

## Effect of Probiotic Microbial Cell Preparation (MCP) on Fasting Blood Glucose, Body Weight, Waist Circumference, and Faecal Short Chain Fatty Acids among Overweight Malaysian Adults: A Pilot Randomised Controlled Trial of 4 Weeks

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### ABSTRACT

**Introduction:** Probiotic microbial cell preparation (MCP) supplementation is one of the approaches to modulate alterations in gut microbiota (GM). This double-blind randomised controlled trial investigated the effect of 4 weeks of MCP supplementation on fasting blood glucose levels (FBG), body weight (BW), waist circumference (WC), and faecal short chain fatty acids (SCFA) among 24 healthy and overweight (with BMI  $\geq 23$  kg/m<sup>2</sup>) Malaysian adults. **Methods:** Twenty-six subjects were randomised to receive either MCP (n= 12) or placebo (n=14), twice daily, for 4 weeks. The probiotic powder contained a mix of six strains namely, *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Lactobacillus casei*, *Bifidobacterium longum*, *Bifidobacterium bifidum* and *Bifidobacterium infantis* ( $3.0 \times 10^{10}$  cfu). FBG, BW, WC, WHR, faecal SCFA, physical activity levels and dietary intake were measured and changes were determined using repeated measures ANOVA. **Results:** Twenty-four subjects successfully completed the 4-week study period. Changes in FBG, BW, WC and SCFA were not significantly different between the groups. Only subjects in the MCP group significantly reduced their energy intake compared to baseline (1671 $\pm$ 476 vs 1386 $\pm$ 447 kcal, P=0.045). **Conclusion:** A 4-week supplementation of the MCP mix powder did not have significant effects on the variables studied. However, the significant reduction in dietary energy intake in the MCP group suggests the potential of probiotics as an adjuvant to dietary therapy for weight loss.

**Key words:** Microbial cell preparation, obesity, overweight, probiotics, randomised controlled trial

## INTRODUCTION

Accumulated evidence shows that alterations in gut microbiota (GM) may contribute to the pathophysiology of metabolic disorders including obesity and type 2 Diabetes Mellitus (DM). One common approach is to modulate GM profile using dietary supplements or food products containing probiotics. Numerous studies in the West have demonstrated that probiotic supplementation improves glucose homeostasis, lipid metabolism and reduces endotoxemia (Duncan *et al.*, 2008). Chronic inflammation reduces glucose tolerance and increases fat deposition in the body as seen in metabolic syndrome (Kovatcheva-Datchary, 2012). Probiotics help by suppressing undesired immune responses, hereby preventing chronic inflammation (Tannock, 2005; Duncan *et al.*, 2008) thus holding promise to improve metabolic dysfunction.

Probiotics produce a large array of glycoside hydrolases and polysaccharide lyases that humans do not encode in their genome (Chan, Estaki & Gibson, 2013). Hence probiotics promote fermentation of undigested carbohydrate in the gastrointestinal tract producing short chain fatty acids (SCFA) as metabolites (Duncan *et al.*, 2007). Currently, studies suggest that there are systemic effects of probiotic-produced SCFA to the host. These include better regulation of blood glucose levels, modulation of satiety and appetite through the gut-hormone link, and fat metabolism and storage (Salazar *et al.*, 2015).

Furthermore, differences in rate and ratio of SCFA production have been reported to be dependent on the type and amount of substrate available for colonic fermentation as opposed to the composition of the type of GM bacteria (Fernandes *et al.*, 2014). This strongly hints that, by acting as a substrate for GM, dietary fibre (DF) intake could have an influence on the rate of SCFA production, with low DF intakes being associated with

low rates of SCFA production (Schwiertz *et al.*, 2010). Apart from nourishing the GM, DF delays gut transit time, thus elongating the colonic fermentation time to yield higher and better SCFA profile (Demigne, Remesy & Morand, 1999). Long transit times in the large intestine have a profound effect on bacterial physiology and metabolism, leading to protein breakdown and amino acid fermentation, thus greatly contributing to colonic SCFA pools (Almada *et al.*, 2015; De Baere *et al.*, 2013).

