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Research Article

The influence of pineapple consumption on gut microbiota in hypercholesterolemic rats

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ABSTRACT

Hypercholesterolemia is a metabolic abnormality that contributes to the development of cardiovascular diseases. The link between gut dysbiosis and metabolic disorders is well established. Targeting the gut microbiota with probiotics or dietary fiber has the potential to lower blood cholesterol and improve gut dysbiosis. Here, we investigated the effects of pineapple consumption on the diversity of gut microbiota in hypercholesterolemic rats. Male Sprague-Dawley rats were fed with a high-cholesterol diet (HCD), in the presence and absence of pineapple (*Ananas comosus* L.) cv. Pattavia powder for 8 weeks. The rats' fecal genomic DNA was extracted and quantified by Nanodrop spectrophotometer. The gut microbiota from the genomic DNA were analyzed by 16S rRNA metagenome sequencing. The findings revealed five enriched phyla with the genus *Romboutsia* being predominant among the microbiota in the rats' guts. Feeding with the HCD resulted in the increased abundance of a pro-inflammatory *Ruminococcus gnavus* (phylum *Firmicutes*) and *Bacteroides* (phylum *Bacteroidota*) whereas a decreased abundance of *Streptococcus* (phylum *Firmicutes*) was observed. Daily consumption of pineapple showed a decrease in the *Ruminococcus gnavus*, *Bacteroides*, and an increase in *Streptococcus* to a level close to that of the control group. We conclude that daily consumption of pineapple has a tendency to attenuate gut dysbiosis in hypercholesterolemic rats by lipid-lowering and anti-inflammation properties.

Keywords:
Hypercholesterolemia, Gut microbiota, Pineapple, Metabolic disorder

1. INTRODUCTION

Hypercholesterolemia, a metabolic condition defined by abnormally high levels of lipids or lipoproteins in the blood, is usually caused by the consumption of an inappropriate high-cholesterol diet (HCD), or is hereditary, familial hypercholesterolemia (FH). Hypercholesterolemia is a key risk factor for the development of cardiovascular diseases (CVD) since patients with hyperlipidemia are nearly twice as likely as healthy persons to develop CVD[1]. The significance of gut microbiota and the consequences for human health, particularly in metabolic illnesses such as obesity, type 2 diabetes mellitus (T2DM), and hypercholesterolemia, has recently received more attention[2-4]. Gut microbiota dysbiosis, the alteration of the composition of microbiota in the intestine, promotes metabolic complications and unhealthy diet induced obesity by several mechanisms including changing energy regulation, altered gut hormone and enzyme regulation, immune dysregulation, and proinflammatory mechanisms (such as lipopolysaccharide (LPS) endotoxins crossing the gut-mucosal barrier to the blood circulation)[5].

Age, the host immune system, genetics, cultural traditions, nutritional consumption, antibiotic use, and geography can all have an impact on gut microbiota[6-9]. Particularly, diet and geographical environment are two essential variables for gut microbiota formation and function[10-12]. Previous study has demonstrated that immigration to the United States from a non-western nation, Thailand, was related to a decrease in gut microbiome diversity

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and functions. These US immigrants lose bacterial enzymes involved in plant fiber degradation. In particular, *Prevotella* strains were replaced by *Bacteroides* strains (both of them belong to phylum *Bacteroidota*). As a result, obesity rates rise through the generations[7]. Moreover, the alteration of diversity and functions of gut microbiota results in different levels of nutritional metabolites such as short chain fatty acids (SCFAs are produced by the bacterial fermentation of dietary fibers), bile acids, and (poly)phenolic metabolites in T2DM and PreDM persons compared to normoglycemic individuals[13-15]. Targeting gut microbiota with dietary fibers might be the solution to reduce metabolic disorders.

Pineapple is a tropical fruit that has plenty of nutrients and can be affordable globally. It is suitable for everyday consumption as a freshly cut fruit product. Pineapple contains dietary fibers and phytochemical substances such as catechin, epicatechin, ferulic acid, and gallic acid, all of which have potent natural pharmacological activities. Several studies have indicated anti-dyslipidemia, anti-obesity, antioxidant, and anti-inflammatory activity from pineapple consumption[16-18]. Recent research has shown that fiber-rich and phenolic-containing fruits including pineapple, apple, pomegranate, and banana, can modulate the balance of gut microbiota[19-20]. So, dietary intervention becomes an intriguing option for shifting the gut microbiota from dysbiosis to the normal. Recently, Seenak et al. have demonstrated that feeding with pineapple powder reduced serum lipid profiles, cardiac oxidative stress, and inflammation in the high cholesterol diet-fed rats[21]. Taken together, pineapple provides lipid-lowering effects as well as dietary fibers targeting gut microbiota. Nevertheless, there have been few investigations into the relationship between pineapple consumption and the gut microbiota. The purpose of this work was to describe gut microbiota in the hypercholesterolemic rats fed with pineapple cultivated in Thailand by using 16S rRNA metagenome sequencing.

2. MATERIALS AND METHODS

2.1. Pineapple preparation

Pineapple (*Ananas comosus* L.) cv. Pattavia cultivated in Thailand was prepared according to Seenak, et al.[21]. Briefly, the pineapples were weighed, sliced, and dried at 60°C for 96 hours using hot air oven. The dried pineapple slices were weighed and ground into fine powder, then stored at -20°C until used. The active compounds, antioxidant activity and approximate analysis were evaluated before feeding to rats.

