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EFFECTS OF L. CASEI STRAIN SHIROTA SUPPLEMENTATION ON FECAL PROFILES AND BODY WEIGHT STATUS OF SCHOOL CHILDREN

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By

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Thesis Submitted to the School of Graduate Studies,Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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October 2017

Chairman: **Associate Professor Barakatun Nisak Binti Mohd Yusof, PhD**
Faculty : **Medicine and Health Sciences**

The concentration of short-chain fatty acids (SCFAs) may be disrupted in overweight and obese children. The effect of probiotic *Lactobacillus casei* strain *Shirota* (*LcS)* on the fecal SCFAs concentration is still unclear. The purpose of this intervention was to investigate the effect of *LcS* on fecal SCFAs and body weight status in school children.

Using the crossover study design, *LcS* was provided for 4-weeks with 4-weeks washout period to the children aged 7 to 10 years old. A total of 22 overweight and obese (OWOB) with mean age = 8.73 ± 1.03 years old; BMI = 24.73 ± 3.91 kg/m^2 kg/m^2 and 55 % boys participated and randomly divided either to intervention or control group. This study recruited normal weight children (NW, n= 20) as a comparison with the OWOB on the fecal profiles and body weight. During the intervention period, the participants received daily 80 ml of probiotic drink at a dosage of 3.0×10^{10} colony-forming unit (CFU) for 4-weeks. The primary outcome measures were fecal SCFAs concentration and body weight status of the children. Other outcome measures included dietary intakes, physical activity level and fecal consistency.

At baseline data of all participants, the concentration of propionate (Mean=43.08 μmol/g; SD= 37.14) was the highest; followed by acetate (Mean= 29.73 μmol/g; SD= 23.30) and butyrate (Mean= 23.19 μmol/g; SD= 17.75) with OWOB reported a higher concentration of butyrate (52%) and propionate (38%) as compared to NW participants ($p>0.05$). After 4-weeks of the *LcS* supplementations, the propionate and the total SCFAs concentration increased significantly than the baseline concentration ($p<0.05$).

The fecal propionate concentration increased by 161 % and total SCFAs concentration increased significantly by 79% from the baseline in the OWOB participants. Meanwhile, the fecal propionate and total SCFAs concentration of the NW participants in the intervention group had increased significantly by 178 % and 79% respectively at the end of supplementations ($p<0.05$). The study also found a significant difference in fecal propionate and total SCFAs of the intervention group in OWOB and NW ($p<0.05$). With regards to the body weight, the mean percentage of body weight change was also significantly increased by 6.4% in OWOB and 7.5% in NW participants at the end of supplementations ($p<0.05$). No significant changes in dietary intakes and physical activity levels of the participants throughout the study ($p>0.05$).

In conclusion, the increased in SCFAs mainly propionic acid after supplementing with a probiotic containing *LcS* may explain its potential as an appetite suppressor. Nevertheless, whether the effect on appetite may translate to weight control in the future warrants future investigationAbstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN PENGAMBILAN *L.CASEI* STRAIN *SHIROTA* PADA PROFIL NAJIS DAN STATUS BERAT BADAN DALAM KALANGAN KANAK-KANAK SEKOLAH

Oleh

NURUL AIN BINTI SAIPUDIN

Oktober 2017

Pengerusi: **Profesor Madya Barakatun Nisak Binti Mohd Yusof, PhD**
Fakulti: **Perubatan dan Sains Kesihatan**

Kepekatan asid lemak rantai pendek (ALRP) boleh terganggu dalam kanak-kanak yang berlebihan berat badan dan obes. Malah, kesan probiotik *Lactobacillus casei* strain *Shirota (LcS)* pada kepekatan ALRP juga masih tidak jelas. Tujuan intervensi ini adalah untuk mengkaji kesan *LcS* pada ALRP dan status berat badan pada kanak-kanak sekolah.

Dengan menggunakan reka bentuk kajian bersilang, *LcS* diberikan kepada kanak-kanak yang berusia antara 7 hingga 10 tahun selama 4 minggu, dengan diikuti tempoh 4 minggu pembersihan dari intervensi. Seramai 22 orang kanak-kanak yang mempunyai berat badan berlebihan dan obes (BO) dengan purata umur = 8.73 ± 1.03 tahun; IJB = 24.73 ± 3.91 kg/m^2; dan 55% lelaki telah menyertai dan dibahagikan secara rawak kepada kumpulan intervensi dan kumpulan kawalan. Kajian ini turut melibatkan 20 orang kanak-kanak yang mempunyai berat badan normal (BN) sebagai perbandingan pada profil najis dan status berat badan dengan kumpulan BO. Semasa tempoh intervensi, subjek menerima 80 ml minuman probiotik setiap hari dengan dos sebanyak 3.0 x 10^{10} unit pembentuk koloni (CFU).

