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Effects of drinking slightly acidic electrolyzed water on the growth health, blood physiology, and intestinal development in mice

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Slightly acidic electrolyzed water (SAEW) as a disinfectant might be ingested by humans and animals along with food and drinking water. The present study explored whether adding SAEW to drinking water affects the drinking water intake, food intake, body weight, intestinal morphology, digestive enzyme activity, and antioxidant enzyme activity of mice. This will provide a theoretical basis for the biosafety of SAEW as a food and drinking water disinfectant. The present study selected and randomly divided 96 4-week-old Kunming mice into three groups: control, 0.5 mg/L SAEW, and 5.0 mg/L SAEW groups. The experiment lasted 60 days. The present study showed that drinking SAEW had no noticeable effect on the food intake, water intake, body weight, and blood biochemical indexes of mice. SAEW significantly increased the plasma superoxide dismutase inhibition rate, glutathione content, and total antioxidant capacity of mice ($p < 0.05$). The fructose-6-phosphate kinase, isocitrate dehydrogenase, and malate dehydrogenase activities of mice in the SAEW group were significantly increased. Furthermore, SAEW promoted the morphological development of the small intestine and increased the activities of amylase, lipase, and protease. Therefore, the 0.5 and 5.0 mg/L SAEW was beneficial for mice, and improved the body's antioxidative, glucose-metabolizing, and digestive ability.

Keywords Slightly acidic electrolyzed water, Mice, Digestive, Antioxidant capacity, Metabolize glucose, Health

The ever-growing human population has increased the food demand[1]. Animal-based protein is important for the human diet. Due to the occurrence of outbreaks during processing, slaughter and production, animal products are often contaminated by pathogens such as *Escherichia coli*[2], *Salmonella*[3,4], *Listeria monocytogenes*[5,6] and *Campylobacter jejune*[7]. Alexis also find that in the case of low demand elasticity for livestock meat, the presence of an animal pathogen causing production losses[8]. Food safety is the concern of people all over the world. In order to guarantee the quality of food, it is urgent to develop a disinfection technology that fewer affects the sensory properties of food. in the meantime, Electrolytic water is a better alternative technology[9].

Slightly acidic electrolyzed water (SAEW) is also known as oxidation potential water. The dilute hydrochloric acid solution, or/and low-concentration sodium chloride solution is electrolyzed in a non-diaphragm electrolytic cell by using electrochemical methods. The pH value and redox potential of the solution can also be changed. Both active chlorine and reactive oxygen species produced in the solution is able to kill microorganisms[10]. Slightly acidic electrolyzed water (SAEW) is a highly safe sterilizing water that consists of an aqueous solution of hypochlorous acid (HOCl)[11].

At present, SAEW is widely used in food and drinking water disinfection[12,13]. SAEW can effectively kill bacteria and microorganisms on the surface of eggshells, with a killing rate of over 70%. It was not only effective in reducing or eliminating *Salmonella enteritidis* and *E. coli*on shelled eggs, but also could maintain the fresh egg quality during storage[14]. The combined ultrasound and SAEW application reduced the bacterial counts of chicken breast during pre-chilling, thereby effectively reducing enterobacteria, mesophilic bacteria, and lactic acid bacteria ($p < 0.05$)[15]. *Vibrio parahaemolyticus* biofilms on crab and shrimp surfaces were eliminated using ultraviolet C irradiation coupled with a mixture of NaClO and SAEW[16]. Pathogenic vegetative cells were

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completely inactivated within 1 min of treatment in SAEW with 20 ppm[17]. The addition of 0.3, 0.5, 0.7 and 1.0 mg/L of SAEW to the drinking water of broilers can effectively improve their production performance, reduce abnormal behaviors[18], improve their immune functions, and also reduce their intestinal *E. coli* and *Salmonella* population[19].

In summary, SAEW can effectively sterilize vegetables, fruits, aquatic products, meat foods, and animal drinking water. However, a great possibility remains that SAEW might enter the human and animal bodies along with food and drinking water. There is no study to date reporting the effects of SAEW entering both animals and humans along with food or drinking water, and also whether it will affect their health. We used the model mice to explore whether adding an appropriate amount of SAEW to drinking water affects the drinking water, food intake, body weight, intestinal morphology, digestive enzyme activity, and antioxidant enzyme activity of mice. This will provide a theoretical basis for the biosafety of SAEW as a food and drinking water disinfectant.

Materials and methods

Ethics statement

Experiments conducted according to the ARRIVE guidelines. Research protocols followed the guidelines stated in the Guide for the Care and Use of Agricultural Animals in Research and Teaching. All procedures and protocols were approved by the Henan University of Animal Husbandry and Economy Institutional Animal Care and Use Committee (HNUAHE 490).

Experimental animals and experimental design

A total of 96 4-week-old Kunming mice (KM mice) (Experimental Animal Center of Zhengzhou, Zhengzhou, China) with an average body weight of 15.2 ± 1.7 g were selected and randomly divided into three groups with four replicates each and eight mice per replicate (four females and four males). The Kunming (KM) mouse is an outbred stock derived from Swiss albino mice with a high heterogeneity of genes and is widely employed in studies on toxicology, immunology, genetics, and pharmacology in laboratories. KM mice have strong disease resistance and adaptability, high reproduction rate and survival rate. And costs are cheap. So, KM mice are suitable experimental animals. The mice were raised in a separate laboratory, at 24.0 ± 1.5 °C, relative humidity of 60.5 ± 1.5%, while avoiding the noise and direct sunlight. During the experiment, the mice in the three groups had free access to both food and water, and were fed SPF grade maintenance mice feed (Synergy Biosciences. Co., Ltd., Hangzhou, China), their litter regularly being changed, and good ventilation. The food intake and water intake of the mice were recorded daily. Individual mice were weighed every 10 days.