2.2. Rats fecal sample collection

Sprague–Dawley rat fecal samples were obtained from the study of Seenak, et al. (approval number: NU-AE610409 by the Animal Ethics Committee of Naresuan University, Thailand)[21]. Briefly, 4-week old male Sprague-Dawley rats, body weight 150-200 grams, were divided into 5 groups (N=5-6 per group). The animals were treated for 8 weeks as follow; control with standard diet and normal drinking water (Control), high-cholesterol diet (standard diet+1.5% (w/w) of cholesterol, 0.37% (w/w) of cholic acid (HCD), high-cholesterol diet with low dose of pineapple (100 mg/kg/day) (HCD+LPA), high-cholesterol diet with high dose of pineapple (200 mg/kg/day) (HCD+HPA), and high-cholesterol diet with simvastatin (40 mg/kg/day) (HCD+Sim). The nutrient profile of dried pineapple was analyzed and reported in Seenak, et al.[21]. The component of each analyte per 100 g dried weight of pineapple included carbohydrate 87.72 g, fat 1.66 g, protein 4.41 g, and dietary fiber 9.07 g. The body weight and serum lipid profile of animal in each group were analyzed and had been published in Seenak, et al.[21]. High cholesterol diet fed rats significantly increased their body weight and serum lipid profiles (total cholesterol, triglyceride, LDL-cholesterol), when compared to the control group[21]. Daily administration of pineapple markedly reduced the body weight and serum level of total cholesterol[21]. After the end of the treatment, animals were sacrificed. The internal organs such as heart, lungs, and colon were collected by aseptic technique and immediately snap-frozen in liquid nitrogen and stored at -80°C until used.

2.3. Gut microbiome analysis

Genomic DNA (gDNA) from each fecal sample was extracted by GeneAll Exgene™ stool DNA mini kit (GeneAll Biotechnology) according to the manufacturer's instruction. The gDNA concentration of each sample was quantified using a Nanodrop spectrophotometer and stored at -80°C until used. Gut microbiome diversity was performed using 16S rRNA metagenome sequencing of the V3-V4 regions of 16S rRNA by Illuminal MiSeq system. The raw sequences were categorized into groups based on the 5′ barcode sequences. The sequences were processed following DADA2 v1.16.0 pipeline (https://benjjneb.github. io/dada2/). The DADA2 pipeline describes microbial diversity and community structures using unique amplicon sequence variants (ASVs)[22]. Microbial taxa were classified from Silva version 138 as a reference database[23]. Alpha diversity index (Chao1 richness, Shannon, and PD whole tree) was computed using DADA2 software. For Beta diversity, non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity and principal coordinate analysis (PCoA) were plotted from Phyloseq data[24]. Linear discriminant analysis effect size (LEfSe)

was performed to identify the unique bacterial species as a biomarker between the experimental groups[25].

2.4. Statistical analysis

The statistical tests were carried out using commercially available software (GraphPad Prism). Pairwise comparison of alpha diversity (Observed ASVs, Chao1, Shannon, and PD whole tree) was calculated using Kruskal-Wallis test ($p<0.05$). Permutational multivariate analysis of variance (PERMANOVA) was performed to evaluate the significant differences for beta diversity among groups at $p<0.05$. Moreover, the Kruskal-Wallis sum-rank test was also used in LEfSe analysis to identify bacterial biomarkers that differed significantly in abundant taxon between sample groups.

3. RESULTS

3.1. Sequence analysis

Rarefaction curve, which represents the species richness (number of different species) within and between sequencing reads, had plateau stage when approximately 10,000 sequencing depths were reached. Our study accurately predicted the genuine bacterial compositions of gut microbiome in rat among the sample groups. (Supplementary S1)

3.2. Diversity analysis

The analysis of alpha diversity (observed species, Chao1, Shannon, and phylogenetic diversity (PD) whole tree) which referred to bacterial diversity within each sample was shown in Figure 1. There was no statistically significant difference between the sample groups in the observed abundance of amplicon sequence variants (ASVs), bacterial richness (Chao1), species diversity (Shannon), and PD whole tree (Kruskal-Wallis, $p>0.05$). This data indicated that the bacterial richness, evenness, and diversity were identical in rats given a conventional diet (control), hypercholesterolemia (HCD), and treatment groups (HCD+LPA, HCD+HPA, HCD+Sim). When compared to hypercholesterolemic rats, feeding pineapple at

