ORIGINAL ARTICLE

THE EFFECTS OF *L.CASEI* STRAIN *SHIROTA* SUPPLEMENTATION ON FAECAL PROFILES AND BODY WEIGHT GAIN OF OVERWEIGHT AND OBESE MALAY CHILDREN

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ABSTRACT

Probiotic Lactobacillus casei strain Shirota (LcS) has been proven beneficial for the treatment of gut-related conditions. However, it is unclear how it influences faecal SCFAs concentrations and how it disrupted in overweight and obese children. This study aimed to investigate the effect of LcS on faecal profiles and body weight gain in overweight (OW) and obese (OB) children. A total of 42 children, comprising 22 OW/OB children (mean age = 8.73 ± 1.03 years old; BMI = 24.73 ± 3.91 kg/m^2; 54.5% boys) and 20 normal weight (NW) children (as a control), were randomly assigned to either receive daily 80 ml of probiotic drink at a dosage of 3.0 x 10^{10} colony-forming units (CFU) for 4 weeks or a control. Faecal samples were taken, and SCFAs were analysed by HPLC. ***Results:*** *Twenty-two OW/OB participants completed the study. At the end of the fourth week of LcS supplementations, the propionate and the total SCFAs concentrations increased significantly over time with evident intervention effect (p<0.05). In the treatment group, the faecal propionate concentration (Mean= 153.57 µmol/g , SD= 142.17) increased by 161 % and total SCFAs concentration (Mean= 201.44 µmol/g , SD= 162.90) increased by 79 % from the baseline. The mean percentage of body weight changed significantly within the treatment and control groups: 6.4% in the control group and 7.4% in the treatment group (p 0.05). Nevertheless, no such significant differences were found between the treatment and control groups (p>0.05). LcS supplementations for the 4 weeks in OW/OB children were able to increase levels of propionate as compared to baseline values.*

Keywords: *Short-chain fatty acids, Probiotic, Bodyweight, Childhood obesity, HPLC*

INTRODUCTION

Childhood overweight (OW) and obesity (OB) have become a serious public health crisis and challenge in many developed and developing countries worldwide, with the potential for a seven-year reduction in life span[1,2].

Recent studies in developed countries, such as the United States, have revealed a significant increase in the prevalence of severe OB, particularly among non-Hispanic African American children[2,3]. Data from the National Center for Health Statistics (NCHS) showed that the prevalence of OB in American school-age children aged 6 to 11 increased from 6.5% to 18.4% between 1980 and 2017[2]. Currently, one-third of the paediatric population in the United States is estimated to be OW or OB[2]. Similarly, there was a significant increase in severe OB among British children aged 2 to 5 years from 2012 to 2013, and this upward trend of OW/OB continued even among South Asian children and black girls attending the English National Child Measurement Program for OW prevention in England[4,5]. Meanwhile in Japan, the national school health statistics have shown the fluctuating prevalence of OB in 11-year-old children. The OB rates were increasing for both boys and girls from 1977 to 2006 (from 6.72% for boys and 6.18% for girls, to 11.82% for boys and 9.65% for girls), then gradually decreasing in 2017 (9.69% for boys and 8.72% for girls), which then re-increased in year 2019 (11.11% for boys and 8.84% for girls)[6]. Another 27-year molecular trend study found that OB rates in Japanese children were rising along with rising proportions of abnormal blood lipid levels (total cholesterol (TC), triglycerides (TG), and HDL cholesterol (HDL-C)), when compared to non-obese children[6].

Consistently, a similar increasing trend in prevalence of OW/OB among school children was found in other developing countries, even in countries with historically high levels of undernutrition such as Bangladesh, Nepal, and India[7-10]. For instance, among Bulgarian and Indian school children aged 6 to 9 years, the prevalence of OW/OB was found at 30.4% and

19.6% in boys and 28.3% and 18.3% in girls, respectively[8,9]. Among Iranian children aged 9 years, the prevalence of OW/OB was 7.1% in boys and 8.6% in girls[10]. In Asian countries like Singapore, the prevalence of OW school children aged 7 to 9 years was around 22.5%[11]. In Thailand, the prevalence of OB among children aged 5 to 12 increased from 12.2% to 15.6% in just two years11.

Invariably, in Malaysia, the prevalence of OW/OB among school children has increased remarkably[12-14]. A recent study among school children in Kuala Lumpur and Selangor reported the prevalence of OW/OB among 8 to 12-year-old children was increased to 30%, which is much higher than a national study in SEANUTS Malaysia (21.6%) and the National Health and Morbidity Survey III (19.9%)[12,13]. Childhood OW/OB was most common in boys between the ages of 5 and 9, and in girls between the ages of 10 and 14. Furthermore, OW/OB occurs more commonly in Malaysian urban (12.1%) than in rural settings (11.2%), in public schools than private schools, and majority among ethnic Chinese (13.0%), followed closely by Indians ethnic (12.6%) and Malays ethnic (11.8%)[14].

Regardless of differences in this so-called "New World Syndrome" trend, the rising OW/OB epidemic among school-age children is cause for concern, mostly among those who live in urban areas. Given that the vast majority of undernourished children with OW/OB live in developing, low- and middle-income countries (LMICs), these phenomena were perceived to be caused by the nutrition transition in LMICs, which had influenced their food systems and food habits, particularly the availability and price of food, and were increasingly exposed to obesogenic environments[7,15]. Aside from lifestyle factors, childhood OW and OB are complicated multi-factorial diseases involving genetic and environmental factors. Other factors include a high calorie intake, altered diet composition, a lack of exercise, and changes in the gut microbiome[16]. Recently, the role of gut microbiota has been increasingly highlighted as an environmental factor that modulates SCFAs production and may lead to obesity development[17-20].