Previous reports demonstrated that drinking SAEW with from 0.3 mg/L[20] Acetyl-CoA Carboxylase (ACC) had positive effects on the growth and health of laboratory animals and layer. This study was to demonstrate whether drinking SAEW of 0.5 mg/L or 5.0 mg/L ACC was beneficial to the growth and health of mice. The experimental group was divided into the blank control group that drank sterilized tap water, 0.5 mg/LSAEW group, and 5.0 mg/LSAEW group. The three experimental groups were provided freshly prepared SAEW and sterilized drinking water at 9:00 AM every day. Storage solution of SAEW was produced using a FX-LAEB non-membrane generator (Fangxin Water Treatment Equipment Co., Ltd., Yantai, China) to electrolyze a NaCl solution (1.0 g/L) containing HCl (100 μg/L). The SAEW generated was diluted in sterile deionized water to obtain final concentrations of 0.5 mg/L and 5.0 mg/L. The experimental period was 60 days, time course of experiment as show as Fig. 1. Eight mice being randomly selected in each group for behavioral experiments on day 0, 30, and 60, respectively. On the 30th and 60th day, eight mice in each group were selected to collect their

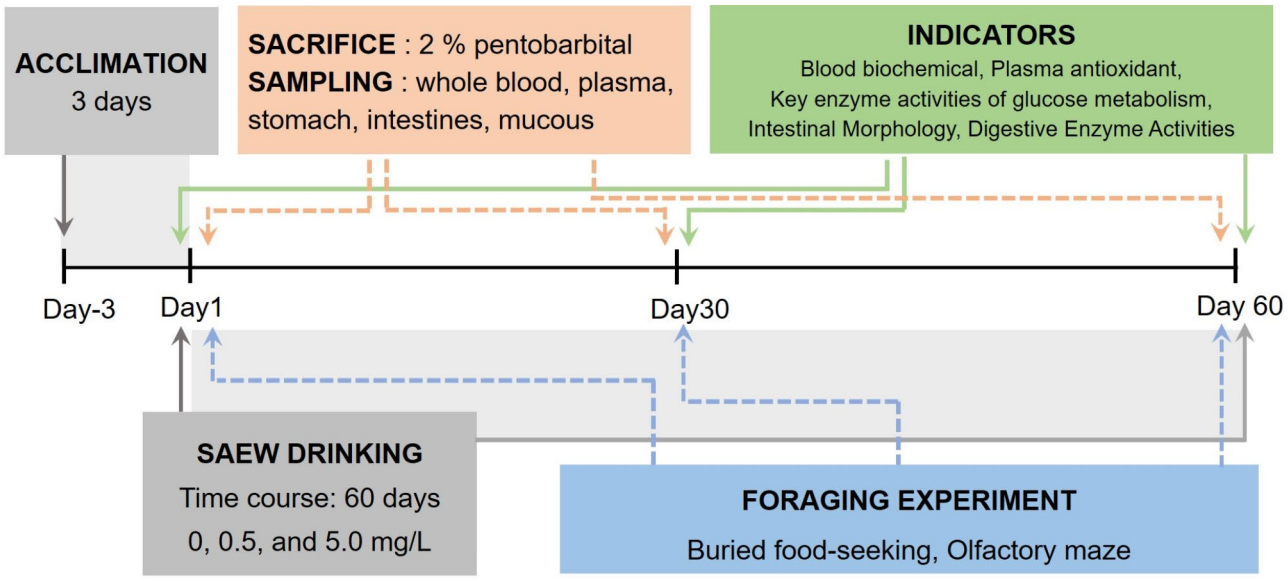

Fig. 1. Time course of experiment.

blood samples, while another eight mice (four females and four males) were selected for sacrifice and sample collection. Time course of experiment as shown in Fig. 1.

Foraging experiment

In order to avoid the stress of mice due to an unfamiliar environment, the foraging test was carried out in the laboratory where mice were raised, with the acclimation experiment being carried out three days in advance. The experiment was carried out at 9:00 every day. Buried food-seeking test and the olfactory maze test are kinds of maze that relies on foraging motivation to induce animals to complete tasks. The animals are fasted for 24 h before the test in order to enhance the test effect and shorten the test time, but they could freely drink water during the fasting period.

In the buried food-seeking test, the cube with a side length of 80 cm is the activity space for mice, with 16 holes with a depth of 2 cm being evenly distributed in the middle, and small mouse food particles were placed in a random hole. The timer was started just after the mouse was put down to stand, and stopped when the mouse grabbed the food with its front paws or gnawed at it. After each mouse was tested, the fecal and urine residues were removed, and the entire experimental device was wiped with an alcohol-soaked cotton ball to remove the odor left by the mouse. The next mouse was tested after it waited for 10 min.