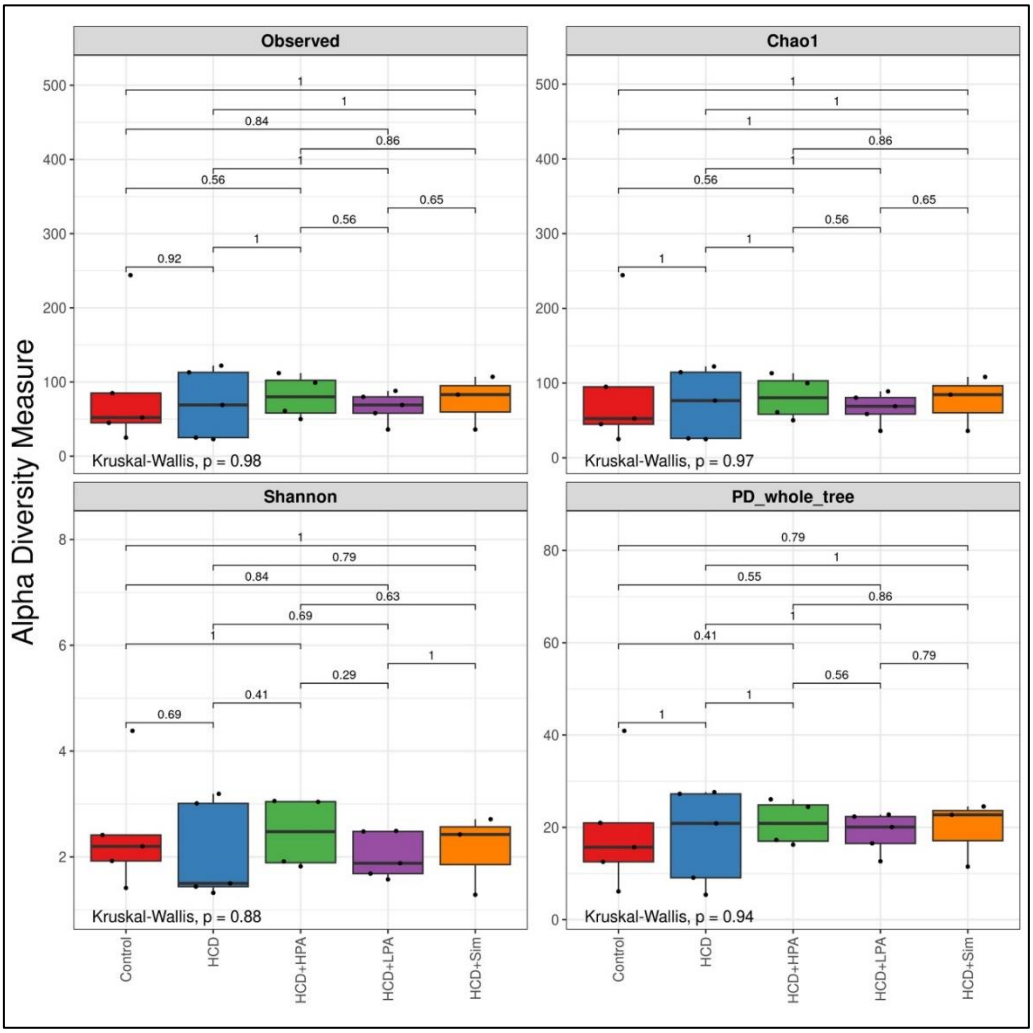

Figure 1. Diversity analysis. Box plots of alpha diversity (observed species, Chao1, Shannon, and phylogenetic diversity (PD) whole tree) in each sample group were shown. The black dots represented individual samples in each group. Alpha diversity values of each sample and quartiles of the distribution (minimum, first quartile, median, third quartile, and maximum of boxes) were demonstrated.