Short-chain fatty acids (SCFAs), the acetate, propionate and butyrate are anions in human faecal which, are presented in a molar ratio approximately in 60:20:20[21]. SCFAs contribute approximately 5 to 10% of the total energy requirements for human energy[22]. In theory, if this were equivalent to a daily intake of 2000 kcal, daily energy would be increased by 1%, resulting in an additional 20 kcal/day and 1 kg of annual weight gain[23]. Higher faecal SCFAs levels were also found in obese individuals than in normal-weight individual[19,24]. With the increase in obesity and the role of the gut microbiota as a vital element in energy homeostasis and weight management, few human trials have been conducted to determine the efficacy of probiotics as a modulator of the composition of gut microbiota and SCFAs concentration in the gut[22,25].

The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) define a "probiotic" as "a living microorganism that can provide beneficial health effects if administered in adequate amounts. Probiotics are also proposed to reduce the rate of body weight gain among children[25]. As in this study, the interest in the role of *Lactobacillus casei* strain Shirota probioticsic to improve body weight status and the level of SCFAs to curb childhood obesity is highlighted. Most of the experimental studies on *LcS* probiotics were only done in animal models and humans adults, which mostly relate the findings with gut microbiota and not SCFAs alone. A pilot study on 12 obese Japanese children discovered that LcS may reduce body weight and improve lipid metabolism by increasing the acetic acid and Bifidobacterium count in the obese children's feces[20].

However, this finding may potentially vary with Malaysian children due to several factors, such as the difference in climate and the dietary practices of the subjects[26]. In addition, the average fibre intake of Malaysian children is reported to be below the recommended amount of 20-30 g/day[12-14]. In the present study, we hypothesised that supplementing OW and OB children with *LcS* probiotic bacteria would increase the release of faecal SCFAs concentration and improve faecal consistency. This could be explained by changes in obese gut microbiota and gut hormones that occur during probiotic ingestion. Therefore, the objective of this study was to determine whether the supplementation of *LcS* probiotics could increase the faecal SCFAs concentration and its consistency in faecal samples from the children after 4 weeks of control and treatment with *LcS probiotics*, specifically among 22 OW/OB Malay children. Likewise, we measured the body weight of the participants to characterise the growth of the children throughout the study.

METHODS

Study Design

The study used a randomised cross-over design with two baseline periods (weeks 0 and 10), period 1 (weeks 1-4), a wash-out period (weeks 6-9), and period 2 (weeks 11-14) (Figure 1). Each period consisted of four weeks of intervention followed by a four-week washout period. During the 4 weeks of the intervention, subjects were randomly assigned to the treatment or control group. The treatment group received daily 80 ml of probiotic drink at a dosage of 3.0 x 10^{10}

colony-forming unit (CFU)/mL of viable *LcS* for 4 weeks (Intervention group: 10 OW/OB and 10 NW; Control group: 12 OW/OB and 10 NW), before crossing over the trial to the control group (Control group: 10 OW/OB and 10 NW; Intervention group: 12 OW/OB and 10 NW) after 4 weeks of wash-out period and vice versa. The duration of intervention of 4 weeks per period was preferred, due to the concentration of faecal SCFAs being found to decrease significantly as early as weeks 4, 6, and 8 during the intervention period with *LcS* probiotics between the treatment and control groups[2]. Since *LcS* can live for less than a week, a 4 weeks of LcS supplementation in each period is enough to show significant changes in the composition of gut microbiota. A 4-week wash-out period is also enough to prevent any carry-over effects from the previous interventions[27-29]. Although a longer period of intervention is suggested, the study may be costly and require high compliance from the subjects.

Sample size rationale

To calculate the sample size in this study, data from previous intervention study using a *LcS* probiotics to observe changes in the concentrations of fecal SCFAs (acetic, propionic and butyric) concentrations (mg/100 ml fecal water) during the test time in the treatment group were referred[28]. As early as weeks 2 and 8 of the intervention study, the concentrations of fecal acetic acid were reduced in the treatment group. The mean (*d*) difference in concentration between the times was 54 mg/100ml[28]. Based on the standard error mean (SEM) information given, the standard deviation (SD) difference between the time was 91.7 Hence, based on a formula for cross-over studies and using the values of d and SD, with α of 0·05 and power of 80%, the required sample size was nineteen subjects[36]. To allow for a drop-out rate of 20%, a total of twenty subjects were needed from the pool of subjects who had participated in the screening and fulfilled the inclusion criteria.

Subjects

A total of 42 school-aged children, comprising 22 OW/OB subjects and 20 NW subjects (as a control and not discussed in this paper), ranging in age from 7 to 10 years old, were recruited from a public primary school in Serdang, Selangor. Prior to the recruitment, 469 school children were screened[31]. The subjects were selected according to the inclusion criteria as follows: Malaysian citizenship, registrated in a selected school, age from 7 to 10 years, and a z-score for BMI-for-age greater than +2.0 SD that indicated overweight, obesity, and severe obesity. As a control, subjects who had a z-score for BMI-for- age between −2.0 and +1.0 SD which indicated normal weight, were also included. Subjects were excluded from the intervention study if they were non-Malaysian (n = 11) or had z-scores for BMI-for-age less than −2.0 SD (n=129), which indicated underweight, thinness, or severe thinness. Further exclusion criteria were as follows (n = 215): on vaccination (polio and others) within 1 month before screening and sample collection, on treatment with antibiotics during the 2 weeks before screening and sample collection, on supplementation with probiotic or prebiotic elements during the 2 weeks before screening and sample collection, on presence of diseases such as metabolic diseases (diabetes mellitus, hypertension, heart disease), cancer, and autoimmune disease during recruitment, and on medication of analgesics, non-steroidal anti-inflammatory drugs. Moreover, subjects who were allergic to fermented milk containing probiotic bacteria, lactose-intolerant, had gastric problems, or had liver or kidney injuries were also excluded (n = 10). About 18 subjects were further excluded due to their failure to participate, and another 55 subjects gave no response. Thus, a total of 42 subjects of the 469 subjects involved in the screening stage eligible and agreed to participate in the intervention[31]. Only 22 OW/OB subjects and 20 normal weight (as a control) agreed to enrol and remained in the study.