In the olfactory maze test, the circular area is the place for free movement range of the mice, while the black area is the food delivery point blocked by an opaque partition. The food was placed at a random food drop point, and the mouse was put down in the central area. After it stood firm, and the timer was started, and it was finally stopped when the mouse grabs the food with its front paws or gnaws at it. After the test of each mouse, the fecal and urine residues were removed, and the odor left by the mouse was wiped with an alcohol-soaked cotton ball. After waiting for 10 min, the next mouse was tested.

Sample collection and storage

The mice were anesthetized by an intraperitoneal injection of 2% pentobarbital sodium solution at a dose of 0.25 mL/100 g. Using orbital blood sampling method. 0.5 mL of whole blood was collected by pipette for determination of biochemical indicators, immediately. In addition, 1.0 mL of whole blood was collected by a heparin anticoagulant tube for the preparation of plasma. The plasma was prepared at 3000 rpm for 15 min at 4 ℃, for determining the antioxidant indexes and activities of key enzymes of glucose metabolism. The mice were then euthanized by cervical dislocation. Segments of their stomach, duodenum, jejunum, ileum, and colon were fixed in 10% neutral buffered formalin for further hematoxylin and eosin (H&E) staining. The mucosa of the duodenum, jejunum, and ileum were scraped and frozen in liquid nitrogen for measurement of the digestive enzyme activity.

Determination of blood biochemical indicators

The Seamaty V7 automatic blood biochemical analyzer (Beijing Biotechnology Co., Ltd., Beijing, China) was used to measure the biochemical indexes of whole blood, including Na^+, Cl^-, albumin (ALB), globulin (GLB), albumin/globulin (A/G), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), and urea. The reagent tray stored at 4 °C was taken out of the refrigerator and left at room temperature for ~ 20 min before use. When measuring the sample, the pipette was first rinsed, its tip was then dipped below the blood level, and was pushed repeatedly for rinsing. After rinsing, 100 µl of the blood sample was drawn and tilted into the reagent tray to avoid air bubbles.

Plasma antioxidant indicators

The inhibition rate of plasma SOD was determined by the kit of Tongren Institute of Chemistry (S311, Tokyo, Japan), which was the colorimetric method. The inhibition rate of SOD was proportional to the activity of SOD. The TAOC was measured using the kit of Nanjing Jiancheng Bioengineering Institute (A015-1-2, Nanjing, China), with the method being the colorimetric method. Malondialdehyde (MDA) content was determined using the kit of Nanjing Jiancheng Bioengineering Institute (A003-1-1, Nanjing, China), using the Thiobarbituric acid (TBA) method.

Key enzyme activities of plasma glucose metabolism

Hexokinase (HK), fructose-6-phosphate kinase(PFK), pyruvate kinase (PK), malate dehydrogenase (MDH) activities were measured using the Nanjing Jiancheng Bioengineering Institute (A077-3-1, A129-1-1, A076-1-1, A021-2-1, Nanjing, China) kit via the colorimetric method. Isocitrate dehydrogenase (IDH) activity was determined using the kit of Shanghai Jimei Gene Medicine Technology Co., Ltd. (MAK062, Shanghai, China) via the colorimetric method. The index determination was performed according to the corresponding kit instructions.

Stomach and intestinal morphology

After fixation in 10% neutral buffered formalin, a single 0.5-cm sample was cut from each stomach and intestinal section, dehydrated with increasing concentrations (70, 80, 95, and 100%) of ethanol (Analytical pure, 925-93-9, Shanghai Yien Chemical Technology Co., Ltd., Shanghai, China), cleared with xylene (Analytical pure, 1330-20-7, Shanghai Yien Chemical Technology Co., Ltd., Shanghai, China), and placed onto a polyfin embedding wax (Analytical pure, 8002-74-2, Shanghai Yien Chemical Technology Co., Ltd., Shanghai, China). Tissue sections (5 µm) were cut, floated onto slides, stained with hematoxylin (Gill #2, Sigma, St. Louis, MO, USA) and eosin (318906, Sigma St. Louis, MO, USA). The stained sections were examined using a CSOIF 4XC20BD bright-dark field inverted metallurgical microscope (Shanghai Optical Instrument No.5 Factory Co., Ltd., Shanghai, China) and images were processed using an Image-Pro Plus 6.0 software (Media Cybernetics, MD, USA). The

morphometric intestinal parameters crypt depth and villus length (μm) were determined on 15 crypt and villus specimens. Finally, the ratios of villus height-to-crypt depth (V/C) were calculated.

Determination of digestive enzyme activities

Amylase, protease, and lipase activities were determined using ELISA kits (A19300, T22470, L17360, 48T, Shanghai Jizhi Biochemical Technology Co., Ltd., China). The method of determination was colorimetry.

Statistical analysis

The results were presented as means with standard error of the mean and analyzed statistically by one-way analysis of variance (ANOVA) using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Differences among all the treatments were separated by a Tukey test for multiple comparisons. Values of $p < 0.05$ were considered significant. All the graphs were made using GraphPad Prism 8.0 (GraphPad, San Diego, CA, USA).

Results

Effects of SAEW on feed intake, water intake and daily weight gain of mice

Drinking SAEW had no significant effect on the average daily feed intake of all the three groups ($p > 0.05$) (Fig. 2A). Furthermore, drinking SAEW during 0–30 d had no effect on the average water intake of mice. However, during 30–60 d, the water intake of mice in the 5.0 mg/L SAEW group was significantly lower than both the control and 0.5 mg/L SAEW groups ($p < 0.05$) (Fig. 2B). The average body weight of the three groups of mice showed no significant differences at 0 d, 30 d, and 60 d during the experiment ($p > 0.05$) (Fig. 2C).