A previous study has shown a 4-week MCP supplementation period to be sufficient to alter the GM profile in patients suffering constipation in Malaysia (Mazlyn *et al.*, 2013) and improve blood glucose regulation (Cani *et al.*, 2008) in other populations. We hypothesised that a 4-week MCP supplementation is sufficient to show changes in fasting blood glucose levels (FBG), anthropometric measures and faecal SCFA in this pilot trial among populations with low DF intake such as Malaysians (Ng, 2010). Hence, the aim of this study is to study the effect of 4-week MCP-supplementation on the FBG, body weight (BW), waist circumference (WC), waist-to-hip ration (WHR) and faecal SCFA among overweight Malaysian adults.

## METHODS

### Ethics approval and study design

This study was approved by the Joint Committee of Research and Ethics of the International Medical University (IMU) at Kuala Lumpur, Malaysia (Project no: MM I 1/2014(06)). The study was designed as a double-blind, randomised controlled trial with parallel group assignment and was conducted at IMU campus in Bukit Jalil, Kuala Lumpur.

### Sample size estimation

The sample size for this study was calculated using the WHO statistical software Epi-info version 5.0, for a power

of 80% at a two-sided 95% confidence level. An allocation ratio of 1:1 was used to block randomise subjects into the placebo and experimental arms. Twenty-four subjects, 12 in each group, were required for the study to detect a 0.5mmol/L difference in FBG between the intervention and control groups. Owing to the short nature of the study, changes in FBG were expected to be superior than other anthropometric measures.

### Subject criteria

Subjects were recruited between October 2014 and January 2015. Informed consent was obtained from subjects prior to their participation during the screening session. The study recruited overweight subjects in the age group of 18-50 years using the WHO Asia-Pacific criteria (BMI  $\geq$  23 kg/m<sup>2</sup> or waist circumference  $>$ 90 cm in men and  $>$ 80 cm in women) with no maximum cut-off (WHO, 2004). Subjects with metabolic or gastrointestinal disorders or on drugs that affect glucose metabolism and GM (such as antibiotics) were excluded. Pregnant and lactating women and subjects with known allergy to probiotic products were also excluded.

### Randomisation and blinding

Recruited subjects were randomised into two groups: MCP (intervention) and placebo (control) using a research randomiser software (<https://www.randomizer.org/>). This study was designed as a triple blind controlled trial with both the researchers, statistician and the participants being blind to the allocation. The blinding procedure was only broken after completion of all analysis.

### Placebo and MCP supplements

Hexbio® B-Crobes Laboratory Sdn Bhd. Ipoh, Malaysia provided the MCP supplement and placebo samples. Both the probiotics and the placebo had a similar appearance and taste; orange-flavoured, in granulated powder form and packed

in an unlabelled sachet. Subjects were given their respective supply of probiotics or placebo supplement sachets in two instalments, one at the start of the study and subsequently at their interim visit. They were instructed to store all sachets in a cool and dry place until consumption. The MCP sachet contained 30 billion colony-forming units of highly compatible, acid- and bile-resistant probiotics strains of *Lactobacillus acidophilus* BCMC® 12130, *Lactobacillus casei* subsp. BCMC® 12313, *Lactobacillus lactis* BCMC® 12451, *Bifidobacterium bifidum* BCMC® 02290, *Bifidobacterium longum* BCMC® 02120 and *Bifidobacterium infantis* BCMC® 02129. The placebo contained all components of the supplements except the probiotics strains. Subjects were asked to consume two sachets daily; one after breakfast and one after dinner, while maintaining their normal diets and physical activity levels throughout the study period. Additionally, subjects were asked to collect and return the empty sachets to the researcher as a measure of compliance.

