0
Soy protein concentrate[b]	100.0	100.0	100.0	100.0	100.0	100.0
Peanut meal[a]	100.0	100.0	100.0	100.0	100.0	100.0
Pork powder[c]	60.0	60.0	60.0	60.0	60.0	60.0
Brewers dried yeast[b]	50.0	50.0	50.0	50.0	50.0	50.0
Wheat flour[a]	184.0	184.0	184.0	184.0	184.0	184.0
Fish oil[d]	60.0	60.0	60.0	60.0	60.0	60.0
Soybean lecithin[a]	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin and mineral mix[a]	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride[a]	5.0	5.0	5.0	5.0	5.0	5.0
Monocalcium phosphate[a]	5.0	5.0	5.0	5.0	5.0	5.0
L-Methionine[e]	2.0	2.0	2.0	2.0	2.0	2.0
Antioxidant[b]	1.0	1.0	1.0	1.0	1.0	1.0
Attractant[a]	5.0	5.0	5.0	5.0	5.0	5.0
Zeolite powder[a]	4.0	3.75	3.5	3.0	2.0	0
Sodium acetate (SA)[e]	0	0.25	0.5	1.0	2.0	4.0
Proximate composition (g/kg)						
Moisture	88.4	84.9	86.7	86.7	88.4	86.2
Crude protein	436.6	438.5	436.8	439.1	439.3	434.2
Crude lipid	106.2	104.6	103.1	104.4	106.8	104.9
Ash	94.7	93.1	94.7	93.1	93.9	93.3

[a]Purchased by Kingkey Smart Agri Technology Co. Ltd. (Shenzhen, China). [b]Purchased by Guangdong Yuequn Ocean Biological Research Development Co., Ltd. (Jieyang, China). [c]Purchased by Guangdong Xunyuan Nutrition Technology Co., Ltd. (Jieyang, China). [d]Purchased by Yongxing Concentrated Feed Co., Ltd. (Guangzhou, China). [e]Purchased by Sangon Biotech Co., Ltd. (Shanghai, China). Vitamin and mineral mix provided the following per kg of diet: vitamin A (5,00,000 IU) 40 mg, vitamin B_1 40 mg, vitamin B_2 93.75 mg, vitamin B_6 20 mg, vitamin B_{12} (1%) 45 mg, vitamin K_3 (50%) 300 mg, inositol 400 mg, calcium pantothenate 250 mg, nicotinic acid 450 mg, folic acid 6 mg, biotin (2%) 10 mg, vitamin D_3 (5,00,000 IU) 15 mg, vitamin E (50%) 300 mg, unite bran 2990.25 mg, NaF 4 mg, KI 1.6 mg, $CoCl_2{\cdot}6H_2O$ (1%) 100 mg, $CuSO_4{\cdot}5H_2O$ 20 mg, $FeSO_4{\cdot}H_2O$ 160 mg, $ZnSO_4{\cdot}H_2O$ 100 mg, $MnSO_4{\cdot}H_2O$ 120 mg, $MgSO_4{\cdot}7H_2O$ 2.4 g, $Ca(H_2PO_4)2{\cdot}H_2O$ 6.0 g, NaCl 200 mg, and zelote power 30.90 g.

ROCHE-P800 biochemical analyzer (Roche, Basel, Switzerland) as previous described [19]. The activities of aspartate aminotransferase (AST) (C010-2-1), alanine aminotransferase (ALT) (C009-2-1), complement 3 (C3) (E032-1-1), complement 4 (C4) (E033-1-1), and immunoglobulin M (IgM) (E025-1-1) in plasma were measured by immunoturbidimetry employing assay kits (Nanjing, China) through the Absorbance Microplate Reader (VersaMax™, SMP500-18170-OULJ, USA).

The frozen liver samples were thawed at 4°C, weighed in a centrifuge tube, and homogenized in 10 volumes of ice-cold physiological saline (1 : 9, *w*/*v*; 8.6 g/kg; pH = 7.4). Then, it was centrifuge for 20 min at 3000 r/min at 4°C to obtain the supernatant for quantifying the superoxide dismutase (SOD) (A001-3-2), peroxidase (POD) (A084-1-1), total antioxidant capacity (T-AOC) (A015-2-1), and malondialdehyde (MDA) (A003-1-2) using commercial kits (Nanjing, China) through the Absorbance Microplate Reader (VersaMax™, SMP500-18170-OULJ, USA).