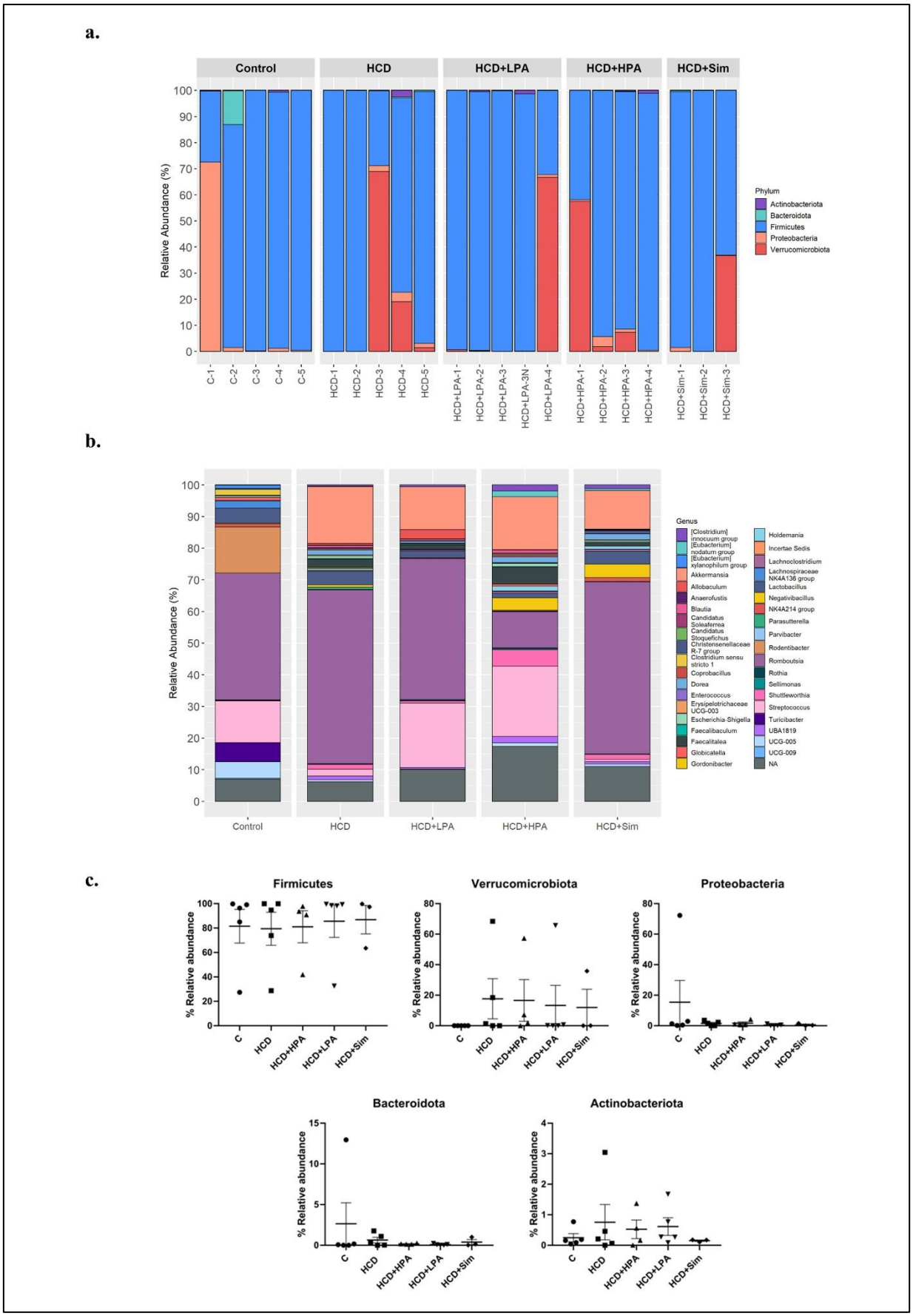

Figure 2. Taxonomic profile of the gut microbiota. The feces of rat fed with a normal diet (Control), high-cholesterol-diet (HCD), HCD with low-dose (HCD+LPA) and high-dose of pineapple (HCD+HPA), and HCD with simvastatin (HCD+Sim) as determined by the relative abundance of bacterial diversity at the phylum (2a and 2c) and genus level (2b). The selected genus/species were demonstrated (2d). Each value was expressed as mean±SEM (n=3-5 per group). *p* value<0.05 was statistically significant difference.

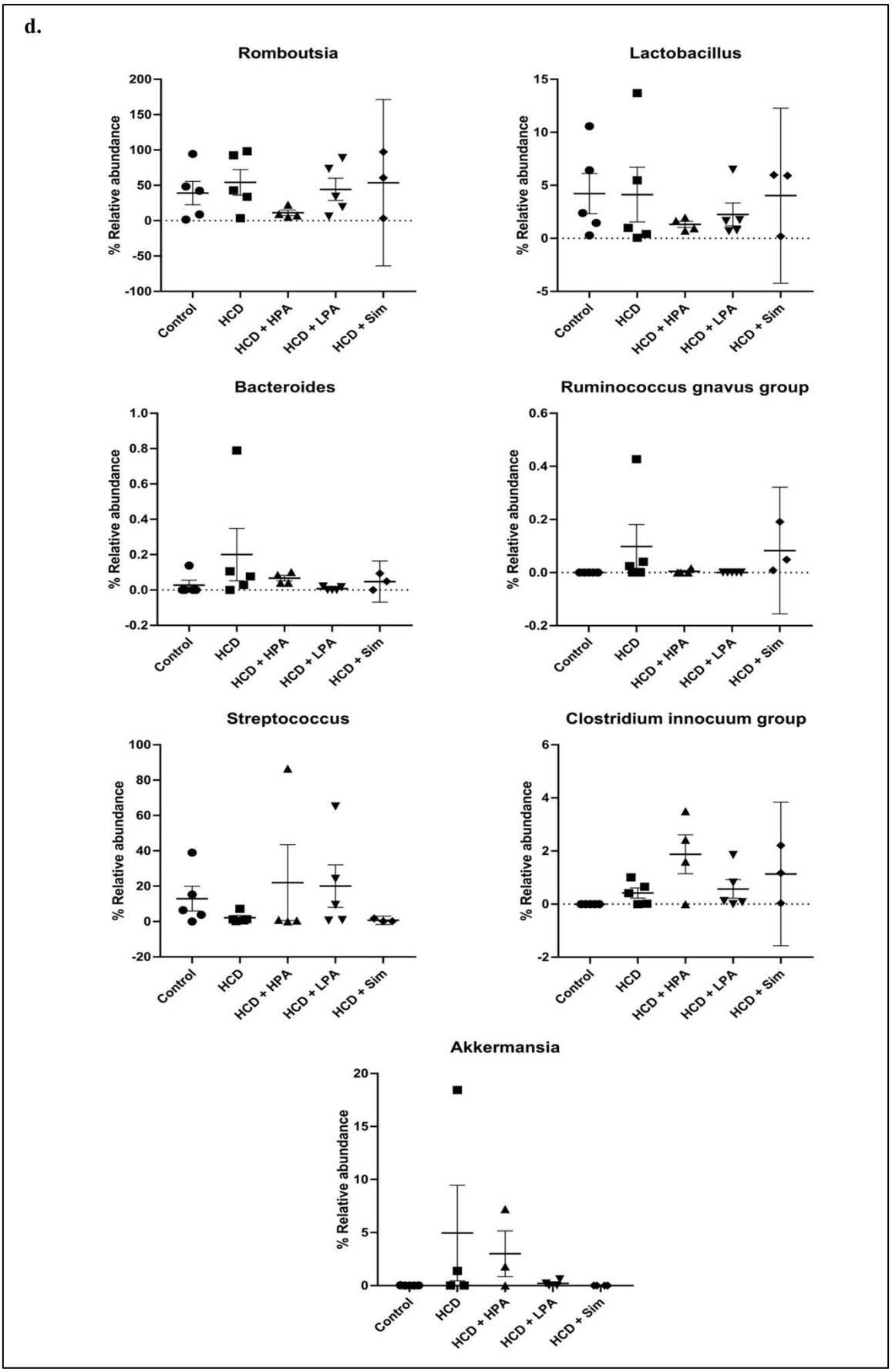

Figure 2. Taxonomic profile of the gut microbiota. The feces of rat fed with a normal diet (Control), high-cholesterol-diet (HCD), HCD with low-dose (HCD+LPA) and high-dose of pineapple (HCD+HPA), and HCD with simvastatin (HCD+Sim) as determined by the relative abundance of bacterial diversity at the phylum (2a and 2c) and genus level (2b). The selected genus/species were demonstrated (2d). Each value was expressed as mean±SEM (n=3-5 per group). *p* value<0.05 was statistically significant difference. (Cont.)