### Measurement of outcomes

#### *Anthropometry, fasting blood glucose, dietary intake and physical activity measurements*

Subjects were followed up fortnightly throughout the 4-week study period. Anthropometric measures, FBG, dietary intake and physical activity levels were measured at baseline (Week 0), interim (Week 2) and end of trial (Week 4). Anthropometric measures included BW (Omron Karada Scan, HBE-375, Japan), WC and hip circumference (SECA Body Meter, SECA 201, Germany) were done according to WHO (2004) protocols and waist-to-hip ratio (WHR) was calculated manually. FBG was measured (Free Style Optium glucometer, MediSense, UK) after a 10-h overnight fast. Furthermore, dietary intake was monitored and analysed using a 3-day food record using the NutriPro software™ (Axxya Systems, USA) while

physical activity level was measured using the validated International Physical Activity Questionnaire (IPAQ)-short form (Craig *et al.*, 2003). Both parameters were measured at the baseline and end-of-trial.

#### *Faecal SCFA analysis*

Faecal SCFA was analysed in a sub-group of eight randomly-selected subjects (4 subjects from each group). Faecal samples were obtained at baseline and at the end of the 4-week trial period. Faecal SCFA analysis was performed using the high-performance liquid chromatography (HPLC) based on the modified protocol from a methodology validated by De Baere *et al* (2013).

#### *HPLC chromatographic conditions and preparation of standard curves*

HPLC method was developed to determine the concentration of organic acids in fecal samples. This was done using Agilent Technologies 1200 Series (Agilent, San Francisco, USA) equipped with a UV-visible detector and a 20- $\mu$ L injector. Compounds were separated on a 30mm X 7.8mm, 9 $\mu$ m particle, SUPELCO Supelcogel C610H Ionic Column (Supelco, Bellefonte, USA) coupled with 0.2% Phosphoric Acid with a pH of 2.03 as the mobile phase. Individual 0.01M of SCFA solution was injected to determine its retention time (RT). Four organic acid standards namely acetic acid, lactic acid, isobutyric acid and propionic acid, were obtained from Sigma Aldrich (Sigma, Germany) to construct standard curves of 8 points ranging from 0.001M until 0.01M was developed individually for each SCFA. Besides, a mixture of four SCFA chromatogram and prepared faecal sample injections are shown in Figure 1 to indicate a clear separation between all organic acids.

#### *Faecal sample preparation*

The faecal sample was collected from eight randomly selected subjects. Four

subjects from each group gave a sample from Day 1 and Day 28 respectively. They were given a bottle with cap and instructed verbally on how to collect the faecal sample. All samples were collected in study site. After collection, the samples were promptly stored in a freezer at -22°C. Due to high volatility of SCFA, all samples were processed individually within a week of collection. A 0.2 g of the sample was weighed in a 10ml falcon tube and diluted with ultrapure water at 1:4 (w/v). The mixture was vortexed for 1 min with the homogenate being centrifuged using Eppendorf centrifuge at 10,000 x g. Two ml of the supernatant was pipetted into a micro centrifuge tube and stored again at -22°C (De Baere *et al*, 2013).