2.7. RNA Extraction and Gene Expression. Total RNA was isolated from the spleen tissue of *T. ovatus* adopting the HiPure University RNA Mini Kit (Magen, Guangzhou, China) according to the manufacturer's instructions and dissolved in DEPC treat water. Then, the quality of total RNA was determined by 1.2% agarose gel electrophoresis, and the concentration of total RNA was detected using NanoDrop 2000 (Thermo Scientific, USA). The absorbance ratios at 260 nm and 280 nm (260/280) of all RNA samples were ranged from 1.8 to 2. The synthesize of complementary DNA (cDNA) was performed using Evo M-MLV RT Kit with gDNA Rraser (AG, Guangzhou, China) and stored at -80°C. The specific primer pairs are listed in Table 2. qPCR determination was conducted as described our previous methods [20]. All samples were determined in triplicate. Relative mRNA levels of target genes were calculated by the $2^{-\Delta\Delta Ct}$ method [21].

2.8. Statistical Analysis. Experimental data were presented as means ± standard error of mean (SEM). The normality and homogeneity of variance were confirmed using the Kolmogorov-Smirnov test and Levene's test before performing the one-way analysis of variance (ANOVA). Then, Tukey's test was conducted to assess differences in the treatments through SPSS 23.0 (SPSS, Michigan Avenue, Chicago, IL, USA). The level of statistical significance was set at $p < 0.05$. A principal component test was conducted to describe the response of dependent variable to dietary SA levels via

Table 2: Primer sequences used for qRT-PCR analysis in this study.

Genes		Sequence	Length (bp)	TM (°C)	Source
TGF-β	F	TATCCCTCTACAACAGCACCA	21	55.61	[41]
	R	GGTCAGCAGGCGGTAATC	18	57.18	
IL-8	F	GAGAAGCCTGGGAATGGA	18	54.90	[41]
	R	GAGCCTCAGGGTCTAAGCA	19	57.32	
TNF-α	F	CGCAATCGTAAAGAGTCCCA	20	55.40	[41]
	R	AAGTCACAGTCGGCGAAATG	20	55.40	
Keap1	F	AGAGGATGGAGATGGCACAG	20	57.45	[41]
	R	CATTGGTTGGTCTTGGGATT	20	53.35	
Nrf2	F	AGCTTGGCCTTCATCAAAT	19	50.85	[41]
	R	GAGTATGGCTGTCCTTCTTCA	21	55.61	
NF-κB	F	TGCGACAAAGTCCAGAAAGAT	21	53.66	[41]
	R	CTGAGGGTGGTAGGTGAAGGG	21	61.47	
β-Actin	F	TACGAGCTGCCTGACGGACA	20	59.50	[48]
	R	GGCTGTGATCTCCTTCTGC	19	57.32	

PAST analysis software, selecting the quadratic regression pattern to evaluate the optimal supplement level of SA for juvenile golden pompano using Microsoft Excel 2016.

3. Results

3.1. Growth Performance. The effects of supplement levels of dietary SA on growth performance are shown in Table 3. FBW, WGR, SGR, and FI of *T. ovatus* fed SA3 and SA4 diets were significantly higher than those fed SA1 diets ($p < 0.05$). Fish fed SA1, SA2, SA3, SA4, and SA5 showed significantly higher FBW, WGR, SGR, and FI than those fed SA6 diets ($p < 0.05$). The peaks of FBW, WGR, SGR, and FI were recorded for SA4 treatments. There were no significant differences in FER, VSI, HSI, K, and survival among all groups ($p > 0.05$). The optimal supplement level of SA was assessed to be 1423.67 mg/kg by a quadratic regression model based on WGR (Figure 1). PCA biplot and loadings showed that strong interdependency between the dietary SA level and dependent variables (FBW, WGR, SGR, and FI), with correlation as 0.9501, 0.9177, 0.9089, and 0.9346, respectively (Figure 2).

3.2. Nutrient Component of Carcass and Dorsal Muscle. The results are presented in Table 4. Supplement levels of dietary SA did not significantly affect whole-body composition of juvenile *T. ovatus*. Crude lipid of dorsal muscle in the SA4 group was significantly higher than that in SA1 treatment ($p < 0.05$). The differences in crude protein, ash, and moisture among all groups were not significant.

3.3. Texture Characteristics of Dorsal Muscle. The results are shown in Table 5. The texture parameters containing hardness, elasticity, chewiness, and adhesiveness did not have statistical differences among the six treatments ($p > 0.05$). Gumminess of dorsal muscle in the SA3, SA4, and SA5 treatments was significantly higher than that in SA1 treatment ($p < 0.05$).