The effect of SAEW on the foraging behavior of mice

The results of the buried food-seeking test and the olfactory maze test are shown in Fig. 3. Adding different concentrations of SAEW to drinking water had no significant effect on the foraging ability of mice ($p > 0.05$).

The effect of SAEW on blood routine indexes in mice

The blood routine indexes of mice are shown in Table 1. Drinking SAEW had no significant effect on the nine indexes, including Na^+, Cl^-, ALB, GLB, A/G, ALT, AST, TP, and urea in blood ($p > 0.05$).

Effects of SAEW on plasma antioxidant indexes and activities of key enzyme of glucose metabolism in mice

It can be seen from Table 2 that the SOD inhibition rate and TAOC in the plasma of the SAEW group were significantly higher than the control group ($p < 0.05$). The GSH activity was significantly higher than the control group ($p < 0.05$), whereas the MDA activity of the three groups had no significant difference ($p > 0.05$). Compared with the control group, the activities of PFK, IDH, and MDH in the plasma of SAEW groups were significantly increased ($p < 0.05$), but there was no significant difference in the HK activity ($p > 0.05$).

The effect of SAEW on the stomach and intestinal morphology

The effect of drinking SAEW on the stomach and intestinal morphology of mice is shown in Fig. 4. Drinking SAEW significantly promoted the morphological development of the stomach, small intestine, and large intestine, with the villus height and crypt depth in the small intestine also being improved. This result is consistent with the small intestine morphology in Table 3. The measured data is consistent. As compared with the control group, the villus height/crypt depth ratio in the duodenum increased by 31.4% in the 5.0 mg/L SAEW group, while the villus height in the jejunum increased by 14.5%.

Effect of SAEW on digestive enzyme activity

The effect of SAEW on the digestive enzyme activity in intestinal digesta is shown in Table 4. The amylase activities in the duodenum and jejunum of mice in the SAEW groups were significantly higher than in the control group ($p < 0.05$). The lipase activities in the duodenum and ileum of mice in the SAEW groups were

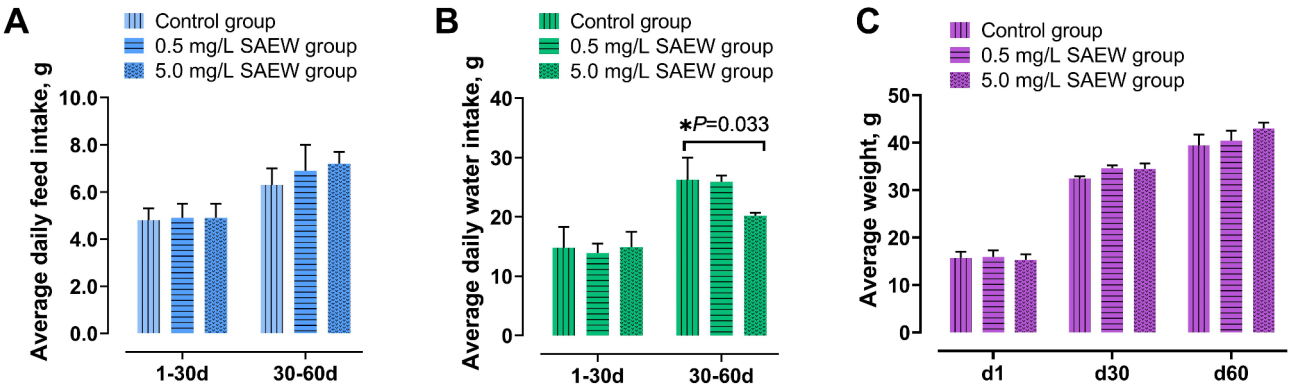

Fig. 2. Effects of SAEW on feed intake, water intake, and daily weight gain in mice.