either a low or high dosage had no effect on gut microbial diversity.

3.3. High-cholesterol diet induced the gut dysbiosis in rats

The five enriched phyla were shown for taxonomic classification of the bacterial community from the rats. The bacteria of phylum *Firmicutes* were highly prevalent in all samples (avg. 82.62±5.52%), followed by *Verrucomicrobiota*, *Proteobacteria*, *Bacteroidota* and *Actinobacteriota*, respectively (Figure 2a and Figure 2c). The *Verrucomicrobiota* was found in HCD group when compared to the control group whereas phylum *Proteobacteria* was highly present in the control group (Figure 2c). This finding indicates that feeding with high cholesterol diet resulted in the change of gut microbiota from phylum *Proteobacteria* to phylum *Verrucomicrobiota*.

Overall, 124 genera were detected among samples. The relative abundance of bacteria in the genus *Romboutsia*, phylum *Firmicutes*, was a predominant microbiota in the gut of rats (Figure 2d). After 8 week of treatment, *Ruminococcus gnavus* group (phylum *Firmicutes*) and *Bacteroides* (phylum *Bacteroidota*) were increased in HCD group (avg. 0.098±.08% and 0.20±0.15%, respectively), when compared to control group (0% for *Ruminococcus gnavus* group and 0.028% for *Bacteroides*). In contrast, feeding with high-cholesterol diet decreased the abundance of *Streptococcus*, which belong to *Firmicutes*, (avg. 2.15±1.28%) in the gut of animals compared to the control group (avg. 12.9±6.98%). These data suggested that consumption of high-cholesterol diet resulted in the gut dysbiosis in rats.

3.4. Pineapple consumption restored the gut dysbiosis in hypercholesterolemic rats

As demonstrated in Figure 2d, *Ruminococcus gnavus* group was decreased in HCD+HPA and HCD+LPA (avg. 0.004% and 0%, respectively), when compared to HCD group (avg. 0.098±.08%). The genus *Bacteroides* was also decreased in HCD+HPA and HCD+LPA (avg. 0.067±0.033% and 0.007±0.003%, respectively), when compared with HCD group. Inversely, the abundance of *Streptococcus* was increased in HCD+HPA (avg. 22.03±11.01%) and HCD+LPA (avg. 20.02±8.95%) compared with HCD group. These findings indicated that pineapple consumption could decrease the abundance of high-cholesterol diet related bacteria and restored the gut dysbiosis to normal in hypercholesterolemic rats. However, our study was unable to indicate an increasing of the probiotic bacteria; *Lactobacillus* and *Akkermansia*, in the HCD-fed rats received either low-or high-dose of pineapple.

3.5. Simvastatin controlled the balance of gut microbiota in hypercholesterolemic rats

Simvastatin, the anti-lipemic drug, decreased the abundance of *Bacteroides*, *Ruminococcus gnavus* group in HCD+Sim (avg. 0.047±0.027% and 0.082±0.055%, respectively), when compared to HCD group (avg. 0.098± .08%) (Figure 2d). These data demonstrated that simvastatin decreased abundance of high-cholesterol diet related bacteria, and controlled the balance of gut microbiota in hypercholesterolemic rats.

3.6. Heatmap analysis

The alteration of bacterial abundance between samples was demonstrated by heatmap (Figure 3). The abundance of bacteria in genus *Turicibacter* and *Eubacterium xylanophilum* group were highly enriched gut microbiome of control rats. On the other hand, unclassified species in family *Lachnospiraceae*, genus *Allobaculum*, *Blautia*, *Lachnoclostridium*, Dorea, and *Ruminococcus gnavus* group increased in hypercholesterolemic rats (HCD). This result suggested that the distribution and abundance of gut microbiome in rats could be changed according to the health status resulting from high-cholesterol diet feeding. Moreover, we found that gut microbiome of HCD-3, HCD-4, and HCD-5 were difference from the control group by increasing of the abundance of genus *Akkermansia*, *Blautia*, *Erysipelotrichaceae UCG-003*, *Faecalitalea*, *Clostridium innocuum* group, *Negativibacillus*, *Dorea*, and *Escherichia-Shigella*. This finding indicated that gut microbiome of these samples was changed. A low-dose of pineapple (HCD+LPA) feeding could decrease the abundance of genus *Erysipelotrichaceae* UCG-003, *Marvinbryantia*, *Candidatus*, *Soleaferrea*, *Eubacterium nodatum* group, *Negativibacillus*, *Eubacterium coprostanoligenes* group, family *Enterobacteriaceae*, and genus *Escherichia-Shigella*, when compared with HCD group. This result showed that low-dose of pineapple feeding could control the increment of gram-negative bacilli, phylum *Proteobacteria* in the gut of hypercholersterolemic rats.