#### *Determination of faecal short chain fatty acids (SCFA) concentration*

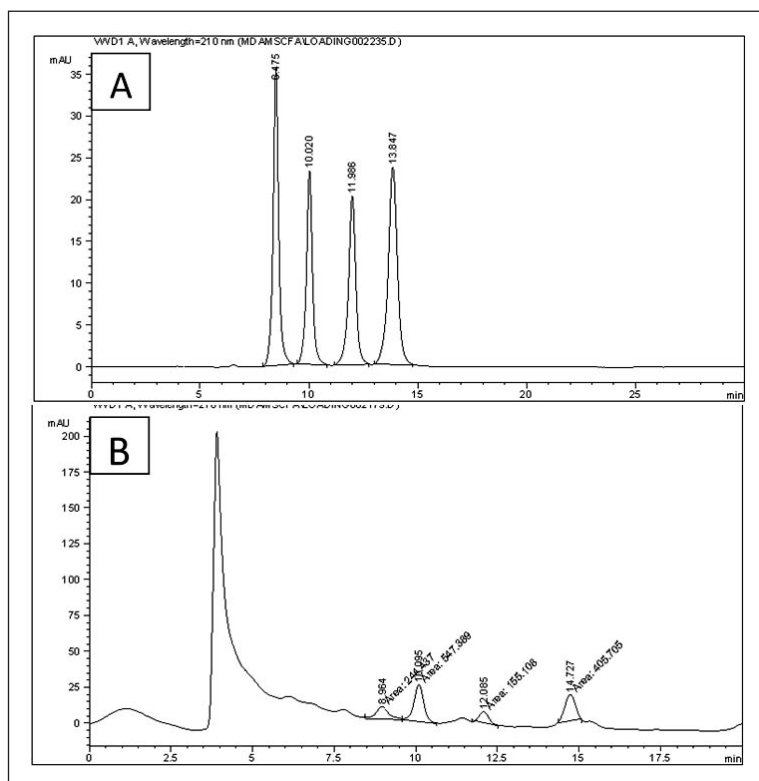
A total of 20 $\mu$ l of each sample was filtered using 0.22 $\mu$ m syringe filter before injection into HPLC. The run-time for each faecal sample was 20 min. Chromatogram of faecal sample was obtained and concentrations of faecal SCFA were calculated. Based on an individual chromatogram, the SCFA peaks was determined by matching its RT. Hence, these peaks were integrated individually using software Agilent ChemStation (Agilent Technologies, 2008).

Faecal concentration in molar was evaluated by solving the peak areas using equations obtained from each SCFA standard curves and was converted into  $\mu$ mol/g which was determined using the equation below:

Faecal SCFA ( $\mu$ mol/g) =

$$\frac{\text{Organic acid in faecal contents (mmol/mL)} \times V_d \times 1000}{\text{Wet weight faeces (g)}}$$

\*  $V_d$  = Total volume of dilution



**Figure 1.** Chromatogram obtained from the injection of Standards Acid (A) and sample stool (B). The peak is clearly separated and distinguishable for quantification. Peaks separation were according to respective SCFA retention time; lactic acid (6 min), acetic acid (10 min), propionic acid (12 min) and eventually isobutyric acid (14 min).

### Statistical analysis

Data were analysed using the SPSS software (IBM, USA). Shapiro-Wilks test ( $P < 0.05$ ) was used to determine the normality of all data sets. The data analysis for this study was performed as per protocol basis. Descriptive statistics for all continuous variables are presented as mean (standard deviation) or median (interquartile range) as found appropriate. For all normally distributed variables, parametric tests were used such as paired and independent *t*-test. For variables that were not normally distributed, appropriate transformation to improve homoscedasticity was attempted to facilitate the use of parametric tests. If efforts failed to improve the normality of the data, appropriate non-parametric tests

were used to analyse such data. Changes in study parameters with time, between groups and between time and group were assessed using repeated-measures ANOVA (RMANOVA). Statistical significance was set at 5%.

### RESULTS

The movement of subjects through the study is presented using a CONSORT diagram (Figure 2). Twenty-six subjects satisfied the inclusion criteria and were randomly allocated into the two groups (Placebo,  $n = 14$ , Probiotics MCP  $n = 12$ ). A total of 24 (12 in placebo and 12 in probiotics MCP) completed the first 2-week period of intervention and necessary measurements

