EFFECT OF NUTRITIVE AND NON-NUTRITIVE SWEETENERS ON GUT MICROBIOTA OF HEALTHY ADULT INDIVIDUALS

EHWARIEME Daniel Ayobola[1], ODUM Edward Ikenna[1] and WHILIKI Onoriadjeren Oscar[2]

[1]Department of Microbiology, Faculty of Science, Delta State University, Abraka, Nigeria.
[2]Department of Science Laboratory Technology, Delta State Polythechnic, Oghara, Nigeria.
*Corresponding author. E-mail: ayoboladaniel@gmail.com

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There have been growing concerns about the health implication of consuming nutritive sweeteners and this has enhanced the utilization of non-nutritive sweeteners (NNSs) due to their low, or zero calorie content. Their effect on gut microbiota is however questionable. This research intends to determine the effect of NNSs at high and low doses, on gut bacteria population and diversity among healthy human subjects. Three groups of individuals were administered Aspertame (Asp), Saccharine (Sac) and Acesulfame potassium (Ace-K) at high and low doses based on body weight, for a period of 56 days. Bacteria load and diversity per gram of stool sample was determined every 7 days throughout the study period. A fourth group was administered, Sucrose (Suc), as control. The log CFU/g of stool sample was observed to increase from 3.64 to 3.80, 3.68 to 3.96 and 3.82 to 4.06 at day 0 to 56 for Asp, Sac and Ace-K respectively, at high dose. Bacteria counts however did not increase or decrease with Sucrose administration. Also, the bacteria counts obtained at low or high doses of NNS were not significantly different ($P>0.05$). Furthermore, bacteria diversity reduced from 7 to 3, 8 to 2 and 8 to 3 at high dose treatment with Asp, Sac and Ace-K respectively, for day 0 to 56. There was no significant difference ($P>0.05$) in bacteria diversity at high or low dose treatment. This study suggests that NNS intake reduces bacteria diversity of gut, while increasing the population of surviving species. Such dysbiosis raises doubt as to whether NNS intake should still be "generally regarded as safe".

Key words: Non-nutritive sweeteners, gut microbiota, dysbiosis.

INTRODUCTION

The consumption of sugars, mainly as sucrose and glucose – fructose syrups, has dramatically increased worldwide and growing concerns about their adverse effects on health and metabolic diseases, such as metabolic syndrome, cardiovascular diseases and type 2 diabetes (T2D), have motivated people to reduce the consumption of sugars (Stanhope, 2016; Lohner et al., 2017). Due to the concern that high sugar intake can increase the risk of developing obesity, type 2 diabetes and cardiovascular diseases, non – nutritive sweeteners are increasingly used to replace sugars (Fowler, 2016).

Non – nutritive sweeteners (NNSs) are defined as sweetening agents that have a higher sweetening intensity and zero calorie content per gram compared with caloric or nutritive sweeteners such as sucrose or corn syrup. NNSs can be of synthetic or natural origins, the latter being increasingly consumed (Lohner et al., 2017). Commercially available artificial NNSs include sucralose, acesulfame potassium (Ace K), saccharin, aspartame, neotame, advantame and naturally occurring NNSs include Steviol glycosides and thaumatin (Carocho et al., 2017; Chattopadhyay et al., 2014; FDA, 2015).

Nutritive sweeteners such as sugars (sucrose, glucose, corn sugar, maltose, honey and high fructose corn syrup) add carbohydrates to food and calories to diets that contain few vitamins or minerals. Sugar alcohol or polyols are a type of nutritive sweeteners, which are low digestible carbohydrates derived from the hydrogenation of their sugar or syrup sources and include, sorbitol, xylilol, isomalt, mannitol, and hydrogenated Starch Hydrolysate (HSH). They are slightly lower in calories than sugar and do not promote tooth decay or cause a sudden increase in blood

glucose (FDA, 2015). Both non nutritive sweeteners and low calorie sweeteners are consumed not only by people with diabetes but also by the general population, because they are used as ingredients in many reduced – calorie foods like soft drinks, dairy products, powdered drink mixes, baked foods, desserts, candy, chocolates, paddings, canned foods, jams and jellies and confectionery chewing gums. In addition, they can be used as table top sweeteners at home, in cafeterias and in restaurants (FDA, 2015). Human exposure to NNSs begins early through breast milk, infant rehydration solutions and medications (Freedman et al., 2010; Sylvetsky et al., 2015).