Parents' information sheets and written consent forms were engaged following the Helsinki Declaration of 1975, as revised in 2008, as well as obtained prior to the study by parents and subjects. The study ethics was approved by the Ethics Committee for Research Involving Human Subjects, University Putra Malaysia (JKEUPM), Malaysia [FPSK_November (13) 03], Ministry of Education, Putrajaya [KP(BPPDP)603/5/JLD.16(1 5 4)] and Department of Education of Selangor, Shah Alam (JPNS.PPN 600-1/49 JLD.32(32)].

Study protocol

For the first 4 weeks of the intervention (Period 1), one group of OW/OB (n = 10) and NW (n = 10) was supplied with fermented milk drinks containing *LcS* (probiotic drinks), and the other group of OW/OB (n = 12) and NW (n = 10) was supplied without probiotic drinks. After 4 weeks of wash-out period, the intervention was crossed over, where subjects who did not receive drinks before the wash-out period were given probiotic drinks, and vice versa, and the intervention continued for another 4 weeks (Period 2). To ensure compliance and reducing biases, drinks were given by the assigned researcher to the subjects on a daily basis once a day (after breakfast) in a school specific site (Health Centre). However, for weekend consumption, two bottles of probiotic drinks were given every Friday afternoon to the intervention group. The drinks were given in an ice box to maintain the refrigerated temperature. To ensure that subjects consumed the drinks and complied with the study protocol, a few reminders were made by calling and/or texting the subjects' parents and/or making a home visit to the subject during the weekend. Throughout the study, subjects

were instructed to maintain their current food intakes as a prior intervention. They were also given a list of restricted foods, vitamin supplements, antibiotics, and medications that may alter the results of the study.

The subjects, with their parents' and the assigned researcher's assistance, were required to fill in the subject's diary daily. The diary incorporated questions about intake of the product (during the treatment period), anthropometry measurements, a food diary, the number of bowel movements, changes in stool consistency, any medications taken, and any symptoms of discomfort, i.e., diarrhea, constipation, vomiting, gas, and illness. Anthropometry measurements included body weight and height and were recorded by the researcher in school, at baseline and every 4 weeks (week 0, week 5, week 10, and week 15). Subjects were weighed on a scale and measured using a stadiometer. BMI was calculated and classified using WHO child growth standards (BMI-for-age), z-scores[32]. The food intake of subjects was examined using a food diary for two days during the week and one during the weekend. Subjects, with their parents' assistance, were asked to record details such as the type and amount of foods and drinks consumed and cooking methods in a food diary section. To assist with the food recording, a set of household measuring cups was also provided to the subjects. To ensure the subjects had recorded their food intake properly, the food diary was frequently examined by researchers in a school. Physical activity was measured by using Physical Activity Questionnaires for Older School Children (PAQ-C) that categorized the subjects into low, moderate and high level of physical activity. The subject's diary was collected every time of faecal samples collection.

Faecal collection

As informed and demonstrated prior to the intervention by the researcher, subjects, with their parents' assistance, were asked to collect the faecal samples in a sterile container[29]. The samples were then given to the researcher at school before being stored in the icebox at – 4 °C, transported to the laboratory, and transferred into sterile 1.5 ml microtubes at an exact weight of 0.3 g. These aliquots were placed at – 6 °C immediately for further analysis. While the excess samples were kept at – 80 °C for storage and analysis[29].

Analysis of SCFAs

The SCFAs (acetic acid, propionic acid and butyric acid) of faecal samples were extracted with little modification by high-performance liquid chromatography[33]. Faecal samples of weight 0.2 g were used and diluted at ratio 1:4 to 1:8 (w/v) in sterile distilled water. Then, the samples were vortex for 1 min and centrifuged at 10,000g for 10 min. The supernatant was filtered through econofilter nylon with a pore size of 0.2 µm (IT Tech Research Sdn Bhd, Malaysia) and stored at -20 °C. A 40 µl of faecal sample extraction was injected directly into HPLC System (Shimadzu LC-10AD Liquid Chromatography) with Shimadzu SPD-6A UV-VIS detector (Shimadzu, Kyoto, Japan). SCFA in faecal samples were separated using an ionic exchange resin, Hi-Plex H column, (Hi-Plex H, 300x7.7mm, Agilent Technologies (Malaysia) at 65 °C. The target compounds were detected by using a UV detector set at a wavelength of 210 nm. Then, 0.01 N H_2SO_4 was filtered through 0.45 µm nylon membrane as a mobile period at a flow rate of 0.6 ml/min. Concentrations of SCFAs were determined by using the external calibration standard curves method and expressed as mean µmol per gram wet weight faeces. Seven calibration standards were prepared at six levels of concentration ranging from 0.01 M to 0.06 M for acetic acid, and 0.02 M to 0.12 M for propionic acid and butyric acid. The calibration curves were constructed by plotting the relative peak area versus the molarity of solution[33].

Faecal consistency

The subjects were informed about and trained on the Bristol Stool Form Scale by the researcher[34]. They were required to use the pictures provided to assess their faecal consistency immediately after defecation.

Statistical analyses

Data analysis was performed with SPSS Statistics 22.0 software. The data is checked for its normality. The non-parametric Wilcoxon signed-rank test was used to compare results from baseline and after 4 weeks of intervention in both the treatment and control groups of OW/OB subjects. The significance level for all tests was set at $p< 0.05$. Since the study was a crossover study and each subject consumed *LcS* probiotic drinks throughout the intervention, data were analysed using a paired-sample t-test to determine whether there was a carry-over effect (between baseline and after 4 weeks of intervention) that could have influenced the outcome.