3.4. Plasma Biochemistry and Immune Response. As shown in Table 6, the biochemistry and immune indices of plasma were significantly affected by supplement levels of dietary SA. TP and HDL-c content in plasma of juvenile golden pompano fed SA-supplemented diets (except for SA2 diets) were significantly higher than those fed SA1 diets ($p < 0.05$). The highest TP and HDL-c were recorded for the SA4 and SA6 groups, respectively. Similarly, fish fed SA-supplemented diets (except for SA2) had significantly lower TG and GLU compared to that fed SA1 diets ($p < 0.05$). AST activities of juvenile golden pompano fed SA2, SA3, SA4, and SA5 diets were significantly lower than those fed SA1 diets ($p < 0.05$). The SA4 group had significantly lower ALT activities compared to the SA1 group ($p < 0.05$). Fish fed SA3, SA4, and SA5 diets had significantly higher C3 and IgM than the group fed SA1 diets ($p < 0.05$). The highest contents of C3 and IgM were recorded for the SA4 and SA3 groups, respectively. C4 contents in the SA2, SA3, and SA4 groups were significantly higher than that in SA1 ($p < 0.05$). There was no significant difference in plasma TC among the groups ($p > 0.05$). PCA presented a strong relationship between dietary SA level and dependent variables (C3, C4, AST, and ALT), and the correlation was, respectively, 0.8735, 0.8385, 0.9283, and 0.8959 (Figure 2).

3.5. Antioxidant Capacity in Liver. Supplement levels of dietary SA significantly influenced the antioxidant capacity of liver (Table 7). The activities of SOD in SA2, SA3, and SA4 treatments were significantly higher than that in SA1 treatment ($p < 0.05$). Fish fed SA4 diets had significantly higher T-AOC compared to that fed SA1 diets ($p < 0.05$). SA-supplemented diets significantly decreased MDA contents ($p < 0.05$). POD activities were not significantly influenced by supplement level of SA ($p > 0.05$). PCA showed a relationship between dietary SA level and dependent variables (SOD, T-AOC and MDA), with correlation as 0.8165, 0.6095, and 0.6710, respectively (Figure 2).

Table 3: Effects of dietary SA levels on growth performance in *T. ovatus*.

Parameters	Dietary SA supplement levels					
	SA1	SA2	SA3	SA4	SA5	SA6
IBW (g)	5.98 ± 0.05	6.01 ± 0.06	6.08 ± 0.01	6.11 ± 0.01	6.09 ± 0.03	6.07 ± 0.05
FBW (g)	80.57 ± 0.53^{b}	83.44 ± 1.10^{bc}	85.97 ± 0.82^{cd}	88.85 ± 0.61^{d}	84.97 ± 1.05^{c}	75.68 ± 0.19^{a}
WGR (%)	1248.53 ± 2.81^{b}	1288.35 ± 11.93^{bc}	1314.86 ± 15.48^{cd}	1353.30 ± 9.67^{d}	1294.84 ± 11.24^{bc}	1147.62 ± 7.50^{a}
SGR (%/d)	4.65 ± 0.00^{b}	4.70 ± 0.02^{bc}	4.73 ± 0.02^{cd}	4.78 ± 0.01^{d}	4.71 ± 0.01^{bc}	4.51 ± 0.01^{a}
FER	0.70 ± 0.00	0.70 ± 0.01	0.70 ± 0.01	0.70 ± 0.00	0.71 ± 0.02	0.71 ± 0.01
FI (g/fish)	105.93 ± 0.36^{b}	110.35 ± 0.23^{bc}	112.16 ± 1.70^{c}	117.42 ± 0.49^{d}	109.39 ± 0.90^{bc}	98.20 ± 1.20^{a}
VSI (%)	8.17 ± 0.54	8.15 ± 0.58	7.62 ± 0.41	7.54 ± 0.11	7.26 ± 0.17	6.91 ± 0.05
HSI (%)	1.75 ± 0.08	1.65 ± 0.18	1.75 ± 0.13	1.60 ± 0.17	1.50 ± 0.13	1.42 ± 0.06
K (g/cm^3)	4.14 ± 0.14	4.31 ± 0.08	4.21 ± 0.04	4.14 ± 0.04	4.11 ± 0.08	4.18 ± 0.10
Survival (%)	100.00 ± 0.00	100.00 ± 0.00	98.33 ± 1.67	100.00 ± 0.00	98.33 ± 1.67	100.00 ± 0.00

Abbreviation: IBW: initial body weight; FBW: final body weight; WGR: weight gain rate; SGR: specific growth rate; FER: feed efficiency ratio; FI: feed intake; VSI: viscerosomatic index; HSI: hepatosomatic index; *K*: condition factor. *Note*: different superscripts within rows indicate significant differences ($p < 0.05$).

