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Evaluation of the prevention of fat accumulation efficacy of sweet potato fermented products *in vivo*

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Abstract

Over-nutrition rather than under-nutrition is an important public health challenge in some developed countries. However, the under-nutrition is a major problem according to the global perspective. Therefore, the research and development (R&D) of agricultural functional materials or products for the prevention of fat accumulation is urgently needed. In this experiment, Sprague Dawley (SD) rats in the normal control group were fed with the normal composition for 8 weeks during the experiment. SD rats in the negative control group and three sweet potato fermented products (SPFP) groups were fed a high fat diet for 8 weeks during the experiment. According to the experimental design, three doses SPFP [250, 500, and 1,000 mg/kg body weight (BW)] will be administered after 4 weeks of feeding the high fat diet. During the experiment, BW of the SD rats was recorded every week and blood, liver, and body fat were collected for analysis of body fat rate, blood lipid content, blood glucose content, liver lipid content, and liver and renal functions. Based on the results, the consumption of SPFP does not affect liver and kidney functions, indicating that SPFP is a safe and edible agricultural material. BW change of the normal control group was significantly lower than that of the negative control group and three SPFP groups ($p < 0.05$). In addition, there was no significant difference in the BW change rate among the groups eat the high fat feed ($p > 0.05$), but the trend of BW change rate in the low and middle doses of SPFP groups was lower than that in the other high fat feed groups. The food utilization rate of the high fat diet group was significantly higher than that of the normal diet group ($p < 0.05$). The body fat rate of the normal control group was significantly lower than that of the high fat feed groups ($p < 0.05$). There was no significant difference between the high fat feed groups ($p > 0.05$). However, the trend showed that the body fat rate of the low and middle doses of SPFP groups were lower than that of the negative control group and the high dose of SPFP group. In addition, the results of other measurement indicators such as blood lipid content, blood glucose content, and liver lipid content did not show any negative effects of SPFP. Based on the above results, although SPFP on the prevention of body fat accumulation was not significantly exhibited, however, the trend shows that the low and middle doses of SPFP can decrease body fat production. Taken these results together, SPFP may has the potential for the prevention of fat accumulation.

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1. Introduction

At present, the over-nutrition rather than under-nutrition is an important public health challenge in some developed countries. However, the under-nutrition is a major problem according to the global perspective. In developing countries, many farmers are highly dependent on root and tuber crops and these are their sources of food, nutrition, and cash income. Currently, the sweet potato has become an important protein source for providing lots of the world's population. The sweet potato also plays a significant role to fight against vitamin A deficiency, is public health significance in developing countries. Additionally, the sweet potato is an excellent source of carotenoid. Therefore, β-carotene is also an important nutrition component in the sweet potato [1].

An imbalance between energy intake and energy consumption can cause obesity. Obesity is a risk factor for the disruption of lipid metabolism. In Taiwan, the rate of over-weight and obesity among adults over the age of 18 is increasing year by year. The average over-weight rate in 2016-2019 reached 47.9%. It is worth noting that, in terms of age groups, the obesity rate of males aged 35-44 is as high as 66.6%. In addition, there will be infertility and increased risk of prostate cancer and other cancers, which should not be ignored [1-2].

Metabolic syndrome related to the disruption of lipid metabolism increases the risk of cardiovascular disease and type 2 diabetes. Metabolic syndrome is a growing social problem, and the efficacy of various functional foods such as green tea, coffee, and soybean on metabolic syndrome has been evaluated. Commonly, the clinical criteria of metabolic syndrome parameters included body weight, triglycerides, high-density lipoprotein cholesterol in serum, and blood pressure [2-3].