The concentration of faecal SCFAs, faecal consistency, and body weight changes were compared between the treatment and control groups of OW/OB subjects using an independent t-test. Furthermore, the mean changes of faecal SCFAs concentration (%) from baseline to 4 weeks of intervention were calculated, and the results were compared between the treatment and control groups of OW/OB subjects using an independent t-test to observe the effectiveness of ingestion of *LcS* probiotic drinks. In addition, a paired sample t-test was used to compare the faecal SCFAs concentration, faecal consistency, and body weight changes between baseline and after 4 weeks of intervention in both the

treatment and control groups of OW/OB subjects.

To test the normality and homogeneity of variance, the Shapiro–Wilk, Levene's, and Fmax statistical tests were used. For all the tests, results were considered significant at $p< 0.05$.

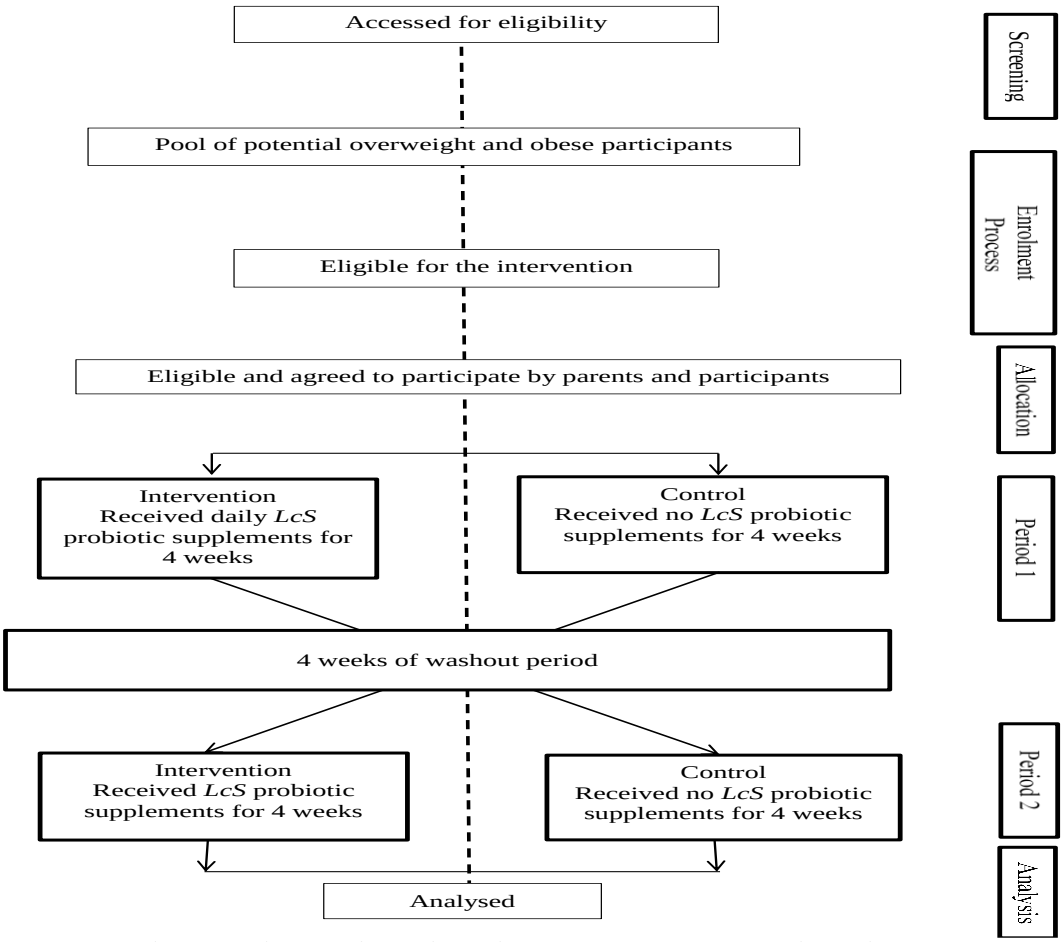

Figure 1: Flow of subjects through each stage of crossover trial. Anthropometry measurements, food diary, and the faecal samples were collected by the researcher in school, during Period 1 (at first baseline week 0, and week 5) and during Period 2 (at the second baseline week 10 and week 15). Period of probiotic ingestion were at week 1-4 (Intervention group: 10 OW/OB and 10 NW; Control group: 12 OW/OB and 10 NW), followed by wash-out period (week 6-9), and week 11-14 (Control group: 10 OW/OB and 10 NW; Intervention group: 12 OW/OB and 10 NW).

RESULTS

Subjects' characteristics at baseline

Table 1 shows the OW/OB subjects' characteristics at baseline. Throughout the study, there were no significant changes in dietary intakes (i.e energy, carbohydrate, protein, and fat intakes) or physical activity level of the subjects in both the control and treatment groups in OW/OB subjects.

Faecal SCFAs concentration

By using an independent-t test, Figure 2 and Figure 3 show no significant difference in faecal SCFAs between the treatment and control groups of OW/OB subjects at baseline and after 4 weeks of intervention (p>0.05), respectively. However, a significant interaction within times (baseline and after 4 weeks of intervention) was found in propionate (p=0.008) and total SCFAs (p=0.005) in treatment group using a paired-sample t-test (Figure 3).