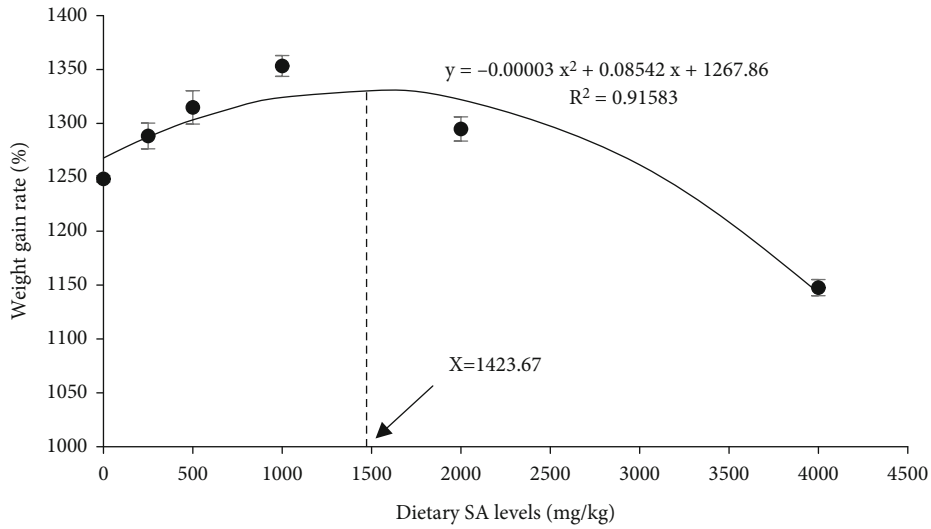


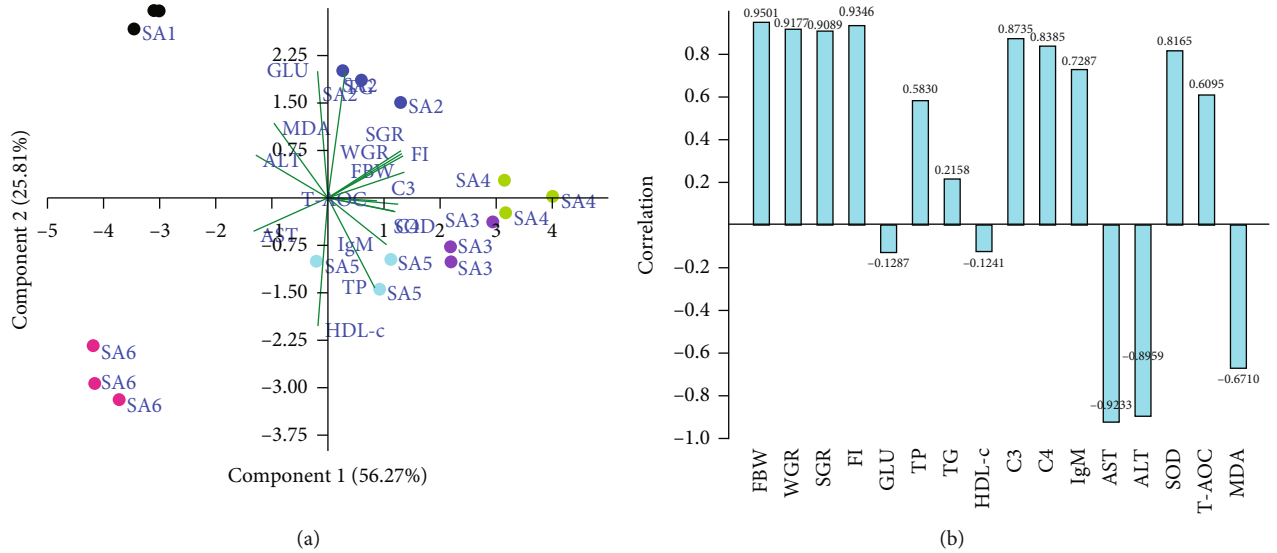
Figure 1: Relationship between dietary sodium acetate (SA) and weight gain rate (WGR). Each point is means ± SEM of three replicate tanks of fish, with 20 fish per tanks.

3.6. Immune-Related Gene Expression in Spleen. The results are presented in Figure 3. Fish fed SA-supplemented diets had significantly lower expression levels of Il-8, TNF-α, NF-κB, and Keap1 genes than that fed SA1 diets ($p < 0.05$). The lowest mRNA level of IL-8 was recorded in SA3 while the lowest expression levels of Keap1, TNF-α, and NF-κB were recorded in SA4. The expression levels of TGF-β genes in SA3 and SA4 were significantly higher than that in SA1 ($p < .05$). The expression levels of Nrf2 genes of juvenile golden pompano fed SA2, SA3, and SA4 diets were significantly higher than that fed SA1 diets ($p < 0.05$).

4. Discussion

Administration of sodium acetate exerts positive effects on growth performance in fish [11, 22]. Acetate also plays a beneficial role in appetite regulation [23]. Studies on zebrafish displayed that SA promoted appetite and FI of zebrafish [12, 24]. Similarly, this study showed appropriate supplement levels of SA significantly promoted WGR and FI of juvenile golden pompano. These findings inferred that moderate SA in diets increased FI through an improvement in appetite, thus promoting growth of juvenile *T. ovatus*. In the current study, the optimal level of dietary SA was evaluated to be 1423.67 mg/kg based on a quadratic regression model of WGR, which was similar to the previous studies on Arctic charr (*Salvelinus alpinus*) [11], zebrafish [12] and Nile tilapia (*Oreochromis niloticus*) [14].