Although the US Food and Drug Administration (FDA), European Food Safety Authority (EFSA), Codex Alimentarius and many national authorities have recognized that both non nutritive sweeteners and low calorie sweeteners are generally safe and well tolerated, there is controversy about the effects of these sweeteners on human health (Suez et al., 2014). The consumption of typically used non – nutritive artificial sweeteners formulations drives the development of glucose intolerance through the induction of compositional and functional alterations to the intestinal microbiota (Suez et al., 2014; Toews and Meerpohl, 2017).

Gut microbiota is a term used to describe the bacteria that take up residence in a host's gastrointestinal tract and is, today, considered to have the function of an organ (Walker and Lawley, 2013; Di Bella et al., 2013). Alteration of this ecosystem can lead to an imbalance in its metabolism and consequently its host's. Through the uptake of indigestible carbohydrates, the gut microbiota produces short - chain fatty acids, which also play a role in the host's health (Udayappan et al., 2014). The study of Suez et al. (2014) showed modifications in the intestinal microbiota after the administration of some sweeteners (especially NNSs). They found positive correlations between NNSs consumption and the Enterobacteriaceae family, the Delta proteobacteria class and the Actinobacteria phylum.

In a related study in healthy non diabetic subjects, 2 weeks of low – calorie sweeteners (LCSs) supplementation was sufficient to disrupt gut bacteria and increase the abundance of those which are normally absent in healthy individuals (EASD, 2018). There are a few clinical studies on the effects of sweeteners on the gut microbiota in human trials and none has been specifically conducted on Nigerians. The genetic variations in humans makes it necessary to evaluate the effects of nutritive and non – nutritive sweeteners on the microbiota of Nigerians which this study seek to determine.

MATERIALS AND METHODS

Human volunteers

Based on individual consent, volunteer individuals enrolled for this study were between the ages of 20 and 40 years. These individuals were healthy and not on any form of medication or special diets prior to research experiment, and remained that way throughout the study period.

Experimental design

Volunteers were shared into 8 groups in all, with 10 individuals in each group. Grouping was based on specific sugar to be administered, as shown in Table 1.

Determination of gut bacteria load

Stool samples from all volunteers were aseptically collected every week beginning from Day 0, and analyzed microbiologically. Also, 1 g stool sample from volunteers were emulsified in 9mL of sterile water and used for a 10-fold serial dilution to determine total bacterial count. Loop-fulls of bacteria from culture plates used for enumeration were also, separately used for bacterial identification. All incubations at 37°C were conducted in duplicates, aerobically and anaerobically (Murray et al., 2016)

Bacterial characterization and identification

Based on differences in colonial and cultural characteristics, bacteria species were further identified by gram staining, as well as various biochemical and serological reactions (Cowan et al., 1993).

Statistical analysis

Bacteria counts from members in each experimental group, was determined by the mean,

Table 1. Volunteers shared in groups.

Groups	Sugar	Recommended daily intake RDI (mg/kg BW)
Group I	High Dose Aspertame (HD-Asp)	50
Group II	High Dose Saccharine (HD-Sac)	5
Group III	High Dose Acesulfame Pottasium (HD-AceK)	15
Group IV	High Dose Sucrose (HD-Suc)	50
Group V	Low Dose Aspertame (LD-Asp)	5.0
Group VI	Low Dose Saccharine (LD-Sac)	0.5
Group VII	Low Dose Acesulfame Pottasium (LD-AceK)	1.5
Group VIII	Low Dose Sucrose (LD-Suc)	5.0

The Accepted Daily Intake (ADI) administered was further calculated based on the formula:

$$ADI = \frac{\text{Body weight (in pounds)}}{2.2} \times RDI$$

(FDA, 2015).

Administration of sugar began at Day 0 and proceeded through to Day 56.

while comparison of HD/LD as well as HD/Control was achieved by T-test, using the SPSS package (23.0). Graphs were used as descriptive.

RESULTS

The log CFU/g of stool samples for individuals fed with HD-Asp and LD-Asp did not significantly differ from each other (P>0.05) though counts increased from 3.64 to 3.80 and 3.66 to 3.78 respectively at high and low dose, from Day 0 to Day 56 (Table 2). Conversely, the bacteria diversity for both treatment cases reduced from 7 (at Day 0) to 3 (at Day 56). For Saccharine at high and low doses treatment, log CFU/g of stool samples increased from 3.68 and 3.70 (at Day 0), to 3.96 and 3.82 (at Day 56) respectively.