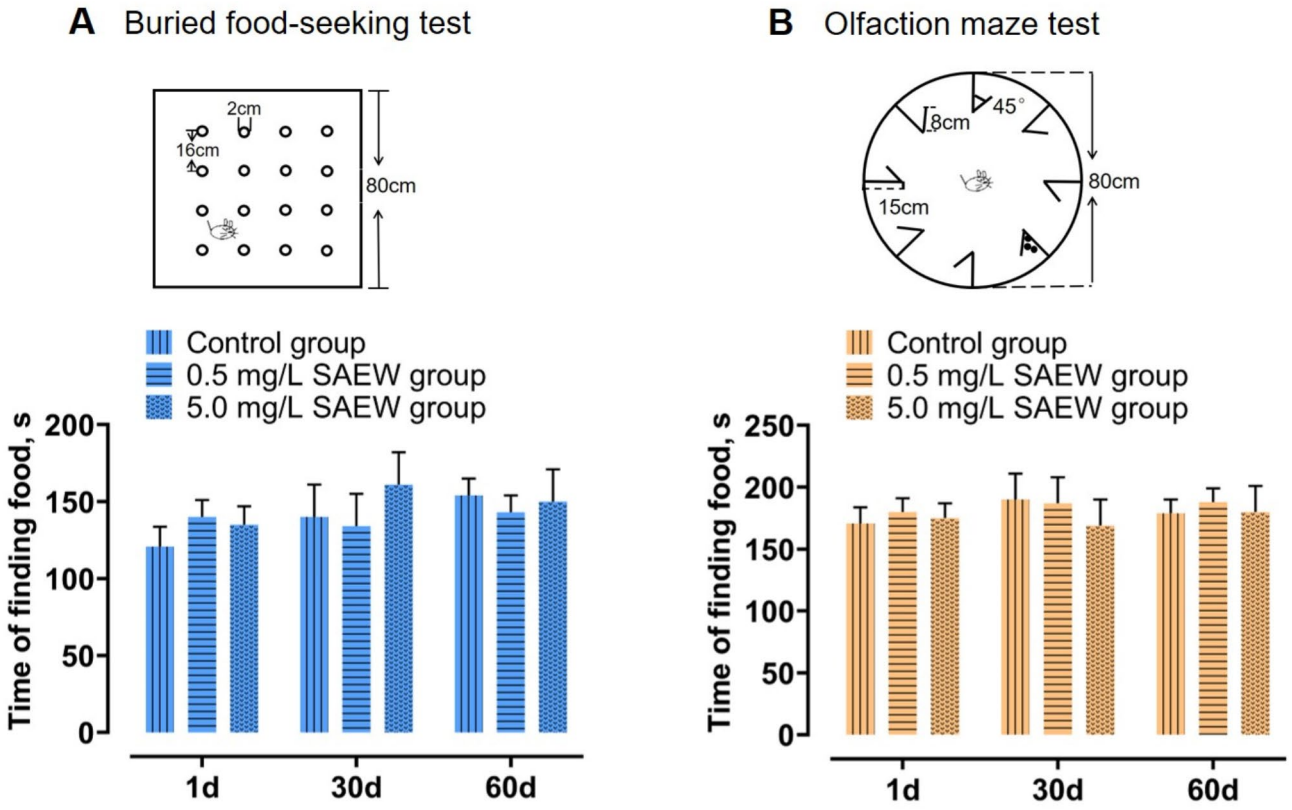

Fig. 3. Buried food-seeking test (**A**) and Olfactory maze test (**B**).

significantly higher than in the control group ($p < 0.05$). The protease activity in the ileum was also significantly higher than the control group ($p < 0.05$).

Discussion

The water intake, feed intake, and body weight of animals are important parameters for judging the growth and health status of animals. Foraging behavior is the basic nature and skill of animals[21]. Although our study results showed that drinking SAEW significantly decreased the water intake of the mice in the 5.0 mg/L group, there was no significant effect on the feed intake, body weight, and foraging behavior of all the three groups. It can be seen that the drinking water containing SAEW does not reduce the animal production performance.

The blood Na^+ and Cl^-concentrations reflect the body's ionic balance and help maintain the osmotic pressure in the body. Their abnormal increase or decrease indicates the imbalance of the osmotic pressure and abnormal ion exchange in animals[22]. ALB and GLB are important indicators of liver and kidney function, and the abnormal A/G ratio indicates either liver or kidney function damage[23]. ALT and AST mainly exist in the cytoplasm and mitochondria of hepatocytes, with their activity levels being directly related to the degree of damage in the hepatocytes or mitochondria. ALT is the most sensitive parameter in acute hepatocyte damage, with it mostly existing in their mitochondria. When the damage of hepatocytes is severe, the AST in the blood increases significantly along with their necrosis and the disintegration of their mitochondria. Therefore, AST can precisely reflect the degree of damage to hepatocytes[24]. TP reflects the synthetic reserve function of the liver, and its decrease indicates that the protein synthesizing ability of the liver has weakened. Urea in blood is a protein derived metabolite, which is excreted via glomerular filtration. It reflects the glomerular filtration rate (GFR)[25]. When the kidneys are substantially damaged, the GFR decreases while the urea concentration increases. In this study, The addition of SAEW did not interfere with the normal metabolism and balance regulation mechanisms of sodium and chloride ions in mice, indicating that it had no negative impact on maintaining the stability of the body's osmotic pressure. Regarding liver and kidney functions, the stability of indicators such as ALB, GLB, A/G ratio, ALT, AST, and TP fully demonstrated that, at normal dosage, SAEW did not cause damage to the liver's protein synthesis, metabolism, and the integrity of hepatocytes, nor did it have an adverse effect on the kidney's filtration function. thereby indicating that SAEW had no adverse effect on the body's ionic balance, liver function, and kidney function.The results of this study suggest that, on the premise of ensuring the normal operation of the body's physiological functions, SAEW is expected to exert its unique effects. However, this study was only a preliminary exploration using a mouse model. In the future, further research on different species, different dosages is needed to comprehensively evaluate the potential impact of SAEW on the health of organisms.