Hardness, elasticity, chewiness, adhesiveness, and gumminess are major indices to assess fillet quality [25, 26]. Decreased lipid content in muscle will enhance the friction between muscle bundle and reduce muscle tenderness [27] as well as decreasing sensory scores for juiciness [28]. In this study, lipid content of muscle significantly increased when fish fed SA4 diets. Furthermore, gumminess of muscle increased significantly in SA3, SA4 and SA5 treatments



(a) (b)

Figure 2: Principal component analysis (PCA): relationship between dietary sodium acetate level and final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), feed intake (FI), glucose (GLU), total protein (TP), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), complement 3 (C3), complement 4 (C4), immunoglobulin M (IgM), aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA).

Table 4: Effects of dietary SA levels on the nutrient component of carcass and dorsal muscle in *T. ovatus*.

Parameters	Dietary SA supplement levels					
	SA1	SA2	SA3	SA4	SA5	SA6
Whole body						
Crude protein (g/kg)	162.46 ± 1.94	166.54 ± 6.29	161.22 ± 2.05	168.47 ± 2.68	167.29 ± 3.00	173.88 ± 2.39
Crude lipid (g/kg)	122.17 ± 1.41	129.27 ± 6.17	126.67 ± 6.01	131.47 ± 4.61	122.76 ± 8.15	130.47 ± 2.15
Ash (g/kg)	34.93 ± 0.94	32.97 ± 1.64	36.16 ± 0.44	35.08 ± 0.54	35.53 ± 1.53	35.30 ± 0.88
Moisture (g/kg)	673.73 ± 0.33	665.83 ± 7.83	670.43 ± 9.68	661.17 ± 4.96	668.40 ± 9.97	656.10 ± 4.78
Muscle						
Crude protein (g/kg)	193.93 ± 1.22	192.13 ± 3.07	196.40 ± 5.86	190.69 ± 3.07	192.96 ± 1.69	194.37 ± 6.55
Crude lipid (g/kg)	41.58 ± 1.79^{a}	45.70 ± 3.15^{a}	47.92 ± 2.12^{ab}	55.47 ± 0.98^{b}	41.03 ± 0.99^{a}	41.70 ± 2.63^{a}
Ash (g/kg)	13.90 ± 0.34	14.21 ± 0.03	13.88 ± 0.34	13.77 ± 0.21	14.14 ± 0.20	14.29 ± 0.20
Moisture (g/kg)	747.83 ± 5.29	746.37 ± 3.98	739.63 ± 5.98	737.00 ± 3.65	749.00 ± 3.20	746.67 ± 5.90

Note: different superscripts within rows indicate significant differences ($p < 0.05$).

Table 5: Effects of dietary SA levels on dorsal muscle texture characteristics in *T. ovatus*.

Parameters	Dietary SA supplement levels					
	SA1	SA2	SA3	SA4	SA5	SA6
Hardness (g)	557.50 ± 7.73	584.00 ± 12.96	558.33 ± 15.30	595.92 ± 26.18	585.92 ± 25.98	522.50 ± 23.30
Elasticity (mm)	1.64 ± 0.12	1.64 ± 0.01	1.72 ± 0.03	1.66 ± 0.05	1.54 ± 0.02	1.66 ± 0.04
Chewiness (mJ)	1.86 ± 0.04	1.89 ± 0.22	2.05 ± 0.27	2.27 ± 0.19	2.32 ± 0.09	2.11 ± 0.11
Adhesiveness (mJ)	0.21 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.23 ± 0.01	0.23 ± 0.02	0.23 ± 0.01
Gumminess (g)	102.03 ± 4.56^{a}	133.07 ± 9.73^{ab}	139.50 ± 12.45^{b}	138.58 ± 9.17^{b}	149.33 ± 4.66^{b}	122.63 ± 7.47^{ab}

Note: different superscripts within rows indicate significant differences ($p < 0.05$).

compared to that in SA1 treatment. A recent study showed muscle lipid content of fish fed SA diets had an increasing trend although this was not significant [29]. These results suggested moderate SA induced muscle lipid accumulation and improved fillet quality of juvenile golden pompano to a certain extent.

Table 6: Effects of dietary SA levels on plasma biochemistry and immune response in *T. ovatus*.