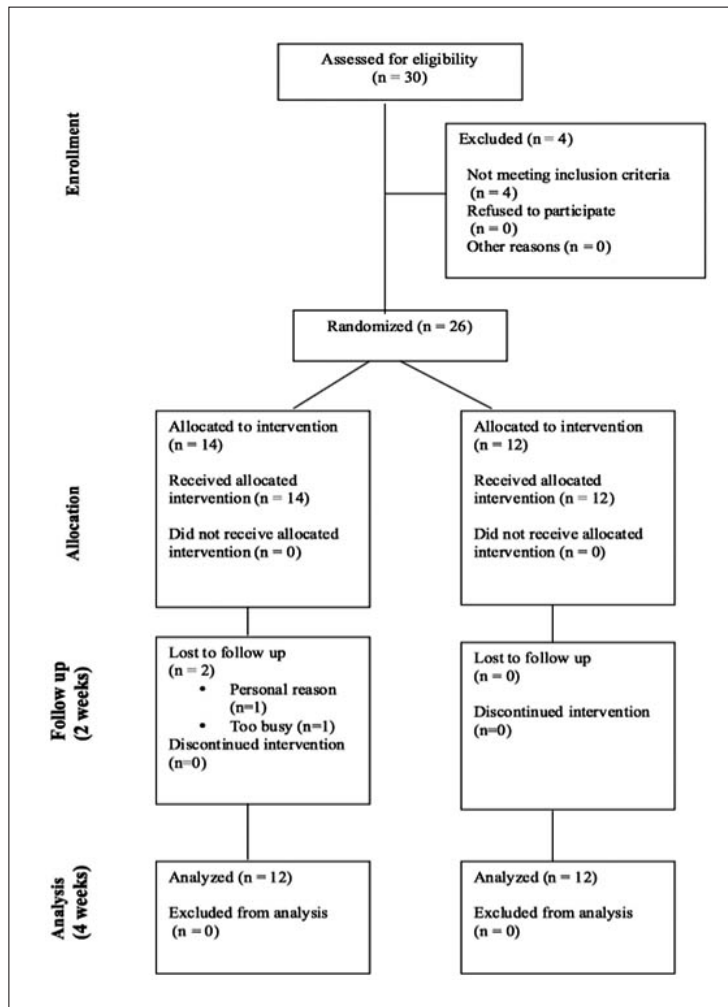


Figure 2. CONSORT diagram depicting the movement of subjects throughout the study

while two subjects in the placebo (6.7%) withdrew from the trial during the first two weeks. The reasons for the withdrawal are provided in Figure 2.

#### Baseline characteristics

The mean age of the subjects in the placebo (30.1±7.2) and MCP (28±8.3) groups were comparable (p=0.072). Twenty-six subjects were enrolled at the start of the study with 61.5% being female (n= 16) and 38.5% male (n= 10). There were eight obese subjects

(30.7%); five (19.2%) were enrolled in control group and three (11.5%) in the MCP group. The mean BMI of both groups was comparable at baseline (placebo: 24.1±5.1, MCP: 25.2±3.2; p = 0.103). Subjects' fasting blood glucose levels, body weight, waist circumference, waist-to-hip ratio and faecal short chain fatty acids were well matched in the randomised groups (Table 1). Twenty-four (92.3%) subjects remained at the end of the study.

**Table 1.** Fasting blood glucose, bodyweight, WC and WTHR in the placebo and probiotic groups at various time points of the study period.

Parameter	Placebo	Probiotic MCP	P value from RMANOVA		
			Time	Group	Time *group
Fasting blood glucose (mmol/L)					
Baseline	5.6 ± 0.5	5.0 ± 0.9	0.001*	0.094	0.307
Interim	5.4 ± 0.5	5.1 ± 0.7			
End-of-trial	5.1 ± 0.5	4.7 ± 0.6			
P-value	0.001*	0.174			
Body weight (kg)					
Baseline	84.0 ± 23.5	78.6 ± 15.1	0.003*	0.314	0.094
Interim	83.7 ± 23.3	79.4 ± 15.8			
End-of-trial	83.8 ± 23.1	78.3 ± 15.3			
P-value	0.525	0.580			
Waist circumference (cm)					
Baseline	94.0 ± 17.1	95.0 ± 10.5	0.469	0.908	0.265
Interim	95.0 ± 16.4	93.1 ± 10.0			
End-of-trial	94.0 ± 16.9	92.9 ± 10.1			
P-value	0.525	0.580			
Waist to hip ratio					
Baseline	0.8 ± 0.1	0.9 ± 0.1	0.491	0.972	0.256
Interim	0.9 ± 0.1	0.8 ± 0.1			
End-of-trial	0.8 ± 0.1	0.8 ± 0.1			
P-value	0.525	0.580			

Legend: Data are shown as mean ± SD; \*P values shown are obtained from using RMANOVA to compare between and within groups at P < 0.05 being significantly different

The compliance rate for the 24 subjects who completed the study was excellent. Those who completed in both the MCP and placebo groups reported 100% compliance and none of the study subjects reported consuming antibiotics or any probiotic products other than the treatment sachet provided during the course of the trial.