Hasil ukuran yang utama ialah kepekatan ALRP di dalam najis dan status berat badan. Hasil ukuran yang lain ialah pengambilan makanan, tahap aktiviti fizikal dan konsistensi najis. Pada data asasnya, kepekatan propionat (purata= 49.63 27μmol/g; SP= 42.03) adalah tertinggi dan diikuti oleh asetat (purata= 29.27μmol/g; SP= 26.67) dan butyrat (purata= 23.19 μmol/g; SP= 17.75) dengan subjek BO dicatatkan mempunyai kepekatan butyrat (52%) dan propionat (38%) lebih tinggi daripada subjek BN (p>0.05). Selepas 4 minggu dari suplemen *LcS*, kepekatan propionat dan jumlah kepekatan ALRP meningkat dengan signifikan

daripada sebelumnya dengan kesan intervensi yang jelas ($p<0.05$). Kepekatan propionat meningkat sebanyak 161% dan kepekatan keseluruhan ALRP meningkat sebanyak 79% dari data asas dalam subjek BO. Sementara itu, kepekatan propionat dan jumlah kepekatan ALRP bagi subjek BN dalam kumpulan intervensi juga meningkat dengan ketara sebanyak 178% dan 79% pada akhir suplementasi ($p<0.05$). Peratusan purata perubahan berat badan juga meningkat dengan signifikan sebanyak 6.4% dalam subjek BO dan 7.5%. dalam subjek BN ($p<0.05$). Secara keseluruhannya, tiada perubahan ke atas pengambilan makanan dan tahap aktiviti fizikal subjek sepanjang kajian dijalankan ($p>0.05$).

Kesimpulannya, selepas pengambilan probiotik yang mengandungi *LcS,* peningkatan dalam ALRP terutamanya propionat mungkin dapat dikaitkan dengan potensi propionat sebagai penahan selera. Walau bagaimanapun, kajian sama ada kesan propionat dalam menahan selera makan dan seterusnya mengawal berat badan perlu dijalankan dengan lebih mendalam di masa hadapan.

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Verily, with hardship there is relief" [Surah Ash-Sharĥ, 94:6].

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I certify that a Thesis Examination Committee has met on 11 October 2017 to conduct the final examination of Nurul Ain binti Saipudin on her thesis entitled "Effects of *L. casei* Strain *Shirota* Supplementation on Fecal Profiles and Body Weight Status of School Children" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Signature: ____________________
Name of Member of Supervisory Committee:
Associate Professor Dr. Syafinaz Binti Amin Nordin

Signature: ____________________
Name of Member of Supervisory Committee:
Professor Dr Amin Ismail

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LIST OF ABBREVIATIONS

BF	Breastfeeding
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BSC	Bristol Stool Chart
CFU	Colony Forming Units
F: B ratio	*Firmicutes* to *Bacteroidetes* ratio
GOS	Galacto-oligosaccharides'
HPLC	High Performance Liquid Chromatography
LcS	*Lactobacillus casei* strain *Shirota*
OWOB	Overweight and Obese
NW	Normal Weight
PAQ-C	Physical Activity Questionnaire for Children
RNI	Recommended Nutrients Intake
SCFA	Short Chain Fatty Acid
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Study of Background

Short-chain fatty acids (SCFAs), the acetate, propionate and butyrate are anions in human fecal in which, presented in a molar ratio approximately in 60:20:20 (Rahat-Rozenbloom *et* al*.,* 2014). The SCFAs contribute about 5 to 10% additional energy to the total energy requirements for the human energy (Tumbaugh *et* al*.,* 2006 & Arora *et* al*.,* 2013). In principle, if this analogue to a 2000 kcal/day, a daily energy would be increased by 1%, which subsequently give an additional 20 kcal/day and 1 kg of annual weight gain (Payne *et* al*.,* 2011).

Several factors that influenced the SCFAs production, absorption and excretion are the compositions of gut microbiota, age, dietary intakes and practices, usage of antibiotics, genetics and other lifestyle or environmental factors of the host (Khan *et* al., 2016). The SCFAs are produced from the fermentation of carbohydrates with the primary source of resistant starches (Wong *et* al., 2006). The amount and type of dietary fibre represent a significant source of fermentable substrates in the colon. High intakes of dietary fibre are associated with a reduced risk of obesity (Kobyliak *et* al., 2016). One of the mechanism by which the fibre may protect against obesity is via the SCFA- mediated modulation of the secretion of gut hormones involved in the regulation of food intake and energy balance (Ríos-covián *et* al., 2016).

Recently, the role of gut microbiota which modulates SCFAs production as one of the mechanisms leading to obesity development has been increasingly upfront (Kobyliak *et* al*.,* 2016; Murugesan *et* al*.,* 2015 & Schwiertz *et* al., 2010). In healthy adults, 80% of the identified fecal microbiota can be divided into three dominant phyla: *Bacteroidetes, Firmicutes* and *Actinobacteria.* The *Firmicutes* to *Bacteroidetes (*F : B ratio) indicates a significant relevance in human gut microbiota composition (Rahat-Rozenbloom *et* al., 2014). In an animal model, studies have shown that the gut microbiota of obese mice had a higher ratio of F: B than the lean mice. The disturbed ratio allowed obese mice produce more SCFAs than the lean mice; hence more energy can be derived from the food (Fernandez *et* al., 2014; Ley *et* al., 2005 & Tumbaugh *et* al., 2006).

In human studies among obese individuals, the results of F: B ratio is conflicting. A higher fecal SCFAs concentration was also seen in the obese individual than a normal weight individual (Schwietrz *et* al., 2010 & Teixeira *et* al., 2013). In one comprehensive study that has been conducted, among 52 lean and 42 overweight adults in Toronto which includes the evaluation on the dietary intakes, BMI, physical activity levels, fecal microbial and fecal SCFAs concentration. The results found an inverse association between *Bacteroidetes* and BMI; a significant positive association between F: B ratio and concentration of SCFAs as well as negative associations between *Bacteroidetes* and SCFAs concentrations. These findings suggested that the SCFAs concentration and F: B ratio are interconnected and could be related to obesity (Fernandes *et* al., 2014).