In Taiwan, among the top ten causes of death in 2020, there are cancer, heart disease, cerebrovascular disease, diabetes, accident injury, hypertensive disease, chronic lower respiratory disease, nephritis, nephrotic syndrome and nephropathy, chronic liver disease and liver cirrhosis. The World Health Organization points out that obesity is a chronic disease and calls for attention to the health hazards of obesity. The health hazards of obese-bearing people are more three times than diabetes-, metabolic syndrome-, and dyslipidemia-, and two times than hypertension-, cardiovascular disease-, knee arthritis-, and gout-bearing people. Studies have confirmed that when an obese-bearing person loses more than 5% of their body weight (BW), it can bring many health benefits, and obesity-related diseases such as hypertension and diabetes will be improved. It is expected that food materials having preventive effects on the disruption of lipid metabolism will be developed in future [1-4]. Therefore, the research and development (R&D) of the agricultural functional materials or products for the prevention of fat accumulation is urgently needed.

2. Material and methods

2.1. Chemicals and Reagents

Phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No. P3813), saline (Taiwan Biotech Co., LTD, Cat. No. 100-120-1101), Zoletil 50 (Virbac, Carros, France), Cholesterol assay kit - HDL and LDL/VLDL (Cat. No. ab65390; abcam, MA, USA), Free fatty acid assay kit - Quantification (Cat. No. ab65341; abcam, MA, USA). Triglyceride colorimetric assay kit (Cat. No. 10010303; Cayman, MI, USA), GOT (FUJI DRI-CHEM SLIDE GOT/AST-PIII; FUJIFILM, Japan), GPT (FUJI DRI-CHEM SLIDE GPT/ALT-PIII; FUJIFILM, Japan), BUN (FUJI DRI-CHEM SLIDE BUN-PIII; FUJIFILM, Japan), CRE (FUJI DRI-CHEM SLIDE CRE-PIII; FUJIFILM, Japan), TCHO (FUJI DRI-CHEM SLIDE TCHO-PIII; FUJIFILM, Japan), TG (FUJI DRI-CHEM SLIDE TG-PIII; FUJIFILM, Japan), GLU (FUJI DRI-CHEM SLIDE GLU-PIII; FUJIFILM, Japan), HDL (HDL-EX N; Denka Seiken, Japan), LDL (LDL-EX N; Denka Seiken, Japan), and T-PER™ Tissue protein extraction reagent (Cat. No. 78510; Thermo Fisher, Taipei, Taiwan) were applied in this experiment.

2.2. Source of Sweet Potato Fermented Products

Sweet potato fermented products were kindly provided by Assistant Professor Dr. Ying-Chen Lu (Department of Food Sciences, National Chiayi University) and Assistant Researcher Po-Hsien Lu (Chiayi Agricultural Experiment Station). Their products contained lactic acid bacteria [4×10^8 colony-forming unit (CFU) / g].

2.3. Experimental Animals and Experimental Design

Adult male 60 Sprague Dawley (SD) rats [6 weeks old; BW between 150-160 g] with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). The environment was

maintained room temperature (24-27 °C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-110060 approved by the IACUC ethics committee. The male SD rats were divided respectively into as the normal control group (n = 12), the negative control group (n = 12), the high dose (1,000 mg/kg BW) of sweet potato fermented product (SPFP) group (n = 12), the middle dose (500 mg/kg BW) of SPFP group (n = 12), and the low dose (250 mg/kg BW) of SPFP group (n = 12). Except for the normal control group, other groups were administrated with the high-fat feed (45% kcal high-fat diet; D12451; OpenSource Diets, New Brunswick, NJ, USA). SD rats in the normal control group were fed with standard laboratory diet (No. 5053, LabDiet®; PMI Nutrition International, St. Louis, MO, USA). All SD rats were administrated with distilled water ad libitum during the experimental period. The clinical behaviors, BW, the percentage of food utilization, the percentage of body fat, the concentrations of blood lipids, liver lipids, and blood sugar, and the liver and kidney function were monitored and performed during the experiment.

2.4. Changes of BW

All SD rats were weighed once a week during the experiment. The calculation formula of BW change is SD rats' BW (g) at the end of the experiment – SD rats' BW (g) at the beginning of the experiment.

2.5. The Rate of Food Utilization

During the experiment period, the feed intake was measured once a day, and the daily feed intake of each SD rat was controlled to be the same. The calculation formula of the rate (%) of food utilization is [SD rats' BW gain (g) / total feed intake (g)] × 100%.