However, bacterial diversity reduced from 8 to 2 (at High dose) and 8 to 4 at low dose treatment for same time interval. There was no significant difference in the bacteria counts at 5% level of significance (Table 3).

Table 2. Bacterial population and diversity in stool sample according to days, with Aspertame treatment

Time (Days)	High dose aspartame		Low dose aspartame	
	Log CFU/g stool	Bacterial diversity	Log CFU/g stool	Bacterial diversity
0	3.64	7	3.66	7
7	3.70	7	3.66	7
14	3.70	6	3.68	7
21	3.74	5	3.72	5
28	3.72	5	3.70	5
35	3.75	5	3.74	4
42	3.78	4	3.78	4
49	3.80	3	3.76	4
56	3.80	3	3.78	3

P > 0.05.

Treatment with high and low doses of Asesulfame K indicates a significant difference in log CFU/g of stool sample bacteria (P<0.05). However, the trend was same as with other NNS, with counts increasing from 3.82 and 3.68 (at Day 0), to 4.06 and 3.80 (at Day 56) for high and low dose treatment respectively (Table 4). Bacteria diversity was equally observed to have reduced from 8 to 3 and 7 to 3 respectively at high and low dose respectively. Sucrose which was used as the control sugar did not produce any increase in bacteria count over the study period (Table 5). Also, there was no decline in bacteria diversity as observed with the

Table 3. Bacterial population and diversity in stool sample according to days, with Saccharine treatment.

Time (Days)	High Dose Saccharine		Low Dose Saccharine	
	Log CFU/g stool	Bacterial diversity	Log CFU/g stool	Bacterial diversity
0	3.68	8	3.70	8
7	3.70	8	3.70	8
14	3.71	6	3.75	8
21	3.80	7	3.74	6
28	3.84	5	3.76	5
35	3.83	5	3.80	5
42	3.92	3	3.80	5
49	3.94	2	3.82	4
56	3.96	2	3.82	4

P > 0.05.

Table 4. Bacterial population and diversity in stool sample according to days, with Ace-K treatment.

Time (Days)	High Dose Ace-K		Low Dose Ace-K	
	Log CFU/g stool	Bacterial diversity	Log CFU/g stool	Bacterial diversity
0	3.82	8	3.68	7
7	3.82	8	3.70	6
14	3.90	8	3.70	6
21	3.92	6	3.72	6
28	3.96	5	3.70	5
35	3.96	5	3.76	4
42	4.02	5	3.76	4
49	4.06	4	3.78	4
56	4.06	3	3.80	3

P < 0.05.

Table 5. Bacterial population and diversity in stool sample according to days, with sucrose treatment.

Time (Days)	High dose sucrose		Low dose sucrose	
	Log CFU/g stool	Bacterial diversity	Log CFU/g stool	Bacterial diversity
0	3.66	7	3.67	8
7	3.65	8	3.66	8
14	3.67	8	3.65	7
21	3.66	7	3.65	7
28	3.65	8	3.65	7
35	3.66	7	3.68	8
42	3.68	7	3.65	7
49	3.66	7	3.67	7
56	3.65	8	3.66	7

P > 0.05.

NNS treatments. However, there was no significant difference in counts either at low or high dose treatment.

Figures 1 and 2 aptly describe the trend in bacteria population with days, for all sugars. There was a steady increase in bacterial population with days for all NNS but not with sucrose treatment. On the other hand, there was a general, steady decline in bacteria diversity with days for all NNS but this trend was also not the case with sucrose. Specific bacteria isolated at Day 0 were *Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, and *Providencia* sp. of these however, only *Prevotella* sp, *Bacteroides* sp and *E. coli* appeared to be left