3.7. The possibly unique bacteria in each group by Linear discriminant Effect Size (LEFSe) analysis

The difference in taxa between groups was determined by Linear discriminant analysis effect size (LEfSe). Bacterial taxa with LDA scores greater than 2 was shown in Figure 4. Bacteria in genus *Turicibacter*, *Lachnospiraceae NK4A136* group, and *Eubacterium xylanophilum* group (belonging to phylum *Firmicutes*) were highly prevalent in control group ($p<0.05$). The *Ruminococcus gnavus* group (phylum *Firmicutes*) was the core gut microbiota in HCD ($p=0.01$). The *Erysipelatoclostridium* and *Clostridium innocuum* group (belonging to phylum

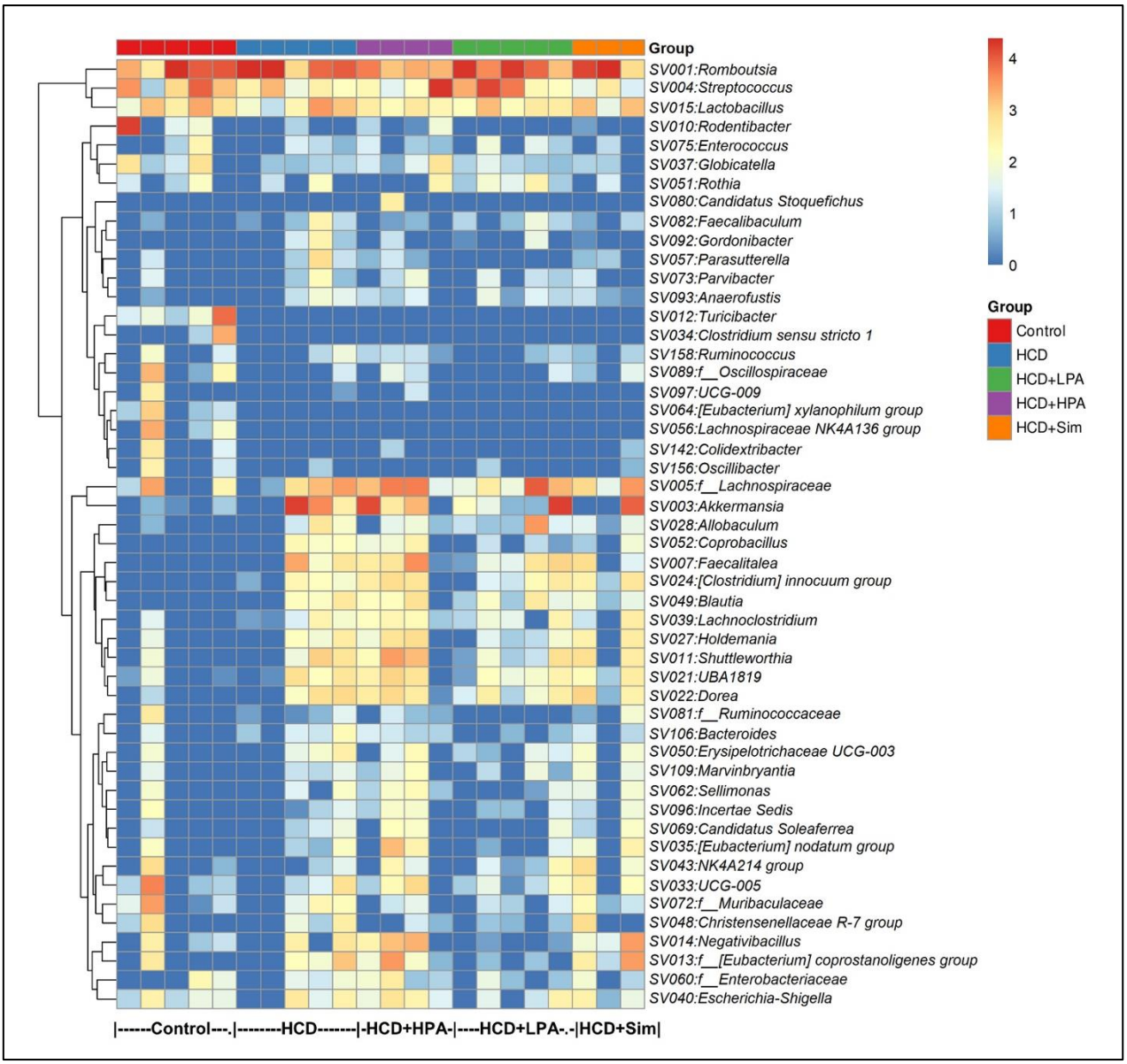

Figure 3. Heatmap demonstrates the relative abundance of prominent bacterial genera from feces of rats fed with regular diet (control), high cholesterol diet (HCD), HCD with low dose pineapple (HCD+LPA), HCD with high dose pineapple (HCD+HPA), and HCD with simvastatin (HCD+Sim).