2.6. The Rate of Body Fat

The SD rats were anesthetized with Zoletil 50. The total fat nears the testis, kidneys and mesentery was carefully removed, weight, and calculate the rate (%) of body fat. The calculation formula of percentage (%) of body fat is [total body fat weight (g) / SD rat's BW (g)] × 100%.

2.7. The Concentrations of Blood Lipids and Blood Sugar

The SD rats were anesthetized with Zoletil 50. At the experimental points, the blood of the celiac aorta was collected and centrifuged (4 °C, 800 ×*g*, and 20 mins). The sera of SD rats were collected and detected the blood lipids [triglycerides, non-esterified free fatty acids, total *cholesterol*, and LDL (low-density lipoprotein) cholesterol and H*DL* (high-density lipoprotein) *cholesterol*] and blood sugar concentrations.

2.8. The Concentrations of Liver Lipids

The SD rats were anesthetized with Zoletil 50. At the experimental points, the liver was collected and homogenized. The lipid concentrations of triglycerides and total *cholesterol* in the liver homogenized tissues were detected.

2.9. The Function Indexes in Liver and Kidney

The SD rats were anesthetized with Zoletil 50. At the experimental points, the blood of the celiac aorta was collected and centrifuged (4 °C, 800 ×*g*, and 20 mins). The sera of SD rats were collected and detected the concentrations of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), uric acid, and creatinine.

2.10. Statistical Analysis

The data were expressed as mean ± SD. All comparisons were made by one-way ANOVA (Analysis of Variance) and Duncan's multiple range test. All significant differences are reported at $^{*}p < 0.05$.

3. Results

3.1. Changes of SD Rats' BW via Administration of Sweet Potato Fermented Products in All Groups

The sweet potato fermented products were orally administrated to SD rats by gavage in the three SPFP groups. In this study, the clinical behavior observation indexes of SD rats in each group were normal during the experiments. During the experiments, the SD rats in each group had smooth hair, normal hair color, and the normal activity. Moreover, all SD rats were survival until the end of the experiments. The survival percentage of SD rats was 100% (60/60) (data not shown). The BW of SD rats was detected on week 0 (W0), W1, W2, W3, W4, W5, W6, W7, and W8. The W0 was SD rats

entering the animal room. The W1 to W4 were focused on the fat accumulation induction. The W5 to W8 were three doses of SPFP administration. During the experiments, the SD rats' BW continued to rise. There was no statistically significant difference in BW between all groups within W1 to W3. Until W4, the SD rats' BW in the negative control group and three SPFP groups were significant higher than the normal control group ($p < 0.05$) (Figure 1A). In addition, the change of BW (g) at the end of the experiment in the negative control group and three SPFP groups were also significant higher than the normal control group ($p < 0.05$) (Figure 1B).