Parameters	Dietary SA supplement levels					
	SA1	SA2	SA3	SA4	SA5	SA6
TC (mmol/L)	4.25 ± 0.07	4.44 ± 0.02	5.01 ± 0.10	4.60 ± 0.24	4.58 ± 0.33	4.55 ± 0.07
TP (g/L)	36.50 ± 0.23^{a}	36.70 ± 0.64^{a}	41.80 ± 0.58^{bc}	44.00 ± 0.92^{c}	42.20 ± 0.06^{bc}	40.40 ± 0.06^{b}
TG (mmol/L)	4.78 ± 0.29^{e}	4.46 ± 0.23^{de}	3.84 ± 0.16^{cd}	3.48 ± 0.08^{c}	2.59 ± 0.08^{b}	1.73 ± 0.11^{a}
GLU (mmol/L)	15.13 ± 0.04^{c}	15.04 ± 0.01^{bc}	14.59 ± 0.03^{ab}	14.60 ± 0.16^{ab}	14.31 ± 0.15^{a}	14.36 ± 0.13^{a}
HDL-c (mmol/L)	2.21 ± 0.03^{a}	2.34 ± 0.05^{a}	2.80 ± 0.02^{c}	2.54 ± 0.01^{b}	2.63 ± 0.03^{b}	3.11 ± 0.03^{d}
AST (U/L)	50.50 ± 1.44^{b}	35.50 ± 1.44^{a}	35.50 ± 2.02^{a}	36.00 ± 0.00^{a}	38.00 ± 3.61^{a}	60.00 ± 1.73^{b}
ALT (U/L)	7.00 ± 0.00^{b}	5.00 ± 0.58^{ab}	5.00 ± 0.58^{ab}	4.33 ± 0.33^{a}	5.00 ± 0.58^{ab}	6.00 ± 0.58^{ab}
C3 (μg/mL)	53.25 ± 1.41^{b}	54.29 ± 0.89^{b}	70.61 ± 2.16^{c}	84.15 ± 2.36^{d}	73.30 ± 1.03^{c}	43.08 ± 2.37^{a}
C4 (μg/mL)	43.98 ± 3.17^{a}	72.49 ± 1.27^{b}	77.58 ± 1.80^{b}	73.83 ± 6.44^{b}	53.99 ± 1.95^{a}	53.92 ± 3.17^{a}
IgM (μg/mL)	62.50 ± 4.12^{a}	71.55 ± 6.75^{a}	171.77 ± 2.51^{c}	117.16 ± 1.66^{b}	111.22 ± 6.13^{b}	71.70 ± 9.41^{a}

Abbreviation: TC: total cholesterol; TP: total protein; TG: triglyceride; GLU: glucose; HDL-c: high-density lipoprotein cholesterol; AST,: aspartate aminotransferase; ALT: alanine aminotransferase; C3: complement 3; C3: complement 4; IgM: immunoglobulin M. *Note*: different superscripts within rows indicate significant differences ($p < 0.05$).

Table 7: Effects of dietary SA levels on hepatic antioxidative abilities in *T. ovatus*.

Parameters	Dietary SA supplement levels					
	SA1	SA2	SA3	SA4	SA5	SA6
SOD (U/mg)	78.96 ± 3.97^{a}	97.27 ± 1.25^{c}	92.31 ± 1.33^{bc}	95.64 ± 1.80^{c}	88.36 ± 2.08^{abc}	85.42 ± 0.67^{ab}
POD (U/mg)	3.16 ± 0.05	3.38 ± 0.22	3.12 ± 0.25	3.40 ± 0.01	3.04 ± 0.09	3.07 ± 0.14
T-AOC (μmol/g)	0.53 ± 0.01^{ab}	0.69 ± 0.02^{bc}	0.69 ± 0.01^{bc}	0.73 ± 0.04^{c}	0.50 ± 0.04^{a}	0.61 ± 0.06^{abc}
MDA (nmol/mg)	1.27 ± 0.07^{b}	0.94 ± 0.06^{a}	0.85 ± 0.00^{a}	0.99 ± 0.01^{a}	0.89 ± 0.04^{a}	1.03 ± 0.03^{a}

Abbreviation: SOD: superoxide dismutase; POD: peroxidase; T-AOC: total antioxidant capacity; MDA: malondialdehyde. *Note*: different superscripts within rows indicate significant differences ($p < 0.05$).

Health status and growth of fish go hand in hand in ensuring maximum outputs during harvest [1]. Biochemical indices of plasma can evaluate the healthy status of fish tissues [19]. TP including albumin and globulin can reflect immune status of organisms [30]. HDL-c is involved in the transport of cholesterol [19]. In the present study, SA-supplemented diets (except for SA2) significantly increased TP and HDL-c contents of fish and significantly decreased TG and GLU levels of fish. These results indicated adding moderate SA in diets had positive effects on hypolipidemic and hypoglycemic for juvenile golden pompano. AST and ALT are considered useful indices to estimate the status of liver damage [31]. Generally, increase of AST and ALT levels indicate dysfunction in liver [19]. In this study, AST and ALT contents first decreased and then increased with the incremental levels of dietary SA, which suggested appropriate SA levels heightened the protection for liver function of *Trachinotus ovatus*.