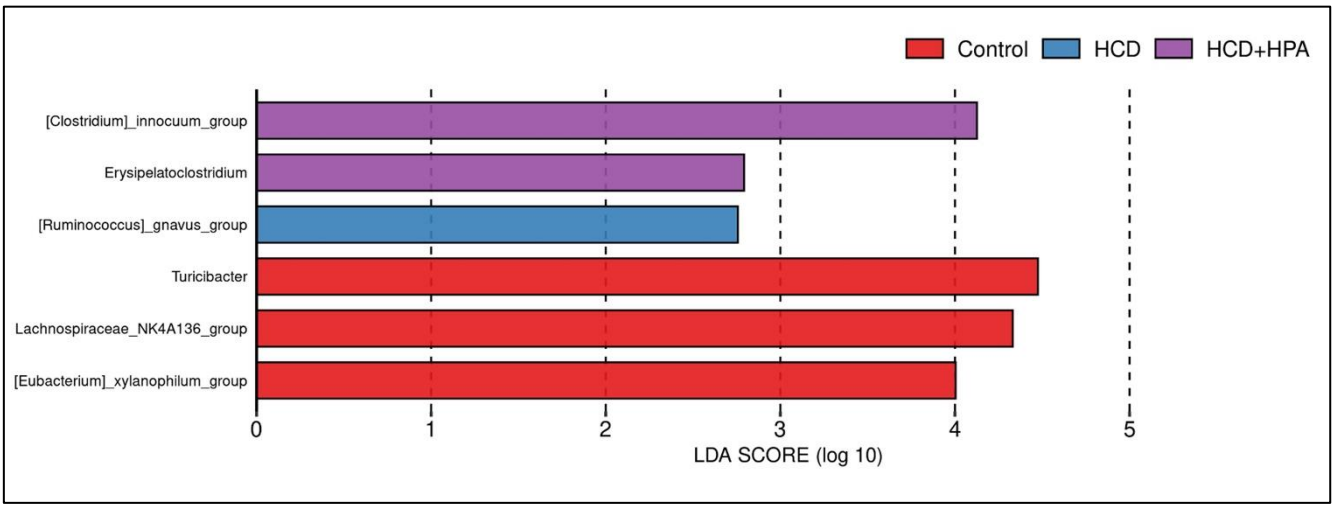

Figure 4. Histogram of LDA score of the species biomarker by Linear discriminant Effect Size (LEFSe) analysis. The unique microorganism found in each group of animals were shown.

Firmicutes) contributed to the dominant biomarkers in HCD feed with a high-dose of pineapple (HCD+HPA) (p=0.02 and p=0.04, respectively). This result indicated that the core microbiota of rats could be affected by feeding with high-cholesterol diet and high-dose of pineapple.

4. DISCUSSION

The effect of pineapple consumption on the gut microbiota of hypercholesterolemic rats was investigated in the current study. Five predominant bacterial phyla were identified in rats. *Firmicutes* was the most abundant phylum, followed by *Verrucomicrobiota*, *Proteobacteria*, *Bacteroidota*, and *Actinobacteriota*, respectively. The genus *Romboutsia*, phylum *Firmicutes*, was predominant in the gut of rats. We discovered the distinct biomarkers in three groups of animals. The core microbiota of rats fed with a normal diet (control) were genus *Turicibacter*, *Lachnospiraceae* NK4A136 group, and *Eubacterium xylanophilum* whereas the biomarkers of high-cholesterol diet fed-rats were the *Ruminococcus gnavus* group. The rats with HCD fed with high doses of pineapple had *Erysipelatoclostridium* and the *Clostridium innocuum* group. Hypercholesterolemic rats showed an increased abundance of the genus *Bacteroides*; however, consuming low and high doses of pineapple or simvastatin, a lipid lowering medicine, could reduce the increment of these bacteria. On the other hand, hypercholesterolemic rats demonstrated a decrease in the abundance of *Streptococcus* whereas feeding with pineapple or simvastatin could restore this bacterium to normal. These data indicate that pineapple consumption modulates the composition of the gut microbiota in hypercholesterolemic rats.

Ruminococcus gnavus (phylum *Firmicutes*) was revealed to be the core gut microbiota in HCD-fed rats, and pineapple intake reduced the quantity of these bacteria. There are a strong association of *Ruminococcus gnavus* with lipid profile, fat mass, and visceral fat area[26-27]. Yan et al. described the species of gut microbiota with a strong correlation with visceral fat including *Escherichia coli, Mitsuokella* unclassified, *Bifidobacterium longum*, *Escherichia* unclassified, *Ruminococcus torques, Dialister succinatiphilus*, *Eubacterium hallii*, and *Ruminococcus gnavus*[26]. Several studies show that the *Ruminococcus gnavus* group is positively correlated with liver inflammation and insulin resistance leading to the impairment of liver function and metabolic disorder[28-29]. Furthermore, *Ruminococcus gnavus* is also associated with advanced coronary artery diseases[30]. In clinical studies, participants with prediabetes and insulin resistance (IR) who received the supplementation with (poly)phenol-dense red raspberries (RRB) showed a reduction in hepatic-IR and plasma cholesterol, and decreased *Ruminococcus gnavus* and increased *Bifidobacterium* spp.[29]. In this regard, our findings are consistent with those of Seenak et al., who discovered that consuming pineapple on a regular basis lowered serum lipid profile, liver enzyme activity, and cardiac-inflammatory cytokines[21]. These findings suggest that reducing the inflammation and decreasing the abundance of the *Ruminococcus gnavus* group resulted in the improvement of gut microbiota in hypercholesterolemic rats.