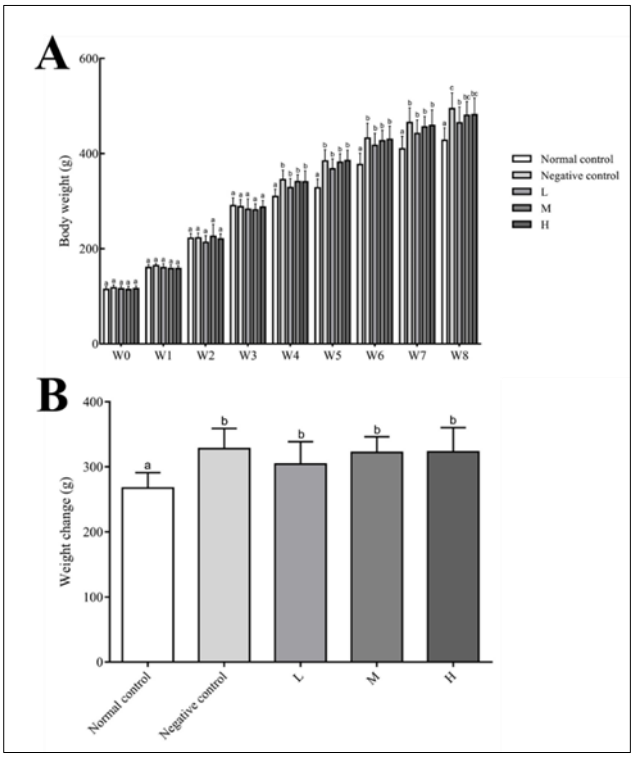

Figure 1 Change of body weight (BW) before and after the treatments of three doses of sweet potato fermented products. (A) Change of BW during the experiment. (B) Change of BW in the end of the experiment. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of sweet potato fermented product group. 'M' is middle dose (500 mg/kg BW) of sweet potato fermented product group. 'L' is low dose (250 mg/kg BW) of sweet potato fermented product group

3.2. The Rate of Food Utilization and the Percentage of Body Fat in All Groups

SD rats' food utilization efficiency (%) in each group were measured during the experiments. The SD rats' food consumption was detected on W1, W2, W3, W4, W5, W6, W7, and W8. During the experiments, the SD rats' food utilization efficiency continued to rise between W1-W2. On W1, the SD rats' food utilization efficiency (%) in the negative control group and three SPFP groups were higher than the normal control group (Figure 2A). The SD rats' food utilization efficiency in the high dose of SPFP group is significantly higher than the normal control group ($p < 0.05$). Between W3 to W8, the SD rats' food utilization efficiencies in the normal control group, the negative control group, and three SPFP groups were continuously decrease. However, the SD rats' food utilization efficiencies in the three SPFP groups were significantly higher than the normal control group ($p < 0.05$), and were not significantly higher than the negative control group ($p < 0.05$) (Figure 2A). In addition, the percentage of SD rats' body fat (%) in the negative control group and three SPFP groups were also significant higher than the normal control group ($p < 0.05$) (Figure 2B). However, the decrease threads of SD rats' body fat in the low and middle doses of SPFP groups were found in comparison with the negative control group and the high dose of SPFP group (Figure 2B).

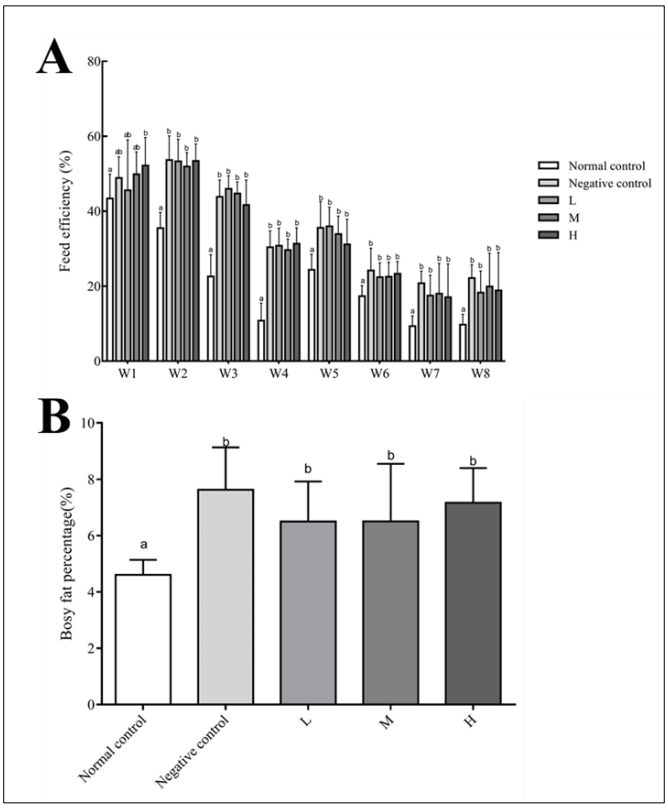

Figure 2 Food consumption before and after the treatments of three doses of sweet potato fermented products. (A) SD rats' food utilization efficiency (%) during the experiment. (B) Change of SD rats' body fat in the end of the experiment. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of sweet potato fermented product group. 'M' is middle dose (500 mg/kg BW) of sweet potato fermented product group. 'L' is low dose (250 mg/kg BW) of sweet potato fermented product group