SCFAs can cause beneficial effects on immune response and inflammation process [32]. Many studies have confirmed vital effects of C3, C4, and IgM on immune system in fish ([19, 33, 34]). A recent study reported butyrate increased complement and IgM levels as well as upregulating the mRNA level of immune-related genes [7]. The study of common carp revealed that alkaline phosphatase and total immunoglobulin contents of skin mucus remarkably increased when fish fed SA-supplemented diets [4]. In the present study, dietary SA level significantly increased C3, C4, and IgM contents, which indicated that appropriate supplement levels of SA enhanced immune response of golden pompano. The immune system and antioxidant system go hand in hand. Oxidative stress means the dynamic imbalance of redox in organisms, and it can cause overproduction of reactive oxygen species (ROS) and decrease of antioxidative enzyme activities as well as contributing to oxidative damage [35]. Oxidative stress is regarded as an important factor leading to disease and death in aquatic animals [36]. In fish, the antioxidant systems include nonenzymatic compounds and antioxidant enzymes (SOD, T-AOC, POD, glutathione reductase, and glutathione peroxidase), which play crucial roles in resisting oxidative stress [37]. SOD can decrease the level of ROS via catalyzing the highly reactive superoxide radical to H_2O_2 [19]. POD is deemed to participate in eliminating H_2O_2, and T-AOC is used to assess antioxidant ability of fish [19, 38]. MDA is a major production of lipid peroxidation, and its content can evaluate the status of lipid peroxidation and cell injury in organisms [39]. In this study, SA-supplemented diets can effectively increase hepatic SOD and T-AOC activities and decrease hepatic MDA contents of golden pompano. These findings indicated that moderate SA levels in diets enhanced antioxidative ability of golden pompano. A study on common carp also showed SA diets significantly upregulated the expression of genes related to antioxidant defence such as glutathione S-

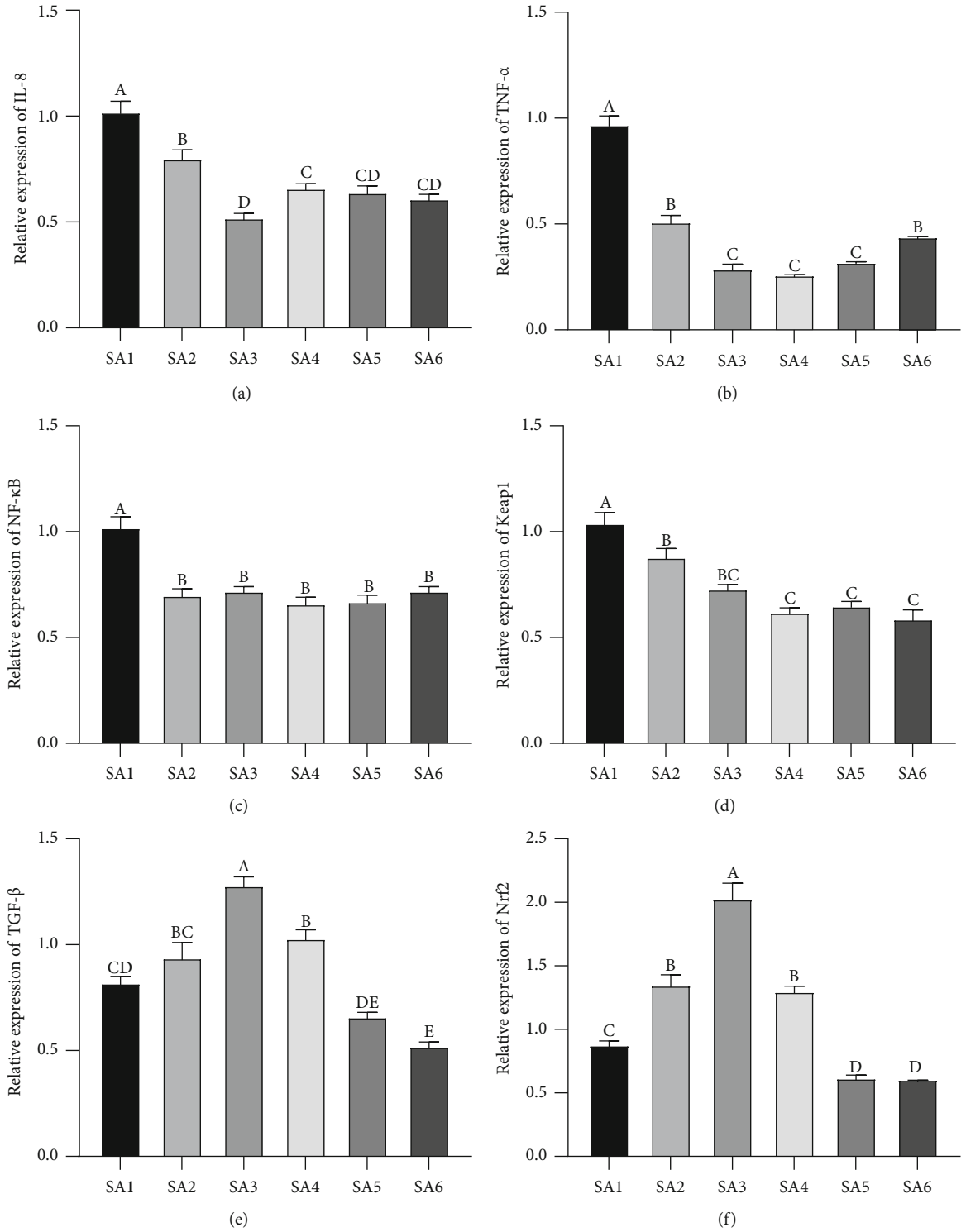


Figure 3: Relative expression of immune-related genes in spleen of juvenile golden pompano fed diets containing graded levels of sodium acetate (SA). Different superscript letters above the bar represent significant differences between treatments ($p < .05$).