The increase in obesity rate and the emerging role of gut microbiota may become a vital element in energy homeostasis and weight management. Few human trials have been conducted to determine the efficacy of probiotic as a modulator to the composition of gut microbiota and SCFAs concentration in the gut (Wang *et* al., 2015 & Arora *et* al., 2013). Probiotic is defined as "a living microorganism that can provide beneficial health effects if administered in adequate amounts (FAO/WHO, 2002).

Probiotics may use to reduce the rate of body weight gain among children (Sanchez *et* al., 2015). Following supplementation of symbiotic drinks which contained seven types of probiotic strains and prebiotic fructooligosaccharides in Iranian overweight and obese children aged 10 years, the study found a significant reduction in BMI, waist circumference and waist-to-hip ratios of the children than the controlled group with placebo (Safavi *et* al., 2013). Moreover, probiotics with three combination bacteria strains which are *Bifidobacterium* strains, four *Lactobacillus* strains and one *Streptococcus* strain have also shown a reduction in BMI among the treated subjects from Canadian (Koleva *et* al., 2015). In a randomized placebo-controlled intervention study in Japan, there was a significantly lower in subcutaneous and visceral fat among the healthy adult participants after supplementation of fermented milk containing *L.gasseri* SBT2055 (200 g/d). The study also found a significant decrease in body weight status and BMI as compared with the control group (Arora *et al.,* 2013 & Cani *et* al., 2009).

Nevertheless, the role of probiotic in weight reduction and the mechanism underneath were still unclear and likely to varied for different probiotic strains. More human trials should be conducted for the overweight and obese individual because probiotic has shown a potential in restoring the altered gut microbiota and intestinal dysbiosis which may lead to a reduction of SCFAs production and increment of hormones to induce weight loss (Sanchez *et* al., 2015 & Arora *et* al., 2013).

1.2 Problem Statement

This study has chosen *Lactobacillus casei* strain *Shirota (LcS)* for several reasons. Firstly, *LcS* is a well-known probiotic strain that has been one of the best-studied probiotics in worldwide. This commercial and accessible probiotic drink carries high bacterial counts in just a small volume (Matsumoto *et* al., 2010). Besides that, *LcS* has shown a high survival rate through the stomach until to the lower gut (Wang *et* al., 2015). *LcS* also has a high resistance to gastric and bile acid (Koebnick *et* al., 2003 & Shirota *et* al., 1966). *LcS* is also predicted to be beneficial in normalizing the balance of gut bacteria and prevent the proliferation of destructive bacteria. By using both methods of PCR and culture, previous studies have found *LcS* in fecal even after seven days of *LcS* probiotic consumptions (Tiengrim *et* al*.,* 2012 & Fujimoto *et* al*.,* 2008).

Currently, studies of *LcS* probiotic strain to human subjects are only limited to modulate parameters such as in immune systems (Dong *et* al, 2012 & Spanhaak *et* al., 1998;), diarrhea (Matsumoto *et* al., 2010 & Matsumoto *et* al., 2006) and constipation (Wang *et* al., 2015 & Koebnick *et* al., 2003 & Shioiri *et* al., 2006). Specifically, in Malaysia, previous studies that relate effects of *LcS* probiotics strain on the fecal profiles among children were very limited except on functional constipation in adults and anti-obesity in mice (Mazlyn *et* al., 2013).

As in this study, the interest on the role of probiotic to improve body weight status and the concentration of SCFAs to curb the obesity epidemic is rising (Kobyliak *et* al., 2016). Nevertheless, most of these experimental studies of *LcS* probiotic were only done in animal models and human adults which mostly relate the findings with gut microbiota and not SCFAs alone. More study is required to be done primarily on the effects of *LcS* probiotic drink on the SCFAs concentration and body weight among school children.

The latest pilot study obtained from 12 obese Japanese children found that the *LcS* may reduce the body weight and improve lipid metabolism by increasing the acetic acid and *Bifidobacterium* count in the fecal of the obese children (Nagata *et* al*.,* 2017). However, this finding may be varied with Malaysian children due to several factors such as the difference in climate and the dietary practices of the subjects (Khan *et al*., 2016). In fact, the study had indicated that the average fibre intakes of Malaysian children are below than the recommended amount of 20 to 30 g/day (Yang *et* al., 2017 & Poh *et* al*.,* 2013, 2016).

To this date, the available statistical data on fecal SCFAs and body weight of Malaysian children are apparently scarce, in addition to the insufficient studies on the beneficial effects of probiotic in controlling overweight among pediatric age group (Safavi *et* al., 2013). Therefore, this study is intended to fill this gap by investigating the effects of *LcS* on fecal profiles and body weight in selected school children.

1.3 Study Significance

The prevalence of overweight and obesity among Malaysian school-aged children is increasing, but their interaction with and without probiotic supplementation is still not adequately addressed. Thus, this study provides the fundamental data on fecal SCFAs and fecal consistency in overweight, obese and normal weight school children during control or intervention with probiotic supplementation.