3.3. The Concentrations of SD Rats' Blood Lipids in All Groups

SD rats' blood lipid concentrations in each group were measured in the end of the experiment. The concentrations of triglycerides, total cholesterol, HDL, LDL, and non-esterified free fatty acids in the negative control group and three SPFP groups were significantly higher than the normal control group ($p < 0.05$) (Figure 3A-E). However, there were non-significant difference for the concentrations of SD rats' blood lipids in the negative control group and three SPFP groups (Figure 3A-E). The decrease threads of SD rats' blood lipid concentrations in the low and middle doses of SPFP groups were found in comparison with the negative control group and the high dose of SPFP group (Figure 3A-E).

3.4. The Concentrations of SD Rat's Liver Lipids in All Groups

SD rats' liver lipid concentrations in each group were measured in the end of the experiment. The concentrations of triglycerides and total cholesterol in the negative control group and three SPFP groups were significantly higher than the normal control group ($p < 0.05$) (Figure 4A-B). However, there were non-significant difference for the concentrations of SD rats' liver lipids in the negative control group and three SPFP groups (Figure 4A-B). The decrease threads of SD rats' liver lipid concentrations in the low and middle doses of SPFP groups were found in comparison with the negative control group and the high dose of SPFP group (Figure 4A-B).

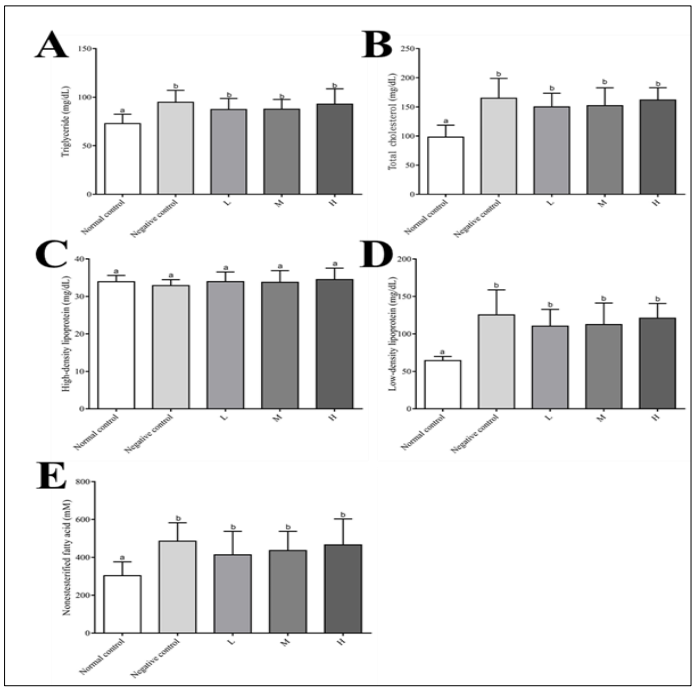

Figure 3 SD rats' blood lipid concentrations before and after the treatments of three doses of sweet potato fermented products. (A) triglycerides (B) total *cholesterol (C) HDL (D) LDL (E)* non-esterified free fatty acids. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of sweet potato fermented product group. 'M' is middle dose (500 mg/kg BW) of sweet potato fermented product group. 'L' is low dose (250 mg/kg BW) of sweet potato fermented product group