transferase and glutathione peroxidase [4]. Similarly, sodium propionate upregulated the expression of SOD and CAT genes in zebrafish [2]. The spleen of fish is a vital organ, which plays an important role in immunity [40]. Our study showed that appropriate supplement levels of dietary SA significantly downregulated expression of Keap1 gene and upregulated expression of Nrf2 gene in spleen of golden pompano. Nrf2, a key transcription factor involved in the combining to antioxidant response elements and regulating the expressions of antioxidative enzyme genes, can protect organisms from oxidative stress [41]. Keap1, serving as a Nrf2-binding protein, can suppress expression of Nrf2 to

attenuate cells antioxidative ability [42]. The results implied that moderate supplement levels of dietary SA promoted antioxidative ability and reduced the oxidative stress of golden pompano. Oxidative stress can cause inflammation and vice versa. Inflammation is an essential organismal defensive mechanism which was induced by chemical, physical, and biotic stimuli [43]. Recent studies found SCFAs involved in regulation of proinflammatory and anti-inflammatory mediators through multiple ways. Sodium butyrate diets stimulated downregulation of the intestinal proinflammatory cytokines (such as IL-1β) and upregulation of the intestinal anti-inflammatory (such as IL-10 and TGF-β) in Pengze crucian carp (*Carassius auratus* Pengze) [7]. Administration of microencapsulated sodium butyrate in common carp is shown to increase the immunoreaction by regulating the expressions of gut proinflammatory and anti-inflammatory cytokines [44]. In the research of common carp, the mRNA levels of immune-related genes in the intestine had an upward trend when fish fed SA diets [4]. In the current study, appropriate levels of SA significantly increased expression of anti-inflammatory TGF-β cytokine and decreased expressions of proinflammatory IL-8 and TNF-α cytokines. The NF-κB signaling pathway participates in innate and adaptive immune responses as well as regulating inflammatory cytokines [41]. Recent studies showed the anti-inflammatory mechanism of SCFAs was associated with the NF-κB pathway [14, 45]. Diets containing 200 mmol/L SA significantly suppressed intestinal NF-κBp65 phosphorylation on Nile Tilapia [14]. Consistent with previous study, our results showed that a significant downregulation was observed on expressions of splenic NF-κB when fish fed SA-supplemented diets. These findings indicated that dietary SA relieved inflammation and enhanced immune response of juvenile golden pompano.

Interestingly, when supplement levels of SA reached SA5 (2000 mg/kg) or SA6 (4000 mg/kg), the growth performance, fillet quality, and immune function presented adverse changes for the health of golden pompano. It has been reported that the administration of exogenous acidifying agents such as acetic acid may cause disorders of acid-base balance and metabolic acidosis [46], which inferred that excessive SA would cause a toxic effect and damage physiological function of juvenile golden pompano. A previous study reported that excessive sodium butyrate decreased the growth performance of juvenile turbot [47], which supported our study. However, dietary SA did not result in the adverse changes in the studies of Arctic charr (*Salvelinus alpinus*) [11] and Nile tilapia (*Oreochromis niloticus*) [14]. This may be because the maximum supplement level of SA in these studies was lower than that in our study, which did not reach the threshold for toxicity. The specific mechanism still needs to be determined.

5. Conclusions

Appropriate supplement levels of dietary SA promoted growth and fillet quality of juvenile *Trachinotus ovatus*. The optimum supplement level of SA was explored to be 1423.67 mg/kg for juvenile golden pompano based on a quadratic regression model of WGR. Additionally, moderate supplement levels of SA improved antioxidative ability and immune response as well as decreasing plasmatic glucose and lipid contents of juvenile golden pompano. Excess SA in diets inhibited the growth and reduced the immune function of juvenile *Trachinotus ovatus*.

Data Availability

Data were available from the corresponding authors by reasonable request.

Conflicts of Interest

All authors declare no conflict of interest.

Authors' Contributions

P. Xun and H. Lin devised the research experiment. P. Xun performed most of the trial work and wrote the manuscript under the direction of C. Zhou. X. Huang, Z. Huang, W. Yu, Y. Yang, and T. Li revised the manuscript. J. Huang and Y. Wu assisted with the diet production and sample determination.

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