Also, this study provides the potential role of probiotic supplementation in influencing the fecal SCFAs concentration in overweight, obese and normal weight school children. The findings is useful for future program and intervention for body weight management among children which related to fecal profiles and consistency.

1.4 Hypothesis

1.3.1. There is no significant different in socio-demographic characteristics, nutritional status, fecal SCFAs concentration and consistency; and physical activity level between normal weight and overweight/obese children at baseline.
1.3.2. There is no significant effects of *LcS* supplementation on fecal profiles and body weight status of the children.
1.3.3. There is no significant difference in dietary intakes and physical activity levels of the children throughout the study.

1.5 Research Questions

1.5.1 What are the socio-demographic characteristics, nutritional status, fecal profiles and physical activity level of the children at baseline?
1.5.2 What are the effects of *LcS* supplementation on fecal profiles and body weight status of the children?
1.5.3 Is there any difference in dietary intakes and physical activity levels of the children throughout the study?

1.6 Objectives

1.6.1 General objective:

To investigate the effects of *LcS* supplementation on fecal profiles and body weight status in school children from Serdang, Selangor.

1.6.2 Specific objectives:

1.6.2.1 To determine socio-demographic characteristics, nutritional status, fecal profiles and physical activity level of the children at baseline.
1.6.2.2 To determine the effects of *LcS* supplementation on fecal profiles and body weight status of the children.
1.6.2.3 To determine the carry-over effect and consistency of dietary intakes and physical activity levels of the children throughout the study

1.7 Conceptual Framework

The study employed a cross-over design consisting of three study periods including baseline, washout and intervention periods. At baseline, data on demographics, nutritional status and fecal SCFAs concentration are collected. Demographic status of the children and their parents included gender, age, ethnicity, monthly household income, parent's educational status and parent's self-perceived body weight index (BMI). The baseline results are significant as to observe the homogeneity of the results to the other current studies. Besides that, baseline data is needed to investigate the effects of probiotic after the intervention period.

As can be seen in Figure 1.1, the concept of this study is primarily to observe the effectiveness of probiotic prior and following its supplementation to the dependent variable of this study. The dependent variables that are observed are fecal profiles (SCFAs and fecal consistency) and body weight status. Meanwhile, covariates data that has been taken for control throughout the study are dietary intakes and physical activity levels. Physical activity level is being assessed by using the physical activity questionnaires for children (PAQ-C). The nutritional status of the children consisted of body weight, height, and the average of energy, macronutrients and dietary fibres intakes. The baseline covariate data must be consistent throughout the study to prevent the carryover effects after the first period of intervention.

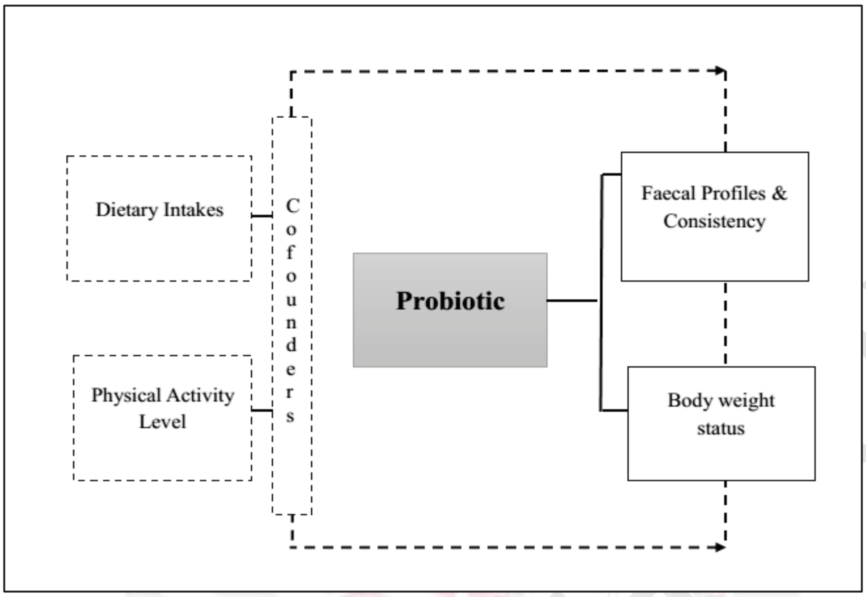

Figure 1.1: Conceptual Framework

REFERENCES

Arboleya, S., Binetti, A., Salazar, N. et al. 2012. Establishment and development of intestinal microbiota in preterm neonates. *FEMS Microbiology Ecology*, Vol.79, no.3, pp. 763–772.

Arellano, C., Bulir, A., Lane, T. & Lipschitz, L. 2009. The dynamic implications of foreign and its variability. *Journal of Development Economics*. 88; 87-102.

Arora, T., Satvinder S., & Raj K. S. 2013. “Probiotics: Interaction with Gut Microbiome and Antiobesity Potential. *Nutrition (Burbank, Los Angeles County, Calif.)* 29(4):591–96.

Azad, M.B., Bridgman, S.L, Becker, A.B., Kozyrskyj, A.L. 2014. Infant antibiotic exposure and the development of childhood overweight and central adiposity. *International. Journal of Obesity*. (London.) 38, 1290–1298.

Backhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A. 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci* USA. 101:15718–23.