Items	Control group	0.5 mg/L SAEW group	5.0 mg/L SAEW group	*p*-value
Na^+, (mmol/L)				
d30	140.25 ± 1.48	148.30 ± 9.90	137.20 ± 8.91	0.344
d60	125.00 ± 1.41	138.45 ± 4.31	130.60 ± 7.64	0.547
Cl^-, (mmol/L)				
d30	127.35 ± 11.10	114.05 ± 3.46	112.20 ± 0.71	0.688
d60	111.60 ± 2.69	110.60 ± 0.85	111.15 ± 1.20	0.733
Albumin, ALB (g/L)				
d30	30.50 ± 2.12	28.05 ± 0.35	29.35 ± 1.20	0.222
d60	30.95 ± 0.64	30.75 ± 0.92	32.85 ± 0.21	0.423
Globulin, GLB (g/L)				
d30	33.80 ± 1.70	32.35 ± 0.07	31.70 ± 2.83	0.600
d60	27.30 ± 2.69	28.15 ± 2.33	26.90 ± 0.14	0.341
Albumin/ Globulin, A/G				
d30	0.84 ± 0.01	0.87 ± 0.01	0.83 ± 0.04	0.086
d60	1.11 ± 0.04	1.10 ± 0.12	1.12 ± 0.01	0.073
Alanine aminotransferase, ALT (U/L)				
d30	85.50 ± 4.95	82.50 ± 4.35	86.00 ± 8.28	0.887
d60	91.50 ± 3.54	82.50 ± 10.61	95.00 ± 6.00	0.788
Aspartate aminotransferase, AST (U/L)				
d30	80.22 ± 3.09	77.32 ± 1.87	78.80 ± 4.57	0.998
d60	69.50 ± 5.50	71.24 ± 3.66	72.56 ± 3.61	0.883
Total protein, TP (g/L)				
d30	64.55 ± 0.78	60.40 ± 0.28	61.05 ± 4.03	0.520
d60	61.15 ± 0.35	58.90 ± 1.41	59.75 ± 0.07	0.589
Urea (mol/L)				
d30	9.92 ± 0.57	9.72 ± 0.11	9.99 ± 0.21	0.822
d60	9.33 ± 0.45	10.03 ± 0.01	9.26 ± 0.87	0.777

Table 1. Effects of SAEW on the blood physiology in mice ($n = 24$). Different lowercase letters in the same row indicate significant differences between groups ($p < 0.05$), while no letters or the same letters indicate insignificant differences ($p > 0.05$). The same notation applies to Tables 2, 3 and 4.

Items	Control group	0.5 mg/L SAEW group	5.0 mg/L SAEW group	*p*-value
Antioxidant-related enzymes				
SOD inhibition rate, (%)	75.53 ± 3.44^b	88.00 ± 10.11^a	89.33 ± 9.09^a	0.007
MDA, (nmol/mL)	1.66 ± 0.34	1.75 ± 0.27	1.75 ± 0.21	0.823
T-AOC, (U/mL)	0.85 ± 0.14^c	1.40 ± 0.22^b	2.05 ± 0.28^a	0.004
GSH, (μmol/L)	81.77 ± 11.09^b	108.88 ± 21.47^a	109.66 ± 14.98^a	0.039
Key enzymes of glucose metabolism				
HK, (U/ L)	74.30 ± 9.33	78.64 ± 7.28	76.55 ± 6.20	0.081
PFK, (U/mL)	4.84 ± 1.24^b	11.07 ± 1.67^a	10.93 ± 2.35^a	0.003
IDH, [nmol/(min•mg)]	6.39 ± 1.02^b	14.14 ± 0.88^{ab}	18.27 ± 2.02^a	0.002
MDH, (U /mg)	29.50 ± 1.33^b	32.27 ± 14.15^a	36.00 ± 6.20^a	0.007

Table 2. Effects of SAEW on the antioxidant-related enzymes and key enzymes of glucose metabolism in plasma of mice ($n = 24$).

The redox reaction is a key physiological and biochemical process in the body, generating various active molecules such as free radicals. A proper level of free radicals is crucial for normal body functions, promoting thyroid hormone synthesis, stimulating phagocyte bactericidal activity, and regulating signal transmission. But excessive free radicals have a strong affinity for major biological macromolecules like proteins, carbs, fats, nucleic acids, etc., causing their cross-linking, degradation or structural changes, leading to abnormal physiological states. Oxidative damage from excessive free radical production is oxidative stress. When humans and animals are in a state of stress for a long time, it causes multiple diseases, like human diabetes, cardiovascular disease, inflammation, etc. The main hazards for farmed animals include reduced growth performance, antioxidant performance, and immunity[26]. The animal body contains diverse antioxidants, such as small molecules, macromolecules, and enzymes. These antioxidants scavenge reactive oxygen species produced in redox reactions