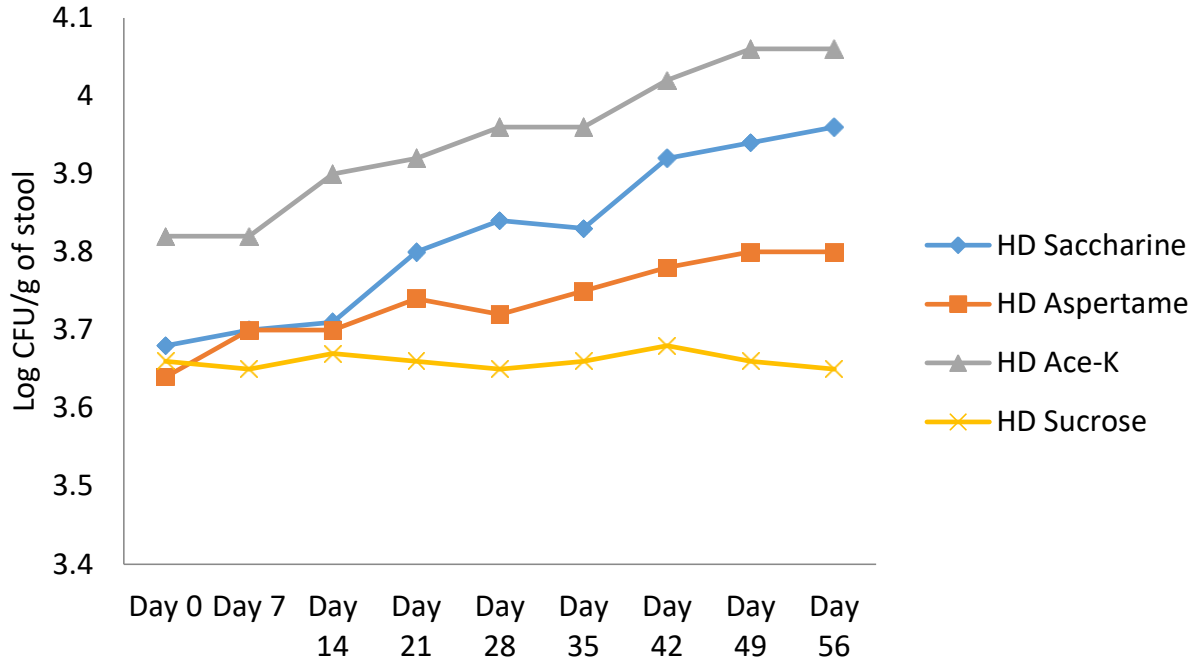

Figure 1. Trend of bacterial population in stool, according to days.

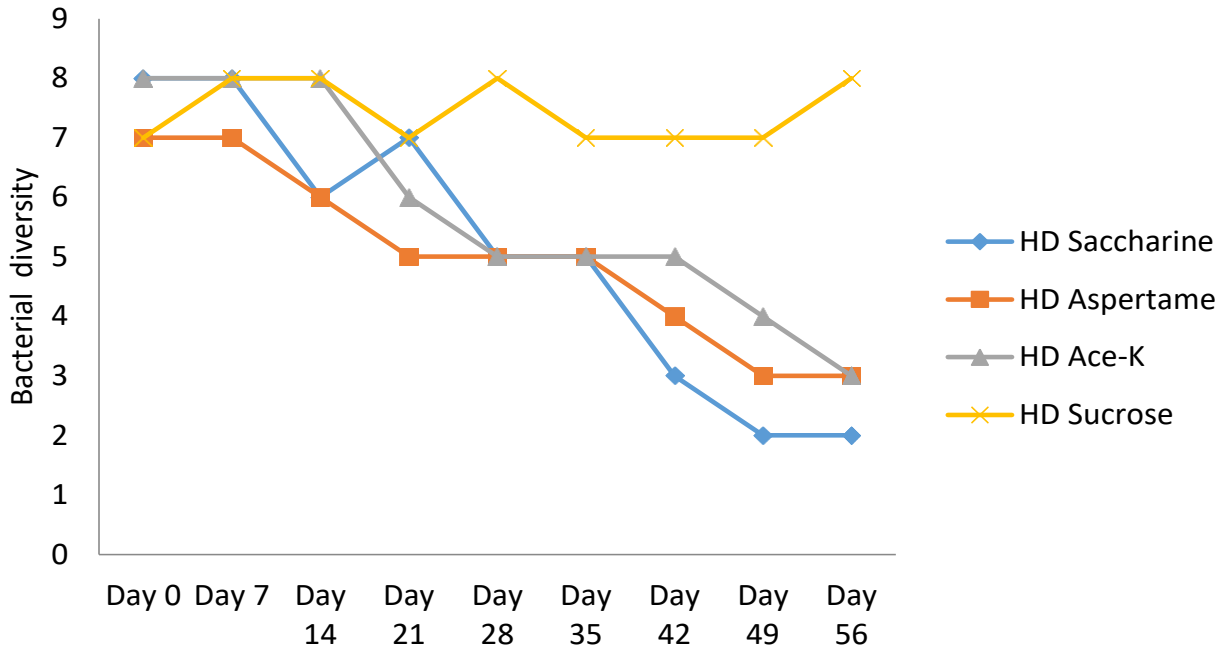

Figure 2. Trend of bacterial diversity in stool, according to days.

as at Day 56. With sucrose treatment on the other hand, isolated bacteria remained similar throughout the study period (Table 6).