Bailey, L.C., Forrest, C.B., Zhang, P., Richards, T.M.; Livshits, A., DeRusso, P.A. 2014. Association of antibiotics in infancy with early childhood obesity. *JAMA Pediatric*., 168, 1063–1069.

Beamish, L.A., Osornio-Vargas, A.R., Wine, E. 2011. Air pollution: An environmental factor contributing to intestinal disease. *J. Crohns Colitis*, 5, 279–286.

Bervoets, L., Van Hoorenbeeck, K., Kortleven, I. et al. 2013. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathogens*, vol.5,article 10.

Bingham, M.O., Harrell, J.S., Takada, H., Washino, K., Bradley, C., Berry, D. 2009. Obesity and cholesterol in Japanese, French, and U.S. children. J Pediatr Nurs.;24: 314-22. doi: 10.1016/j.pedn.2008.01.002.

Biro, F.M. &Wien, M. 2010. Childhood obesity and adult morbidities. *American. Journal Clinical Nutrition*., 91, 1499S–1505S.

Bjursell, M.; Admyre, T.; Göransson, M.; Marley, A.E.; Smith, D.M.; Oscarsson, J.; Bohlooly-Y, M. 2011. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Amer. J. Physiol. Endocrinol. Metab*. 2011, 300, 211–220.

Cani, P.D., Delzenne, N.M. 2011. The gut microbiome as therapeutic target. *Pharmacol. Ther*.; 130, 202–212.

Cani, P.D.; Delzenne, N.M. 2009. Interplay between obesity and associated metabolic disorders: New insights into the gut microbiota. *Curr. Opin. Pharmacol*., 9, 737–743.

Chambers, E. S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K. G., Zac-varghese, S. E. K., Frost, G. 2015. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut.* 1744–1754. http://doi.org/10.1136/gutjnl-2014-307913

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T.G., Cryan, J.F. 2013. The microbiome-gut-brain axis during

early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry,* 18, 666–673.
De Onis, M., Blössner, M., & Borghi, E. 2010. Global prevalence and trends of overweight and obesity among preschool children. *Am. J. Clin. Nutr*. 92, 1257–1264.
Den Besten, G., van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.J., Bakker, B.M. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res*.; 54, 2325–2340.
Department of Statistics. 2010. Population Distribution and Basic Demographic Characteristics Report 2010 (Updated 05/08/2011); Department of Statistics: Kuala Lumpur, Malaysia.
Dı´az E.G., Folgueras T.M. 2011. Systematic review of the clinical efficacy of sibutramine and orlistat in weight loss, quality of life and its adverse effects in obese adolescents. *Nutr Hosp*; 26: 451-7.
Dong H., Rowland I., Yaqoob P. 2012. Comparative effects of six probiotic strains on immune function in vitro. *Br J Nutr*.; 108: 459-70
Everard A., Belzer C., Geurts L., Ouwerkerk J.P., Druart C., Bindels L.B., et al. 2013. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci* U S A. 110:9066–7
Ewaschuk, J.B., Zello, G.A., Naylor, J.M., Brocks, D.R. 2002. Metabolic acidosis: separation methods and biological relevance of organic acids and lactic acid enantiomers. *J Chromatogr B Analyt Technol Biomed Life Sci*., 781(1-2):39-56.
FAO/WHO (Food and Agriculture Organization/World Health Organization). 2002. Guidelines for the evaluation of probiotics in food: Report of a Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food; Food and Agriculture Organization/World Health Organization: London, ON, Canada.
FAO/WHO/UNU. Expert consultation on energy and protein requirements. Geneva: World Health Organization; 1985. (WHO Technical Report Series No. 724).
Fernandes J., Su, W., Rahat-Rozenbloom, S., Wolever, T.M.S., and Comelli, E. M. 2014. Adiposity, gut microbiota and fecal short chain fatty acids are linked in adult humans. *Nutrition and Diabetes,* vol. 4, article e121.
Filippo, C. D., Duccio C., Monica D., Matteo R., and Jean B.. 2010. “Impact of Diet in Shaping Gut Microbiota Revealed by a Comparative Study in Children from Europe and Rural Africa. 107(33):14691–96.
Firouzi S., Barakatun-Nisak M.Y., Ismail A., Majid H.A., Azmi K.N. 2013. Role of probiotics in modulating glucose homeostasis: evidence from animal and human studies. Int J *Food Sci Nutr*.; 64: 780-6.
First Data Bank. (2005). Nutritionist Pro. *San Bruno*, CA 94066.
Flint H.J. 2011. Obesity and the Gut Microbiota. *J. Clin Gastroenterol*.; 45(Suppl): S128-132. doi: 10.1097/MCG.0b013e31821f44c4.
Florentino, R.F., Villavieja, G.M., Laña, R.D. 2002. Regional study of nutritional status of urban primary schoolchildren. 1. Manila, Philippines. *Food Nutr Bull.*;23:24-30.

Freedman, D.S., Mei, Z., Srinivasan, S.R., Berenson, G.S., Dietz, W.H. 2007. Cardiovascular risk factors and excess adiposity among overweight children and adolescents the Bogalusa Heart Study. *J Pediatr.*;150:12-7.e2. doi: 10.1016/j.jpeds.2006.08.042.

Frost G., Sleeth M.L., Sahuri-Arisoylu M., Lizarbe B., Cerdan S., Brody L. et al. 2014. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun.*;5:3611. doi:10.1038/ncomms4611.