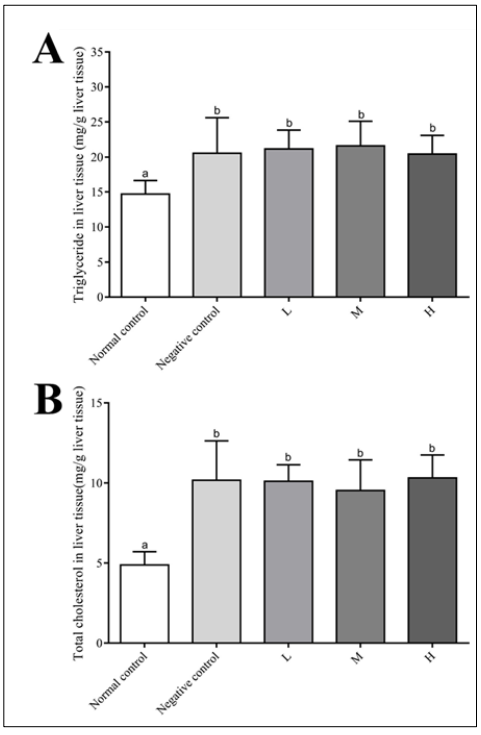

Figure 4 SD rats' liver lipid concentrations before and after the treatments of three doses of sweet potato fermented products. (A) triglycerides (B) total *cholesterol*. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of sweet potato fermented product group. 'M' is middle dose (500 mg/kg BW) of sweet potato fermented product group. 'L' is low dose (250 mg/kg BW) of sweet potato fermented product group

3.5. The Concentrations of Blood Sugar in All Groups

SD rats' blood sugar concentrations in each group were measured in the end of the experiment. The concentrations of blood sugar in the negative control group and three SPFP groups were significantly higher than the normal control group ($p < 0.05$) (Figure 5). However, there were non-significant difference for the concentrations of SD rats' blood sugar in the negative control group and three SPFP groups (Figure 5). The decrease threads of SD rats' blood sugar concentrations in three SPFP groups were found in comparison with the negative control group (Figure 5).

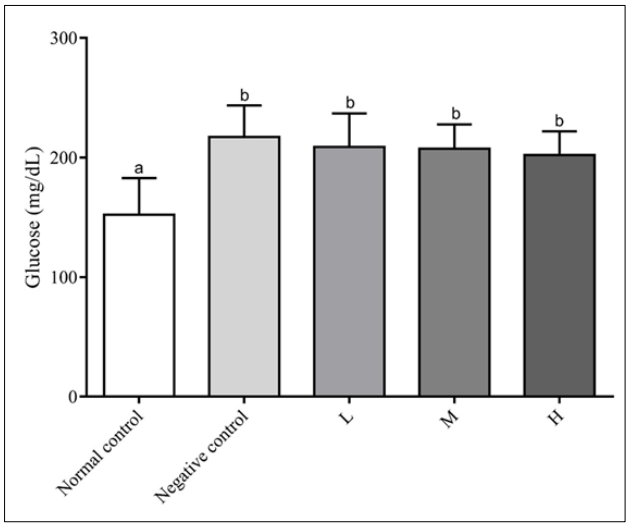

Figure 5 SD rats' blood sugar concentrations before and after the treatments of three doses of sweet potato fermented products. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of sweet potato fermented product group. 'M' is middle dose (500 mg/kg BW) of sweet potato fermented product group. 'L' is low dose (250 mg/kg BW) of sweet potato fermented product group

3.6. The Levels of Function Indexes in Liver and Kidney in All Groups

SD rats' function indexes (GOT, GPT, creatine, and uric acid) in liver and kidney in each group were measured at the end of the experiment. SD rats' function indexes in liver and kidney in all groups were non-significant difference ($p > 0.05$) (Figure 6).