Table 1: Subjects' characteristics at baseline

Variables	Mean ± SD
Sex (n)	
Male	12
Female	10
Age (years)	8.73±1.03
Weight (kg)	47.76± 12.09
Height (m)	140.87 ± 8.71
BMI (kg/m^2)	24.73±3.91

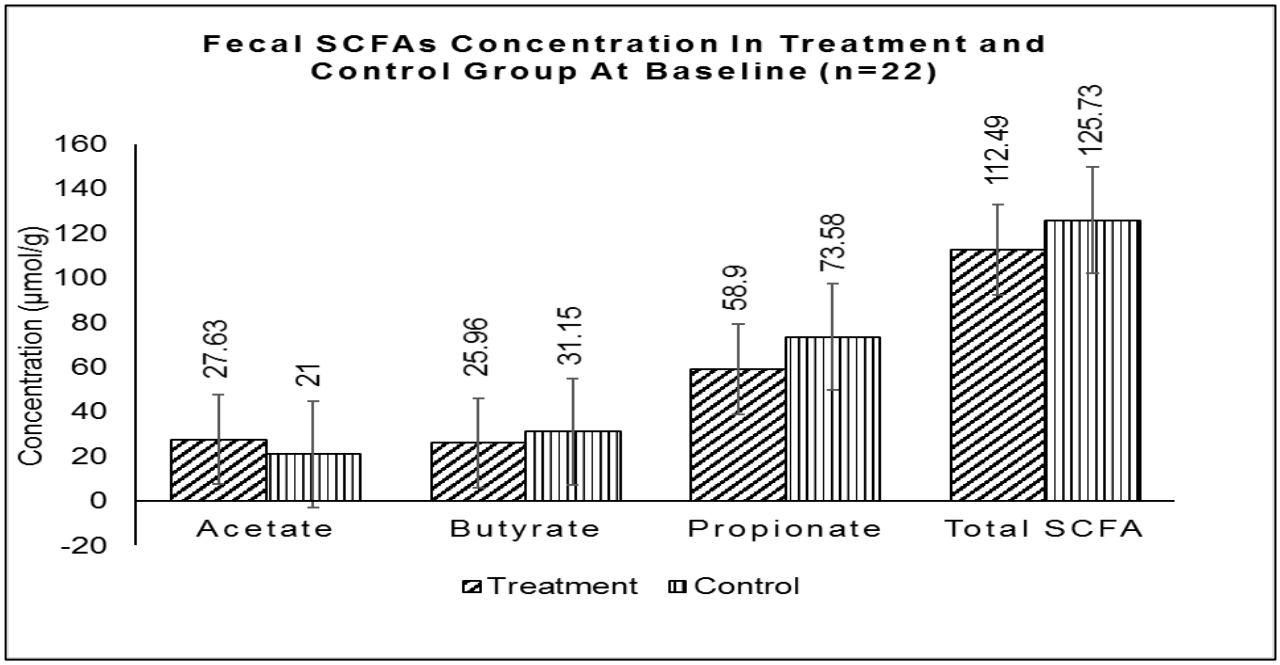

Figure 2: Faecal SCFAs concentration in treatment and control group at baseline of OW/OB subjects

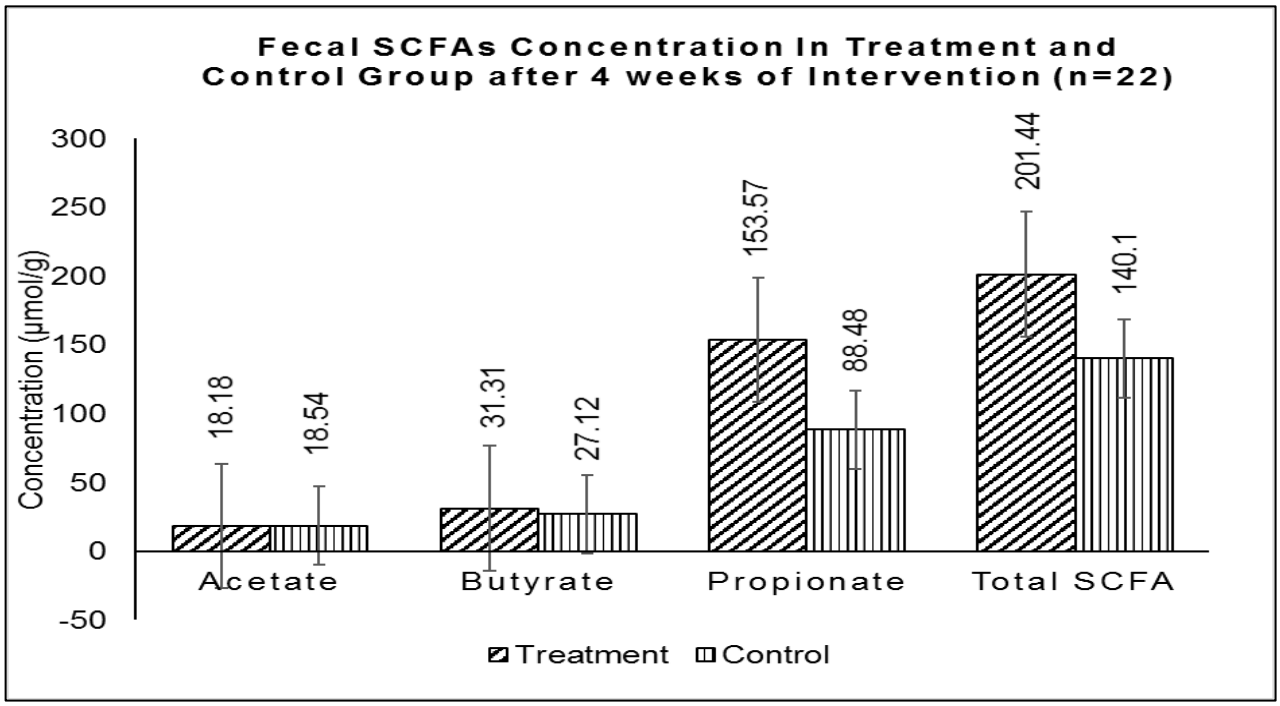

Figure 3: Faecal SCFAs concentration in treatment and control group after 4 weeks of intervention of OW/OB subjects

Figure 4 shows the mean percentage change of SCFAs between the baseline and after 4 weeks of intervention in the control and treatment groups of OW/OB subjects. The most prominent values that significantly increased over time were propionate and total SCFAs by using a paired sample t test, (p < 0.05). In treatment group, faecal propionate ($M_{propionate}$=153.57µmol/g, SD=142.17) and total SCFAs (M_{TSCFAs}=201.44 µmol/g, SD=162.90) concentration had increased about 161% and 79% than baseline respectively ($M_{propionate}$=58.90µmol/g, SD=49.11; M_{TSCFAs}=112.49µmol/g, SD=59.30).