#### Effect of MCP on study parameters

##### Fasting blood glucose levels

FBG levels in the MCP group and the placebo group remained statistically comparable throughout the study (Table 1). However, RMANOVA showed only the placebo recorded a significant decrease over the 4 weeks (-0.6 ± 0.4 mmol/L, p=0.001), with no significant change in the MCP group (-0.3 ± 0.8 mmol/L, p=0.174).

Besides, the change in FBG level from baseline was not significantly different between the placebo and MCP groups after two weeks (-0.2 ± 0.4 vs 0.1 ± 0.6 mmol/L, p=0.327) as well as after 4 weeks (-0.6 ± 0.4 vs -0.3 ± 0.8 mmol/L, P=0.342). Finally, no significant differences were noted between the group across the trial period.

##### Body weight (bw), waist circumference and waist-to-hip ratio (WTHR)

BW in MCP and placebo groups was comparable at all points during the study (Table 1). Statistically significant reductions in BW were observed at the end of 4 weeks in both groups. However, these changes were not significantly different between the placebo and MCP groups after 2 weeks (-0.4 ± 1.3 vs. 0.8 ± 2.3

**Table 2.** Changes in fecal SCFA concentration in probiotics MCP and placebo group.

Timepoints	Concentration	
	Placebo (n=4)	Probiotics MCP (n=4)
Lactic acid ( $\mu\text{mol/g}$ )		
Baseline	115.3 $\pm$ 95.6	123.4 $\pm$ 16.6
End-of-trial	98.6 $\pm$ 63.3	120.5 $\pm$ 36.6
P-value	0.679	0.855
Mean change	16.7 $\pm$ 72.9	2.8 $\pm$ 28.4
Acetic acid		
Baseline	242.0 $\pm$ 107.7	175.7 $\pm$ 19.5
End-of-trial	145.7 $\pm$ 58.1*	144.7 $\pm$ 26.
P-value	0.034*	0.034*
Mean change	96.1 $\pm$ 52.0	31.0 $\pm$ 16.7
Propionic acid		
Baseline	36.8 $\pm$ 16.4	33.6 $\pm$ 27.0
End-of-trial	29.2 $\pm$ 5.4	22.7 $\pm$ 11.8
P-value	0.525	0.269
Mean change	7.6 $\pm$ 21.3	10.9 $\pm$ 16.1
Isobutyric acid		
Baseline	204.4 $\pm$ 116.3	233.4 $\pm$ 46.1
End-of-trial	138.4 $\pm$ 89.2	187.6 $\pm$ 72.3
P-value	0.338	0.094
Mean change	66.0 $\pm$ 116.0	45.9 $\pm$ 38.0

Legend: Data are shown as mean  $\pm$  SD; P-values shown were obtained from using Student's t-test to compare between groups (independent samples); \*P <0.05 is deemed significant.

kg,  $p=0.608$ ) or after 4 weeks ( $-0.3\pm 1.5$  vs  $-0.3\pm 1.7$ kg,  $p=0.503$ ). Similarly, changes in WC and WTHR between the MCP and placebo groups were not significant within or between groups (Table 1).

#### *Fecal short chain fatty acids (SCFA) concentration*

There was no significant difference in faecal SCFA concentrations after 4 weeks in both placebo and MCP groups as well as within the groups (Table 2) with the exception of acetic acid which was significantly reduced after 4 weeks in both groups ( $p=0.034$  for both groups).