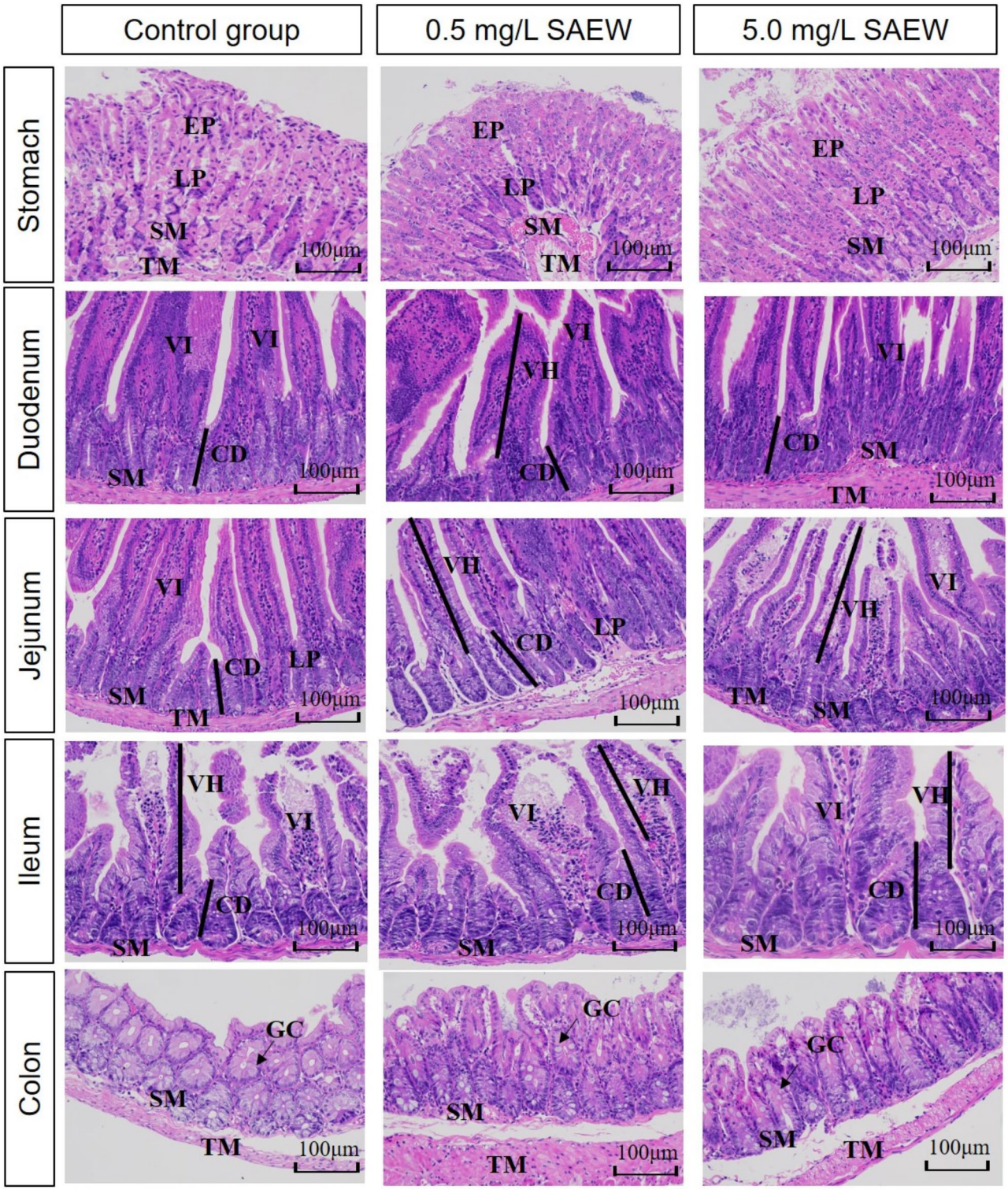

Fig. 4. Photomicrographs of stomach and intestinal tract cross-cutting of mice stained with hematoxylin-eosin under light microscopy. Abbreviation: villi (VI), tunica muscularis (TM), villus height (VH), crypt depth (CD), sub-epithelial mucosa (SM), goblet cell (GC), lamina propria (LP) and epithelium (EP).

to protect against oxidative stress-induced damage[27]. SOD, MDA, TAOC, and GSH are key elements of the body's innate antioxidant defense. SOD, an antioxidant enzyme, can dismutate superoxide anion into $H_2O_2$and neutralize its toxicity. MDA, the end-product of lipid peroxidation, indicates the body's peroxidation level. TAOC represents the body's overall antioxidant capacity, while GSH scavenges free radicals within the body[28]. Zhao[29] reported that Stevia residue extract (SRE) scavenged various free radicals and significantly increased TAOC

Item	Control group	0.5 mg/LSAEW group	5.0 mg/L SAEW group	*p*-value
Duodenum				
Villus height (μm)	53.92 ± 22.37[b]	66.74 ± 24.64[a]	69.16 ± 19.78[a]	0.006
Cypt depth (μm)	46.79 ± 10.65	49.72 ± 14.90	44.01 ± 11.03	0.067
Villus height/crypt depth ratio	1.21 ± 0.07[c]	1.38 ± 0.08[b]	1.59 ± 0.10[a]	0.030
Jejunum				
Villus height (μm)	42.12 ± 1.30[b]	48.83 ± 2.86[a]	48.27 ± 1.32[a]	0.038
Crypt depth (μm)	27.51 ± 3.03	30.92 ± 1.02	30.21 ± 1.55	0.349
Villus height/crypt depth ratio	1.55 ± 0.16[b]	1.65 ± 0.17[a]	1.61 ± 0.21[ab]	0.032
Ileum				
Villus height (μm)	38.56 ± 12.49	38.91 ± 14.62	38.29 ± 16.62	0.971
Cypt depth (μm)	28.87 ± 13.12	28.92 ± 15.07	27.01 ± 14.34	0.889
Villus height/crypt depth ratio	1.35 ± 0.05	1.35 ± 0.07	1.40 ± 0.08	0.764

Table 3. Effects of SAEW on the intestinal morphology in mice ($n = 24$).