Studies of Huang et al. revealed that daily consumption of pineapple peel containing high levels of water-insoluble fiber-rich fraction (WIFF) improved the intestinal function of hamsters and increased the growth of *Lactobacillus* spp. and *Bifidobacterium* spp.[31]. Gomez-Garcia and colleagues demonstrated that the bioactive compound found in pineapple peel and stems can be applied as a prebiotic enhancer for promoting the growth of probiotics such as *Lactobacillus* spp. and *Bifidobacterium* spp.[32]. In contrast, our study could not find an increase in *Bifidobacterium* spp. from the hypercholesterolemic rats fed with pineapple. Furthermore, feeding with pineapple resulted in a decrease in *Lactobacillus* spp. The discrepancy between our study and others may be due to the proteolytic activity of protease during the pineapple preparation procedure. Both groups of researchers removed the enzymatic fraction from the pineapple crude juice to decrease the proteolytic activity and increase, as a consequence, the prebiotic potential. From their study, we conclude that our study uses the pineapple meat without the removal of enzymatic activity for making the powder, so it may not be suited for becoming a prebiotic to promote the probiotic microorganisms. Furthermore, our study could not observe the increasing abundance of *Akkermansia*, a beneficial genus in the phylum *Verrucomicrobiota*, after feeding HCD-fed rats with pineapple. Several studies have revealed the negative association of this genus, especially *Akkermansia muciniphila*, with metabolic disorders, obesity, and inflammation[33-34]. However, the abundance of *Akkermansia muciniphila* in inflammatory bowel diseases (IBD) such as colorectal cancer (CRC) is higher than in healthy individuals, both in patients and mice, especially in the early stage of CRC[35]. The hypercholesterolemic rats had recovered from the inflammation after receiving pineapple or simvastatin[21], but could not restore the abundance of *Akkermansia muciniphila*. The controversial nature of *Akkermansia muciniphila* needs to be further investigated.

Streptoccoccus spp. (phylum *Firmicutes*) was decreased in the HCD group and elevated after receiving both low and high doses of pineapple. A randomized controlled clinical trial has demonstrated that daily co-administration of multi-strain probiotics, such as *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bifidobacterium lactis* to obese participants resulted in a significant reduction in weight and serum cholesterol after 8 weeks[36]. Previous study has shown that fiber extraction from apple pomace could be

used as a prebiotic to activate the growth of the probiotic strains *Streptococcus faecalis* and *Lactobacillus sporogenes*[37]. These data support that consumption of dietary fibers from fresh fruits, such as pineapple, can promote the balance of gut microbiota.

Bacteroides spp. (phylum *Bacteroidota*) is involved in obesity, hypertension, diabetes mellitus, and metabolic disorders[2,7]. The gut microbiota play an essential role in the fermentation of dietary fibers and endogenous intestinal mucus. This fermentation results in the growth of the particular bacteria that generate short chain fatty acids (SCFAs) and gases[38]. The major SCFAs include acetate, propionate, and butyrate, which are an essential energy source for colonocytes. Clinical trials have demonstrated that higher production of SCFAs correlates with lower diet-induced obesity[39] and reduction of insulin resistance[40]. Westernized, high-fat diet consumption has shown the loss of cellulose-hydrolysis bacteria, *Prevotella*, and increases in *Bacteroides*. This has resulted in low SCFAs in Westernized diets, leading to gut dysbiosis[7,10]. Our findings were consistent with previous research, which found that this bacterium was more prevalent in rats on a high-cholesterol diet than in rats receiving a normal diet. After receiving both low and high doses of pineapple or simvastatin, this bacterium was restored to virtually normal levels, so the consumption of fresh pineapple is an alternative option for reducing gut dysbiosis.

The *Clostridium innocuum* group and *Erysipelatoclostridium* were the core gut microbiota in HCD-fed rats treated with high doses of pineapple (HPA). Both of them are gram-positive, spore-forming anaerobes in the phylum *Firmicutes* that significantly impact the metabolism and functioning of the human gastrointestinal tract. The *Clostridium innocuum* group has been reported to be associated with weight regulation. Weight loss was seen in high-fat diet-induced rats treated with carnosic acid from rosemary[41]. From the same study, *Erysipelatoclostridium* was increased as a marker of anti-obesity. Our findings concerning the *Clostridium innocuum* group correlate with Seenak et al.[21]. The rats in HCD+HPA showed the low body weight in a time dependent manner when compared with the HCD group. This assumes that intake of pineapple attenuates the microbial change in hypercholesterolemic rats. However, *Clostridium innocuum* has been reported as a diarrheal pathogen in humans that causes a *Clostridium difficile* infection-like antibiotic-associated diarrheal illness[42-43]. The fundamental mechanisms are as yet unclear.

The limitation of this study was the small number of animals in each group. With just 3-5 Sprague-Dawley rats in each group, there may not be a significant difference between the experimental and control groups. However, we still observed a tendency for the modification of gut flora following pineapple ingestion.

5. CONCLUSION

We discovered that consuming fresh pineapple on a regular basis can help to alter gut microbiota in hypercholesterolemic rats. Pineapple anti-inflammation properties play an important part in gut microbial homeostasis.

6. ACKNOWLEDGEMENT

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Conflict of interest
None to declare.

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Ethics approval
None to declare.

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Author contribution
PP, SK, NN, and WM conceptualized and designed the experiments; PP, SK, NN, and WM conducted the experiments; PP and SK interpreted and analyzed the data; PP and SK drafted the manuscript. All authors read and approved the final manuscript.

Supplementary data
Supplementary S1. Rarefaction analysis of 16S rRNA gene sequences obtained from rat fecal samples.

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