DISCUSSION

This study investigates the effect of both nutritive sweetener (Sucrose) and non-nutritive sweeteners (Aspertame, Acesulfame potassium and Saccharin) on gut microbiota in a human trial over a period of 56 days at low and high concentrations. The results show that there is no significant difference in the bacterial population of stool among individuals that consumed the nutritive or non-nutritive sweeteners, either at low or high concentrations over the experimental

Table 6. Succession of gut microbiota over 8weeks period.

Time (days)	Bacteria genera isolated at high doses of test sugars			
	Ace-k	**Aspartame**	**Saccharine**	**Sucrose**
0	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.
7	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.
14	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.
21	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Escherichia coli*, *Providencia* sp.
28	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.
35	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.
42	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.
49	*Prevotella* sp., *Bacteroides* sp., *Enterobacter* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Escherichia coli*	*Bacteroides* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.
56	*Prevotella* sp., *Bacteroides* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Escherichia coli*	*Bacteroides* sp., *Escherichia coli*	*Prevotella* sp., *Bacteroides* sp., *Clostridium* sp., *Citrobacter* sp., *Lactobacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Providencia* sp.

period. Only Ace-K displayed exception to this observation. This indicates that, even at low concentration, whatever effect these sugars have on gut bacteria, will still be manifested. So far, all the NNSs showed a selective bacteriocidal effect on some gut bacteria species, while sucrose had no bacteriocidal effect at all. Nutritive sweeteners when consumed in moderation with mixed meal and as part of a healthy overall diet does not have adverse health effects which is why sucrose had no bacteriocidal effects on the bacterial diversity. The NNSs on the other hand, were inhibitory on the gut bacteria.

Aspertame has been reported to disintegrate into, Diketoperazine which may be accountable for its toxicity to gut microbiota (Prodoliet and Bruelhart, 1993). There is evidence pointing to a mechanism whereby non-nutritive sweeteners exert bacteriastatic effects through inhibition of metabolic enzymes by altering nutrient transportation, or process that are essential for growth (Omran and Coughlin, 2013). The abundant bacterial diversity following exposure to non nutritive sweeteners over the experimental period were *Prevotella* sp, *Bacteroides* sp and *E. coli*. These bacteria were obviously enhanced selectively by the NNSs.

The result of this study is consistent with previous studies which utilized animal models. The study of Suez et al. (2014) showed modification in the intestinal microbiota after administration of some non-caloric artificial sweeteners (NASs) from data collected on 172 randomly selected individuals. They found positive connections between NASs consumption and the Enterobacteriaceae family, the Delta proteobacteria class and the Actinobacteria phylum. In another study, a two weeks supplementation with non nutritive sweeteners in capsules showed decrease in levels of *Eubacterium cylindroides*, as well as in levels of the beneficial and fermentative Bifidobacterium, Lactobacillus, Bacteriodes and Butyrivibrio populations (EASD, 2018)

Gut bacteria helps in the breakdown of complex molecules in meats, vegetables and plant cellulose. The implication of alteration of the microbiota following consumption of NNSs increases the risk of infections with opportunistic pathogens as the gut microbiota play a critical role in the development of a robust and balanced immune system. Immune tissues in the gastrointestinal tract constitute the largest and most complex faction of the human immune system (Robles & Guarner, 2013). The gut microbiota resist intestinal over growth of externally introduced population that would otherwise cause disease. It has been documented that people who suffer from certain diseases (such as inflammatory bowel disease, irritable bowel disease, and allergy) have a microbiota that is different from that of healthy people (Backhed et al., 2012). The result of the study shows that the consumption of NNSs consistently for a period of 2 weeks event at low doses will have bacteriocidal effects on the gut microbiota which can negatively affect the health status of such individuals. Therefore, NNSs should be consumed discreetly.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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