#### *Diet and physical activity*

Dietary intake was also comparable between groups throughout the study (Table 3). Nevertheless, subjects in MCP group significantly reduced their energy

intake by approximately 300 kcal/day, after 4 weeks of MCP supplementation when compared to baseline intakes ( $p=0.045$ ). No other significant change in dietary pattern was found in either groups and none of the dietary changes were significantly different between groups. Subjects were all self-reported non-smokers and no alcohol consumption was reported in this group. Physical activity levels among subjects in both the groups were comparable and the majority of the subjects had low or moderate activity levels. No major change in physical activity levels were noted during the monitoring period.

## DISCUSSION

This study tested the hypothesis that four weeks of probiotics MCP supplementation will alter the GM sufficiently to improve



FBG, BW, WC and WTHR as well as faecal SCFA production among overweight and obese Malaysian adults. The primary endpoint of probiotics supplementation in this study was its effects on FBG levels. This study tested one of the commonly marketed Malaysian probiotic MCP products that contained *bifidobacteria* and *lactobacilli*; these microbial strains have been reported to be effective in promoting intestinal health (Firouzi *et al.*, 2017; Tannock, 2005).

Firouzi *et al.* (2017) found that MCP helps to improve glycolated hemoglobin (HbA1c) and fasting insulin in diabetics. However, in our study, no significant change was observed between overweight and obese subjects taking MCP and the placebo at the end of the 4-week trial. However, it is interesting to note that in this study, the MCP group began to show relatively stronger effects in reducing FBG levels compared to the placebo from after Week 2 (Table 1). These findings are in agreement with two reviews that suggest the time taken for MCP supplementation to influence FBG levels and anthropometric measures is six weeks (Delzene, Neyrinck & Cani, 2011; Samah *et al.*, 2016). This is because the probiotic colony should be established within the GM prior to exerting metabolic effects. Probiotics are known to establish themselves in the host GM within the first two weeks of supplementation (Taverniti *et al.*, 2014). This time lag could be the result of indigenous GM being resistant to the introduction of exogenous strains including the commensal probiotics (Koebnick & Saad, 2014) and ensuing competition of the probiotic strains with other bacteria including the pathogens to colonise the hosts' GM (Corr *et al.*, 2007).

Furthermore, the establishment of the probiotic colony may in turn be dependent on factors like the availability of DF which based on an individual's intake. Apart from nourishing the GM, fermentation of DF produces SCFA in the

gut which signals the regulating stability of GM (Delzene & Cani, 2010). The SCFA also possess a bacteria-static effect on competing pathogens by lowering the pH of the mucosa layer and facilitating the colonisation by probiotics bacterial strains (Demigne *et al.*, 1999). However, it is argued that 95% of the colonic SCFA is absorbed into the bloodstream while faecal SCFA is the remaining 5% (REF).

The unexpected reduction in FBG seen in the placebo group has precedents in literature. Similar improvements in FBG levels in the placebo group were documented by Koebnick *et al.* (2003). This placebo effect was also observed in the first two weeks of the study that used the probiotic fermented milk with *Lactobacillus casei* Shirota® and a nutrient drink as the placebo. Therefore, the paper argued that the "white coat effects" existed within the first two weeks in both groups due to frequent contact with the research team. Probiotic effect is only seen after its establishment in the GM which was expected to be on week 3 and week 4 of intervention. Hence, the gradual improvement of FBG levels of the placebo group seen in our study also suggests that the duration of the intervention period had been insufficient to distinguish the differences between the two groups.

There was no significant BW change after a 4-week probiotics MCP supplementation. However, longer trials (>6 weeks) were found to show reduced abdominal adiposity measured by WC and WTHR (Delzene *et al.*, 2011; Kootte *et al.*, 2012). Kadooka *et al.* (2013) showed that a 12-week probiotic supplementation lowered effects on body weight and abdominal obesity. Given that a trend for reduction in energy intake was evident in our probiotic group, we expected to see anti-obesity effects of probiotics MCP in this population, if the duration of supplementation was extended. Also, the effect of probiotic supplementation as an

adjunct to low calorie diets therapy needs a further study. The results of this study suggest that compliance to a reduced calorie intake may be facilitated by MCP supplementation.