Fujimoto J, Matsuki T, Sasamoto M, Tomii Y, Watanabe K. 2008. Identification and quantification of Lactobacillus casei strain Shirota in human feces with strain-specific primers derived from randomly amplified polymorphic DNA. *International journal of food microbiology.* 126(1–2):210–5.

Guthold R, Cowan M.J., Autenrieth C.S., Kann L, Riley L.M.. 2010. Physical activity and sedentary behavior among schoolchildren: a 34-country comparison. *J Pediatr.*;157:43-49. e1.

Haines, L., Wan, K.C., Lynn, R., Barrett, T.G., & Shield, J.P. 2007. Rising incidence of type 2 diabetes in children in the UK. *Diabetes Care*, 30, 1097–1101.

Heaton, K. W. & Lewis, S. J. .1997. 'Stool form scale as a useful guide to intestinal transit time'. *Scandinavian Journal of Gastroenterology*, vol.32, no.9, pp.920 - 924.

Hemalatha, R. 2017. Effect of Probiotic Supplementation on Total Lactobacilli , Bifidobacteria and Short Chain Fatty Acids in 2 – 5-Year-Old Children." *Microbial Ecology in Health and Disease* 28(1).

Hong Y.H., Nishimura Y., Hishikawa D., Tsuzuki H., Miyahara H., Gotoh C., et al. 2005. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology.*;146(12):5092-5099.

Huazano-garcía, A., & López, M. G. 2013. World largest Science , Technology & Medicine Open Access book publisher Metabolism of Short Chain Fatty Acids in the Colon and Faeces of Mice After a Supplementation of Diets with Agave Fructans

Huda-Faujan, N., Abdulamir A.S, Fatimah A.B., Muhammad Anas O., Shuhaimi M., Yazid A.M. & Loong Y.Y. 2010. "The Impact of the Level of the Intestinal Short Chain Fatty Acids in Inflammatory Bowel Disease Patients versus Healthy Subjects." *The open biochemistry journal* 4(Ic):53–58.

Kadooka, Y., Sato, M., Imaizumi, K., Ogawa, A., Ikuyama, K., Akai, Y., Okano, M., Kagoshima, M., Tsuchida, T. 2010. Regulation of abdominal adiposity by probiotics (Lactobacillus gasseriSBT2055) in adults with obese tendencies in a randomized controlled trial. *Eur. J. Clin. Nutr.*; 64, 636–643.

Kalliomaki, M., Collado, M. C., Salminen, S. and Isolauri, E. 2008. Early differences in fecal microbiota composition in children may predict overweight. *The American Journal of Clinical Nutrition,* vol.87, no.3, pp. 534–538.

Khan, Muhammad Jaffar, Konstantinos Gerasimidis, Christine Ann Edwards, and M. Guftar Shaikh. 2016. "Role of Gut Microbiota in the Aetiology of Obesity : Proposed Mechanisms and Review of the Literature." 2016.

Khor, G.L., Chee, W.S., Shariff, Z.M., Poh, B.K., Arumugam, M., Rahman, J.A., Theobald, H.E. 2011. High prevalence of vitamin D insufficiency and its association with BMI-for-age among primary school children in Kuala Lumpur, Malaysia. *BMC Public Health,* 11, 1–8.

Kimura, I., Ozawa, K., Inoue, D., et al. 2013. The gut microbiota suppresses insulin-mediated fat accumulation via the short- chain fatty acid receptor GPR43. *Nature Communications.* 4, article no. 1829.

Koebnick, C., Wagner, I., Leitzmann, P., Stern, U., and Zunft, H. F. 2003. Probiotic beverage containing Lactobacillus casei Shirota improves gastrointestinal symptoms in patients with chronic constipation, *Can. J. Gastroenterol*., 17, 655–659.

Koleva, Petya T., Sarah L. Bridgman, and Anita L. Kozyrskyj. 2015. “The Infant Gut Microbiome: Evidence for Obesity Risk and Dietary Intervention.” 2237–60.

Kobyliak, N., Conte, C., Cammarota, G., Haley, A. P., Styriak, I., Gaspar, L., Kruzliak, P. 2016. Probiotics in prevention and treatment of obesity : a critical view. *Nutrition & Metabolism*. http://doi.org/10.1186/s12986-016-0067-0

Koo, Hui Chin et al. 2016. “Are Malaysian Children Achieving Dietary Guideline Recommendations ? SEANUTS ”*Asia Pacific Journal of Public Heath*, 1-13.

Kotzampassi K., Giamarellos-Bourboulis E.J., Stavrou G. 2014. Obesity as a consequence of gut bacteria and diet interactions. ISRN Obes.;651895. doi:10.1155/2014/651895.

Kowalski, K. C., Crocker, P. R. E., & Faulkner, R. A. 1997. Validation of the Physical Activity Questionnaire for Older Children. *Pediatric Exercise Science*, 9, 174-186.

Lau X.C., Chong K.H., Poh B.K., Ismail M.N. 2013. Physical activity, fitness and the energy cost of activities: implications for obesity in children and adolescents in the tropics. *Adv Food Nutr Res*, 70: 49-101.

Ley, R. E., Backhed, F., Turnbaugh, P. Lozupone, C. A., Knight, R.D. and Gordon, J. I. 2005. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 31, pp. 11070–11075.