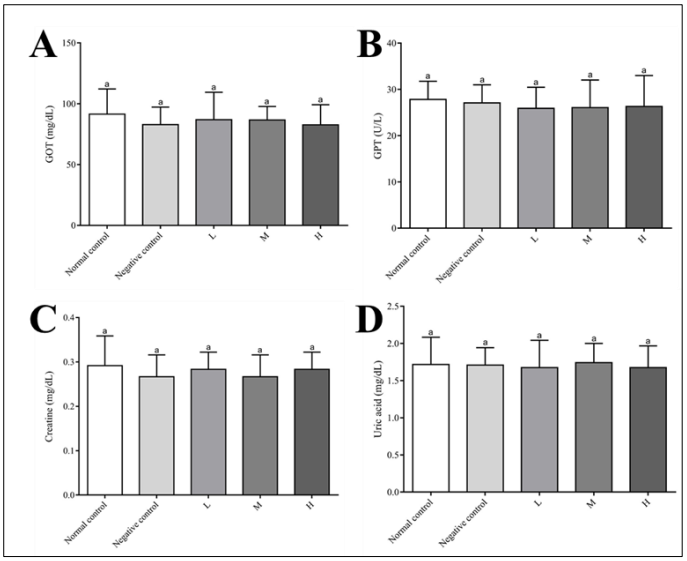

Figure 6 SD rats' levels of function indexes in liver and kidney before and after the treatments of three doses of sweet potato fermented products. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of sweet potato fermented product group. 'M' is middle dose (500 mg/kg BW) of sweet potato fermented product group. 'L' is low dose (250 mg/kg BW) of sweet potato fermented product group

4. Discussion

The sweet potato contains many nutrients as protein, carbohydrates, minerals, carotenoids, dietary fiber, vitamins, and very little fat. According to literatures, the sweet potato is a staple food source for many people. Its protein contents in leaves and roots respectively range from 4.0%-27.0% and 1.0%-9.0%. The sweet potato has been verified for involved many physiological regulatory functional components as β-carotene and anthocyanins. Globally, vitamin A deficiency causes temporary and permanent eye impairments and increased mortality. Plant foods do not contain vitamin A, however, they contain precursors of vitamin A as β-carotene or carotenoids, which converts to vitamin A via human body. The control of vitamin A deficiency is via improving dietary quality and quantity and diversification for human consumption. Dietary diversification includes the production of such as orange-fleshed. Because sweet potato is a β-carotene-rich crop, its major carotenoid is all trans-β-carotene, which exhibits highest pro-vitamin A activity. Therefore, sweet potato could be also considered as an excellent novel health care source for the functional food market. Moreover, the sweet potato possesses the high levels of anthocyanin and β-carotene. Therefore, it is a promising and healthier alternative to synthetic coloring agents in human food systems. Additionally, sweet potato also possesses other nutrients as starch and protein etc. can create new economic and employment activities for farmers and can add nutritional value to human food systems. The sweet potato production and potential are re-positioned for value-added products, will contribute substantially to utilizing its benefits and many uses in human food system [5-7].

An abnormal or excessive fat accumulation can cause obesity that affect people's health. Therefore, R&D of agricultural functional materials or products for the prevention of fat accumulation is urgently needed. Commonly, the body mass index (BMI; kg/m^2) is a simple index of weight-for-height that is commonly used to classify obesity in adults in life. However, BMI is just a rough guide. Currently, there are many detection methods for the detection of intracellular lipid droplets as Nile red stain, BODIPY 493/503 (4, 4-Difluoro-1, 3, 5, 7, 8-Pentamethyl-4-Bora-3a, 4a-Diaza-s-Indacene) stain, BODIPY 665/676 [(E, E)-3, 5-Bis (4-phenyl-1, 3-butadienyl)-4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacene] stain, 1,6-diphenylhexatriene stain, DAPI (4', 6-diamidino-2-phenylindole) stain, Hoechst stain, Sudan III stain, and Oil-red O stain [8-13]. In this study, many evaluated indexes were applied as BW of the SD rats was recorded every week and blood, liver, and body fat were collected for analysis of body fat percentage, blood lipid content, blood glucose content, liver lipid content, and liver and renal functions during the experiment. Although SPFP on the prevention of body fat accumulation was not significantly exhibited, however, the trend shows that eating low and middle doses of SPFP can decrease body fat production. Taken these results together, SPFP may has the potential for the prevention of fat accumulation.

5. Conclusion

During the experiment, BW of the SD rats was recorded every week, and at the end of the experiment, blood and body fat were collected for analysis of body fat percentage, blood lipid content, blood glucose content, liver lipid content, and liver and renal functions. Based on the above results, although SPFP on the prevention of body fat accumulation was not significantly exhibited, However, the trend shows that eating low and middle doses of SPFP can decrease body fat accumulation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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