This study also documented the effect of MCP supplementation on four faecal SCFA, namely, lactic acid, acetic acid, propionic acid and isobutyric acid. The faecal SCFA concentration of our subjects before intervention was at the lower end of the known range reported for SCFA for healthy Malaysian adults (Huda-Faujan *et al.*, 2010). This difference is expected as overweight and obese individuals are said to have a GM profile that is hypothetically different from normal individuals (Miele *et al.*, 2015).

Acetic acid was the only faecal SCFA to show a significant change when compared to its respective baseline value. Both groups recorded significant reduction in faecal acetic acid contents ( $P=0.042$  for both groups). This reduction in acetic acid can be explained by a few possibilities. Faecal SCFA production in various populations is reportedly in the order of acetate > propionate > butyrate with a molar ratio of approximately 60:20:20, respectively (Almada *et al.*, 2015). These colonic SCFA contribute approximately 5–10 % of human energy requirements (Huda-Faujan *et al.*, 2010). In the colon, preference for oxidation is in the order of butyrate > acetate > propionate, glucose and glutamine (Fleming, 1991). With acetate being the mostly commonly produced SCFA and the second most preferred substrate for oxidation, we can expect apparent changes in its levels after probiotic supplementation. Secondly, the plasma membrane SCFA transport protein expressed primarily in colonic epithelial cells, facilitates SCFA uptake by colonic enterocytes to satisfy in excess of 95 % of their energy requirements (Schwartz *et al.*, 2010). This uptake by the enteric colonocyte is enhanced by *bifidobacteria*

through increased gut permeability (Ewaschuk *et al.*, 2008). Thus the probiotic supplement used in this study could have increased absorption of the SCFA into the enterocytes from the lumen, leading to a reduction in acetic acid concentrations.

Despite participants being briefed to retain their usual dietary intake during this study, a small reduction in overall calorie intake which influenced a reduction in dietary fibre intake, though statistically insignificant, was observed among both groups of subjects. Trial participation is suggested to be the reason for the reduction in calorie intake (MacNeill *et al.*, 2006). This reduction could have had a consequence on the total SCFA production as literature shows that acetic acid production might be lowered when dietary fibre intake is reduced (Demigne *et al.*, 1999; Delzenne *et al.*, 2011a; 2011b). The authors argue that, it is possible that faecal SCFA concentration may not appropriately reflect MCP effects on colonic fermentation. Instead, it is suggested that absolute faecal SCFA contents rather than individual SCFA may better reflect probiotic effect on colonic fermentation (Huda-Faujan *et al.*, 2010; De Baere *et al.*, 2013). Furthermore, this preliminary study investigated only a limited number of SCFA and this is probably insufficient to capture the SCFA changes that reflect the synergistic effect of MCP supplementation.

A few limitations to this study are acknowledged. This sample size of  $n=24$  in this study was calculated only to detect a change in FBG, the primary outcome variable, between the study groups, at the end of the 4-week study period. However, the study was not powered to detect changes in other parameters. Besides, this study is one of the few preliminary studies to study the effect of probiotic MCP of faecal SCFA profile in a population with low dietary intakes of fibre. Future studies should focus on monitoring changes in GM through fecal microbial analysis to

investigate the colonisation of the gut by the intended species. Nevertheless, such efforts are warranted to better understand GM functionality, metabolism and their health benefits.

## CONCLUSION

Overall, this study showed that Malaysian participants showed good compliance to MCP supplementation. Subjects on the MCP supplement significantly reduced their energy intake at the end of a 4-week supplementation period. MCP could therefore be a promising adjunct to reduced calorie diets for weight loss among Malaysians. Such a synergistic effect should be further verified among overweight and obese Malaysian subjects. The trend of a reduction in FBG in the MCP group was apparent only after two weeks of supplementation with the effect size becoming more apparent after 2 weeks of supplementation suggesting a longer period of supplementation may be needed to beneficially modulate FBG and body weight among overweight and obese Malaysian adults.

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