Faecal Consistency

Table 2 shows a significant main effect for time obtained for faecal consistency (p=0.008) with confidence levels after the treatment (M=3.45 units, SD=0.51) being significantly higher than before the treatment (M=2.95 units, SD=1.13) of OW/OB subjects. However, there is no significant effect between the treatment and control groups, and its interaction (time*group) was also not found (F(1, 42) = 1.264, p=0.267; (F(1, 42) = 7.851, p=0.008).

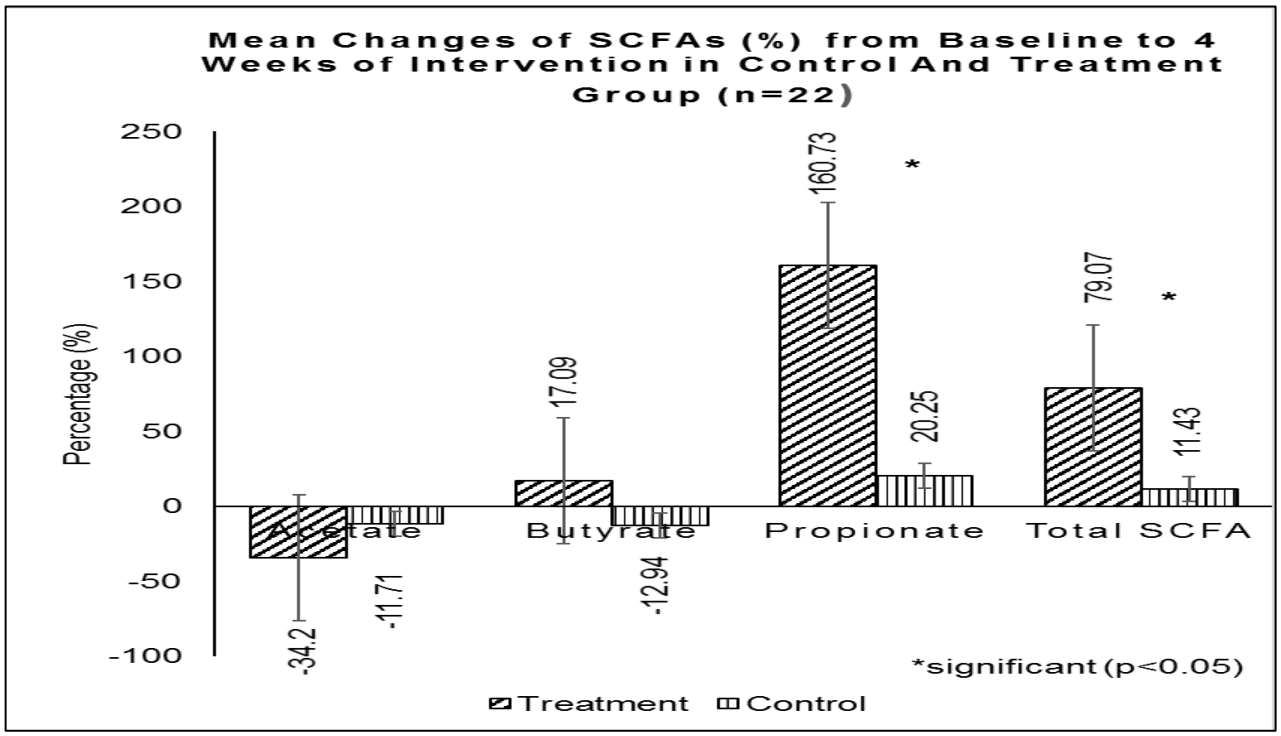

Figure 4: Mean change of SCFAs (%) from baseline to 4 weeks of intervention in control and treatment group of OW/OB subjects

Table 2: Effects of the *LcS* probiotic on faecal consistency in treatment and control group

Treatment		Control		*p*-value		
Baseline	After 4 weeks	Baseline	After 4 weeks	Time[a]	Group[b]	Time* Group
2.95 ± 1.13	3.45 ± 0.51	2.95 ± 1.13	3.45 ± 0.67	*p*<0.05	ns	ns

The values are presented as mean ± SD. SD: standard deviation
Bristol Stool Form Scale: 1, hard lumps like nuts; 2, sausage-shaped but lumpy; 3, like a sausage but with cracks on its surface; 4, like a sausage or snake, smooth and soft; 5, soft blobs with clear-cut edges; 6, fluffy pieces with ragged edges, a mushy stool; 7, watery, no solid pieces.
[a] *p<0.05 indicated a significant difference based on paired-sample t test as compared at baseline and after 4 weeks of intervention*
[b] *ns=no significant based on an independent-t test as compared between treatment and control groupof OW/OB subjects*

Body Weight Changes

Table 3 shows that, there was no significant effect of *LcS* probiotic supplementation on body weight status between the treatment and control groups (*p*>0.05) of OW/OB subjects. However, there was a significant difference in terms of time for both groups (*p*<0.05). The mean percentage of body weight change after 4 weeks of intervention as compared to baseline in the control group was 7.69% higher than in the treatment group, which was only about 6.43%.