Ley, R. E., Turnbaugh, P. J., Klein, S., and Gordon, J. I. 2006. Microbial ecology: human gut microbes associated with obesity. *Nature*, vol. 444, no. 7122, pp. 1022–1023.

Macfarlane, G., Macfarlane, S. 2007. Models for Intestinal Fermentation: Association between Food Components, Delivery Systems, Bioavailability and Functional Interactions in the Gut. Current Opinion in Biotechnology; 18(2) 156-162.

Mallappa, R.H. Rokana, N.,Duary, R.K. Panwar, H., Batish, V. K., and Grover, S. 2012. Management of metabolic syndrome through probiotic and prebiotic interventions. *Indian J Endocrinol Metab*. 16(1): 20–27. doi: 10.4103/2230-8210.91178

Matsumoto K., Takada T., Shimizu K., Kado Y., Kawakami K., Makino I., Yamaoka Y., Hirano K., Nishimura A., Kajimoto O., Nomoto K. 2006. The effects of a probiotic milk product containing Lactobacillus casei strain Shirota on the defecation frequency and the intestinal microflora of sub-optimal health state volunteers: a randomized placebo-controlled cross-over study.

Matsumoto K., Takada T., Shimizu K., Moriyama K., Kawakami K., Hirano K., Kajimoto O., Nomoto K. 2010. Effects of a probiotic fermented milk beverage containing Lactobacillus casei strain Shirota on defecation frequency, intestinal microbiota, and the intestinal environment of healthy individuals with soft stools. *J Biosci Bioeng* 110: 547–52.

Mazlyn Mena M,, Nagarajah Lee Hl, A. Fatimah, Norimah Ak, and Goh Kl. 2013. "Stool Patterns of Malaysian Adults with Functional Constipation : Association with Diet and Physical Activity."

McComb, J., Chusilp, K. 2003. The prevalence of childhood obesity in primary school children in urban Khon Kaen, northeast Thailand. *Asia Pac J Clin Nutr*.; 12:66-72.

Meimandipour, A., Shuhaimi, M., Hair-Bejo, M., Azhar, K., Kabeir, B.M., Rasti, B. & Yazid, A.M 2009. *In vitro* fermentation of broiler cecal content: the role of lactobacilli and pH value on the composition of microbiota and end products fermentation. *Journal of Applied Microbiology* Volume 49, Issue 4, Pages 415-420. DOI:10.1111/j.1472-765X.2009.02674.x

Membrez, M., Blancher, F., Jaquet, M. et al. 2008. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *The FASEB Journal*, vol.22, no.7, pp. 2416– 2426.

Mills, Edward J. et al. 2009. "Design , Analysis , and Presentation of Crossover Trials." 6:1–6.

Mohd Ismail. M.N., Norimah, A.K., Poh, B.K., Nik Shanita, S., Nik Mazlan, M., Roslee, R., Nurrnnajiha, N., Wong, J.E., Nur Zakiah, M.S., Raduan, S. 2009. Prevalance and trends of overweight and obesity in two cross-sectional studies of Malaysian children, 2002-2008. MASO Scientific Conference on Obesity; 12-13 August; Kuala Lumpur, Malaysia.

Murakami, K., Miyake, Y., Sasaki, S., Tanaka, K., Arakawa, M. 2012. Characteristics of under- and over-reporters of energy intake among Japanese children and adolescents: The Ryukyus Child Health Study. *Nutrition,* 28, 532–538.

Murugesan S., Ulloa-Martínez M., Martínez-Rojano H., Galván-Rodríguez F.M., Miranda Brito C., Romano M.C. et al. 2015. Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children. Eur *J ClinMicrobiol Infect Dis*.; 34:1337–1346. doi: 10.1007/s10096-015-2355-4.

Nagata, S., Chiba, Y. Wang, C. & Yamashiro, Y. 2017. "The effects of the Lactobacillus casei strain on obesity in children: a pilot study". *Beneficial Microbes* 0 (0)-Pages 1-10.

Narang, I. & Mathew, J.L. 2012. Childhood obesity and obstructive sleep apnea. *J. Nutr. Metab.*134202.

National Coordinating Committee on Food and Nutrition (NCCFN). 2005, 2017. Recommended Nutrient Intakes for Malaysia. Putrajaya; National Coordinating Committee on Food and Nutrition, Ministry of Health: Putrajaya, Malaysia.

National Coordinating Committee on Food and Nutrition (NCCFN). 2013. Malaysian Dietary Guidelines for Children and Adolescents—Summary; Ministry of Health, Putrajaya, Malaysia.