Items	Control group	0.5 mg/L SAEW group	5.0 mg/L SAEW group	*p*-value
Amylase (U/mL)				
Duodenum	910.11 ± 29.0[c]	1152.21 ± 38.02[a]	1056.77 ± 22.90[b]	0.026
Jejunum	879.56 ± 38.87[b]	1070.02 ± 67.89[a]	1076.66 ± 37.24[a]	0.034
Ileum	681.07 ± 43.22	686 ± 61.10	699.22 ± 71.37	0.245
Lipase (U/L)				
Duodenum	167.89 ± 23.81[b]	185.66 ± 10.75[a]	188.00 ± 24.53[a]	0.038
Jejunum	197.09 ± 11.15	199.08 ± 14.03	196.15 ± 10.24	0.845
Ileum	81.29 ± 4.92[b]	114.77 ± 10.08[a]	107.40 ± 10.10[ab]	0.039
Protease(U/mL)				
Duodenum	251.51 ± 10.09	321.60 ± 20.35	291.55 ± 23.65	0.322
Jejunum	181.61 ± 10.17	178.54 ± 9.16	180.45 ± 100.11	0.941
Ileum	141.89 ± 9.23[a]	151.88 ± 9.12[b]	156.32 ± 11.13[b]	0.042

Table 4. Effects of SAEW on digestive enzyme activity in intestinal Chyme ($n = 24$).

content and SOD activity, SRE can improve antioxidant status in mice. Zhang[30] found that the supplementation of chlorogenic acid in piglet diets increased GSH activity, and improved the antioxidant status of piglets. Similarly, our study found that drinking SAEW could significantly increase the plasma SOD inhibition rate, GSH content, and TAOC of mice. It is possible that slightly acidic electrolyzed water contains a variety of reactive oxygen species, and the increase in the concentration of reactive oxygen species in the animal body can stimulate the enhancement of antioxidant gene expression to some extent. These suggested that drinking SAEW can improve the antioxidant capacity of mice. These findings strongly suggest that incorporating SAEW into the drinking water of mice can effectively improve their antioxidant capacity. This improvement in antioxidant capacity may have far-reaching implications for the overall health and well-being of the mice, potentially protecting them from a variety of oxidative stress-related diseases and conditions, and promoting their normal physiological functions and growth.

HK catalyzes the phosphorylation of glucose to glucose-6-phosphate, which consumes one ATP molecule. PFK catalyzes the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, by using one ATP molecule. The tricarboxylic acid (TCA) cycle occurs in the mitochondria. In this cycle, isocitrate is oxidatively decarboxylated to form α-ketoglutarate. This is the first decarboxylation reaction in the tricarboxylic acid cycle that is catalyzed by IDH and accompanied by the generation of NADH. MDH catalyzes the dehydrogenation of malate to oxaloacetate, which is accompanied by NADH formation. Changes in the activities of key enzymes of glucose metabolism in the blood reflect the physiological state of glucose metabolism in the body to a certain extent[31,32]. In this experiment, as compared with the control group, the plasma PFK, IDH, and MDH activities of mice in the drinking SAEW group significantly increased, thus indicating that SAEW improved the body's ability to metabolize glucose. Improving the glucose metabolism ability means that the body's cells can acquire and utilize glucose for oxidative decomposition more quickly, thereby enhancing energy supply. SAEW enables more efficient decomposition of nutrients such as carbohydrates in food into monosaccharides like glucose, and promotes the subsequent intracellular metabolism of glucose. This is due to SAEW's regulation of the activities of enzymes in the intestine, such as amylase, lipase, and protease, which is consistent with the results of this study.

The intestine is the main site of nutrient absorption, and the health of villi is a key factor influencing it. In the present study, drinking SAEW significantly increased the villus height/crypt depth ratio, thereby promoting intestinal development. Given the scarcity of existing research in this area, the present study postulates that

SAEW might augment the length of intestinal villi by exerting an impact on the processes of proliferation and apoptosis within intestinal epithelial cells. Insufficient production of gastric acid and pancreatic enzymes limits the digestive and absorptive capacity of the intestinal system[33]. Hao[34] found that SAEW reduced the effects of enteric pathogens on piglet water, feed intake and diarrhea. SAEW also increased the amylase, lipase, and protease activities compared with the control group. Wang[35] found that SAEW can adjust the intestinal pH of broiler chickens. SAEW may increase the activities of amylase, lipase and protease by regulating the intestinal pH.

Conclusions

Ingestion of slightly acidic electrolyzed water (SAEW) had no noticeable effect on the food intake, water intake, body weight, and activity of mice. Furthermore, it could significantly increase the plasma SOD inhibition rate, GSH content, and TAOC of mice. The PFK, IDH, and MDH activities of mice in the SAEW groups were significantly increased. It also promoted the morphological development of the small intestine and increased the activities of amylase, lipase, and protease. These indicate that SAEW significantly improved the antioxidant, glucose-metabolizing, and digestive abilities of mice.

Data availability

Data is provided within the manuscript.

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Author contributions

All of the authors have contributed to and reviewed the manuscript. Conceptualization, Z.S. and L.X.; Methodology, Z.S., S.G.; Software, Z.Y.S.; Validation, P.C. and X.W.; Formal Analysis, Z.S.; Investigation, Z.Y.S; Resources, L.X.; technical support, Z.Y.;Data Curation, X.W.; Writing – Original Draft Preparation, Z.S. ,S.G.and Z.Y.S.; Writing – Review & Editing, L.X. and P.C.; Visualization, Z.S.; Supervision, L.X.; Project Administration, L.X. and Z.S.; Funding Acquisition, L.X. and Z.S.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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