Table 3: Effects of the *LcS* probiotic on the mean of body weight, BMI and BMI-for age of treatment and control groups

	Treatment		Control		p-value		
	Baseline	After 4 weeks	Baseline	After 4 weeks	Time[a]	Group[b]	Time* Group
Weight (kg)	51.3± 12.3	54.6± 12.8	50.7± 13.4	54.6± 12.5	*p*<0.05	ns	ns
BMI	25.7 ± 3.5	25.5 ± 3.6	25.4±4.1	25.3±4.1	*p*<0.05	ns	ns
BMI-for-age	2.9 ±.7	2.8 ±0.7	2.8±0.9	2.7±0.8	*p*<0.05	ns	ns

The values are presented as mean ± SD. SD: standard deviation
[a] *p<0.05 indicated a significant difference based on paired-sample t test as compared at baseline and after 4 weeks of intervention*
[b] *ns=no significant based on an independent-t test as compared between treatment and control groupof OW/OB subjects*

DISCUSSION

Acetate, propionate, and butyrate constitute almost 90–95% of the SCFAs present in the colonic lumen during fermentation and in the general systemic circulation[35]. The main products of SCFAs in the gut are mostly acetate>propionate>butyrate in a molar ratio of 60:20:2020[35]. Many factors influence SCFAs production, absorption, and excretion in the host gut, including the composition of the gut microbiota, age, dietary intakes, antibiotic use, genetics, and other lifestyle or environmental factors of the bacteria[35,36]. SCFAs can be produced by commensal microbes in the distal colon from the active fermentation process of soluble dietary fibres such as pectins, inulin, fructans, xylan, as well as resistant starches. SCFAs are rapidly absorbed in the caecum and colon. Only about 5 to 10% of SCFAs are being excreted in the faecal[37]. Once absorbed, SCFAs are metabolized at three major sites which include cells of the caecum-colonic epithelium, liver cells and muscle cells in the body[38]. There are a few physiological functions of SCFAs. Acetate is involved as a substrate in the synthesis of cholesterol and suppressor of appetite[38]. Butyrate supplies almost 70% energy to the colonic epithelium by undigested carbohydrate fibers[38]. Propionate acts as a precursor to gluconeogenesis, synthesis of protein and lipogenesis in the liver[38]. While together with a combination of acetate and propionate undergo adipogenicity via GPCR43[18].

Overall, this study found a significant difference in terms of time (not group) after 4 weeks of intervention on faecal propionic acid and total SCFAs (p<0.05) of OW/OB subjects. Both faecal propionic acid and total SCFAs concentrations in the treatment group increased by 161% and 79% from baseline to 4 weeks of *LcS* supplementation, respectively. This increase is not clear whether it was linked to the maturation of the children or because of other reasons. However, this study had suggested OW/OB children may be able to produce greater propionate-producing bacteria after the supplementation of *LcS* probiotic. The propionate-producing bacteria such as *Akkermansia muciniphila* may improve the metabolic function in mice[38]. Furthermore, the gut microbiota of the obese itself may be more efficient in removing energy content from diet intakes as compared to normal weight children[39]. This eventually will influence the energy storage, adiposity, as well as body weight gain, of the children.

Recent reports have shown a significant increase in the amount of faecal SCFAs after a few weeks of being supplemented with fermented milk beverages containing *LcS*[18,40]. Similarly, a study of 14 boys and 9 girls from Japan found a significant increase in faecal SCFAs concentrations and a decrease in faecal pH during the 6-month *LcS* supplementation period[25]. The consistent consumption of *LcS* - containing probiotics among the children may alter the composition of gut microbiota, maintain the gut environment as well as gut homeostasis[13]. Other researchers, however, found the opposite effect in Europeans, finding a decrease in acetate and propionate concentrations during *LcS* administration[40,41]. Likewise, in one study on 12 obese and 22 non-obese children from Japan, faecal acetic acid concentration was reported to be higher during *LcS* ingestion as well as higher in the obese group than the control group[20]. In this study, acetic acid concentration was lower after *LcS* supplementation in both treatments (34%). However, this observation resulted in no significant difference to prove whether *LcS* can decrease acetic acid concentration or not. The previous study may have produced different results due to the inclusion of longer treatment periods with *LcS* probiotics as well as a well-planned diet and exercise therapy[20]. A longer treatment period may be the major influence on the increment of acetate concentration in that study.

It has been suggested that such SCFAs concentration inconsistencies may be caused by a complex interaction between different dietary factors in diverse countries and populations, resulting in differences in microbiome diversity, gut transit time, and mechanisms in colonic microbiota that modulate human faecal SCFAs concentration[21,42]. In addition, the quantity and relative concentration of SCFAs metabolites produced in the gut has , also been closely associated with breastfeeding practices during infancy, dietary intakes from indigestible carbohydrates, particularly dietary fibers and resistant starch, as well as gut microbiota composition, diversity, and activity[42]. Thus, by understanding these factors in the multiethnic population worldwide, these inconsistencies of SCFAs production before and after probiotic ingestion could be further understood and explored.

The trend in SCFAs is not limited to a specific body weight status, but is more related to gut microbiota dysbiosis, genetics, environmental factors, and the children's particular dietary intake. In the future, our findings could possibly provide a certain association between the gut microbiota, and gut hormones with faecal SCFAs concentrations. This is because previous research has linked higher faecal SCFAs concentrations to higher gut microbiota and gut hormone levels in OW/OB children, even after *LcS* probiotic ingestion[20,29]. Most of these findings agreed with the idea of relating higher production of various SCFAs by the colonic microbiota to fermentation of dietary fibre, which is subsequently absorbed by the host. The colonic microbiota uses dietary

fibre as its primary energy source to produce beneficial metabolites known as SCFAs for the host's health[22].

One mechanism that may cause to increment of faecal SCFAs after *LcS* ingestion is by increasing the total lactobacillus counts together with its pre-existing abundance of indigenous lactobacillus in the intestine. This will then lead to reduce pH stool and defeat the proliferation of destructive bacteria[43]. *LcS* ingestion was also had shown significantly related to a higher number of Bifidobacterium counts[41]. Similarly, Wang et al. discovered higher concentrations of Bifidobacterium *spp*. in the intervention group of OW/OB and NW children who consumed *LcS* probiotics, but the mechanism by which Lactobacillus in *LcS* probiotics may stimulate the proliferation of Bifidobacteria was still unknown[25]. Nevertheless, Bifidobacterium counts may reflect the homeostasis of the gut microflora and SCFAs production. Lactobacillus and Bifidobacterium synergistic effects may provide protection against dysbiosis, an advanced mucosal gut barrier, and colonisation resistance[43].

The supplementation of Lactobacillus strain probiotic is found to increase the production of propionate and butyrate while reducing the concentration of acetate[29]. The study has also suggested that *LcS* can restore a proper microbial balance in the gut by the formation of propionate, and stimulate butyrate-producing bacteria to produce butyrate in the OW/OB cecum[29]. When *LcS* restored the imbalanced gut microflora, it may also act as a modulator to energy intake and metabolism by producing higher SCFAs from bacterial fermentation of resistant starch, dietary fibre and other indigestible polysaccharides[17]. The increment of SCFAs production may then lead to an increment in energy substrates, satiety, and food intake regulation. SCFAs are believed to activate the G-protein-coupled receptors GPR41 and GPR43 as well as stimulate the secretion of hormones such as peptide tyrosine tyrosine (PYY) and glucagon-like peptide-I (GLP-1)[17]. As a result, gut motility and gut transit time will be suppressed, leading to greater nutrient absorption. On the other hand, propionate and butyrate also have been linked to the reduction of food intake and may protect from diet-induced obesity and insulin resistance in mice[37]. The function of SCFAs in metabolism and body weight regulation has not yet been fully explained, as various factors may affect gut microbiota composition and body weight gain in children.

Another factor that may increase propionate excretion in the faecal is by reducing propionate absorption in the distal small intestine and colon of obese children, since only 5 to 10% of SCFAs are excreted in the faecal[29,43]. More than 95% of the major three are rapidly absorbed in the human colon and caecum. SCFAs will then be metabolized at 3 different sites in the human body, which are cells of the caecum-colon epithelium, liver cells, and muscle cells. In this process, butyrate is seen to have an important function for generating energy to the colonic epithelium, whereas most of the acetate will enter the systemic circulation and peripheral tissue. While propionate is involved in the gluconeogenesis in portal circle[29,43]. Due to this rapid assimilation, faecal SCFAs concentration is reported to not properly correlate with production and concentration in the gut of the human[43]. There is little known about the effect of maturation on SCFA levels in children's faeces that could be explained here. Therefore, the results obtained in the study suggest that it would be relevant to follow cohorts of healthy children for a long time to get a better understanding of this.

Faecal consistency was also investigated during this study. A previous randomized placebo-controlled study found that *LcS* probiotic has improved the defecation frequency and faecal consistency of the adults who have defecation problems and constipation[40]. Furthermore, *LcS* probiotic consumption may improve defecation and stool consistency in chronic constipation patients[44]. These findings suggest that taking *LcS* probiotics may help improve the frequency and consistency of bowel movements in constipated patients. *LcS* probiotics have also been shown to improve faecal consistency in healthy humans and children, with an increase in bowel movements, softened feces, and an increase in bifidobacterial percentage in *the gut*. As a result, this study suggests *LcS* as a probiotic strain that is beneficial to humans[40].

Both the treatment and control groups of OW/OB subjects did show an increment in body weight and a significant difference within the time of the study ($p<0.05$) but no significant effect of *LcS* on body weight was found between the treatment and control groups. This study suggested that it might be because of normal human physiological growth factors that are occurring in children. Furthermore, as reported in another study, the treatment period with *LcS* probiotics during this study was too short to show any significant effect on body weight reduction[20]. In this study, bodyweight gain may also reflect a negative status for the children's energy expenditure. This could be due to an increase in food consumption or a decrease in energy output[45]. However, this study found no significant difference in diet intake between the treatment and control groups. Hence, the body weight gain resulting from the study might also be because of lower energy expenditure and changes in the children's gut barrier.

Several administrations of bacteria strains in probiotics have also been shown to demonstrate

body weight loss and a reduction in fat mass of human studies[46]. In general, probiotics may alter the composition of gut microbiota, affect appetite and satiety, and affect body weight composition and metabolic functions[29,46]. Aside from that, an animal model study discovered that *LcS* probiotics reduced body weight by decreasing leptin levels and adipocyte size[47]. A previous study showed that supplementation with probiotics produced far better results than using treatments such as orlistat for weight management. *LcS* administration has also been shown to significantly reduce fat mass and ALT levels in the liver compared to other drugs[47].

CONCLUSION

In conclusion, at baseline, subjects' characteristics in this study were homogenous. After 4 weeks of intervention, both the treatment and control groups of OW/OB subjects significantly increased propionate, total SCFAs, and faecal consistency. The mean percentage of body weight changed significantly within the treatment and control groups, by 7.7% in the control group and 6.4% in the treatment group ($p < 0.05$). Nevertheless, no such significant were found between the treatment and control groups ($p > 0.05$). *LcS* probiotic supplementations for the 4 weeks in OW/OB were able to increase levels of faecal SCFAs concentration especially propionate, may be corresponding to higher obese gut microbiota and gut hormones that might be presented. This effect can be explained far more fully if a future study includes the gut microbiota profiles, intermediate metabolites, and gut hormone levels such as PYY, GLP-1, and ghrelin that seem beneficial for appetite suppression and body weight management in OW/OB prevention. Thus, this study suggested a longer term of probiotic ingestion with larger and more multiethnic subjects for future investigation.

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Conflict of interests
The authors declare no potential conflict of interest.

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