Neish, A.S. 2009. Microbes in gastrointestinal health and disease. *Gastroenterology*.;136:65-80.
Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Gakidou, E. 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, *384*. doi:10.1016/S0140-6736(14)60460-8
Omar, J.M., Chan, Y.-M., Jones, M.L., Prakash, S., Jones, P.J.H. 2013. Lactobacillus fermentum and Lactobacillus amylovorusas probiotics alter body adiposity and gut microflora in healthy persons. J. *Funct. Foods*; 5, 116–123.
Patil, D.P.; Dhotre, D.P.; Chavan, S.G.; Sultan, A.; Jain, D.S.; Lanjekar, V.B.; Gangawani, J.; Shah, P.S.; Todkar, J.S.; Shah, S.; et al. 2012. Molecular analysis of gut microbiota in obesity among Indian individuals. *J. Biosci*., 37, 647–657.
Payne, A N., Chassard, C., Zimmermann, M., Müller, P., Stinca, S., & Lacroix, C. 2011. The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. *Nutrition & Diabetes*, *1*, e12. doi:10.1038/nutd.2011.8
Pessione, E. 2012. Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Front Cell Infect Microbiol.* ; 2:86.
Poh, Bee Koon et al. 2013. "Nutritional Status and Dietary Intakes of Children Aged 6 Months to 12 Years: Findings of the Nutrition Survey of Malaysian Children (SEANUTS Malaysia)." *The British journal of nutrition* 110 Suppl :S21–35.
Poh, Bee Koon et al. 2016. "Nutritional Status and Dietary Intakes of Children Aged 6 Months to 12 Years : Findings of the Nutrition Survey of Malaysian Children (SEANUTS Malaysia) *British Journal of Nutrition* (2013).
Poh, B.K.; Ismail, M.; Zawiah, H.; Henry, C. 1999. Predictive equations for the estimation of basal metabolic rate in Malaysia adolescents. Malays. *J. Nutr.,* 5, 1–14.
Pryde, S., Duncan, S., Hold, G., Stewart, C., and Flint, H. 2002. The Microbiology of Butyrate Formation in the Human Colon. FEMS Microbiology Letters; 217(2) 133-139.
Puddu, Alessandra, Roberta Sanguineti, Fabrizio Montecucco, and Giorgio Luciano Viviani. 2014. "Evidence for the Gut Microbiota Short-Chain Fatty Acids as Key Pathophysiological Molecules Improving Diabetes."
Rahat-Rozenbloom S., Fernandes J., Gloor G.B., Wolever T.M. 2014. Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. *Int J Obes (Lond)*.
Rangan, A., Flood, V., Gill, T. 2011. Misreporting of energy intake in the 2007 Australian Children's Survey:Identification, characteristics and impact of misreporters. *Nutrients*, 3, 186–199.
Ríos-covián, David et al. 2016. "Intestinal Short Chain Fatty Acids and Their Link with Diet and Human Health.7(February):1–9.

Sabanayagam, C., Shankar, A., Chong, Y.S., Wong, T.Y., Saw, S.M. 2009. Breast-feeding and overweight in Singapore school children. *Pediatric Internatiol Journal*.; 51:650-6.
Safavi, M.; Farajian, S.; Kelishadi, R.; Mirlohi, M.; Hashemipour, M. 2013. The effects of symbiotic supplementation on some cardio-metabolic risk factors in overweight and obese children: A randomized triple-masked controlled trial. *Int. J. Food Sci. Nutr*.; 64, 687–693.
Salonen A., De Vos W.M. 2014. Impact of diet on human intestinal microbiota and health. *Annu Eev Food Sci* T5: 239–62.
Samuel, B.S.; Shaito, A.; Motoike, T.; Rey, F.E.; Backhed, F.; Manchester, J.K.; Hammer, R.E.; Williams, S.C.; Crowley, J.; Yanagisawa, M, 2008. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, GPR41. Proc. Nat. Acad. Sci. USA, 105, 16767–16772.
Sanchez, M., Panahi, S., & Tremblay, A. 2015. Childhood Obesity: A Role for Gut Microbiota? 162–175. http://doi.org/10.3390/ijerph120100162
Scheppach W. 1994. Effects of short chain fatty acids on gut morphology and function. *Gut*. 35(1): S35–S38.
Schwiertz, A., Taras, D., Schafer, K., Beijer, S., Bos, N.A., Donus, C., Hardt, P.D. .2010. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity*, 18, 190–195.
Sharifah, W.W., Hana, H., Ruzita, A.T., Reilly, J.J. 2014. Objectively Measured Habitual Physical Activity and Sedentary Behaviour in Obese and Non-Obese Malaysian children. *J. Trop. Pediatr*., 60,161–163.
Sharif, Razinah et al. 2016. "Results From Malaysia ' S 2016 Report Card on Physical Activity for Children and Adolescents.13(Suppl 2):201–5.
Shioiri T., Yahagi K., Nakayama S., Asahara T., Yuki N., Kawakami K., Yamaoka Y., Sakai Y., Nomoto K., Totani M. 2006. The effects of a synbiotic fermented milk beverage containing Lactobacillus casei strain Shirota and transgalactosylated oligosaccharides on defecation frequency, intestinal microflora, organic acid concentrations, and putrefactive metabolites of sub-optimal health state volunteers: a randomized placebo-controlled cross-over study. *Bioscience Microflora* 25: 137–46.
Shirota, M., Aso, K., and Iwabuchi, A.: 1966. Alteration of the constitution of the intestinal flora by oral administration of L. acidophilusstrain Shirota to healthy infants, *Jpn J. Bacteriol*., 21, 274–283
Soekirman, Hardinsyah, Jus'at, I., Jahari, A.B. 2002. Regional study of nutritional status of urban primary schoolchildren. 2. West Jakarta and Bogor, Indonesia. *Food Nutr Bull*.; 23:31-40.
Spanhaak, S., Havenaar, R., & Schaafsma, G. 1998. The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune parameters in humans.
Sumarni, M. G., Muhammad Amir, K., Ibrahim M. S., Mohd Rodi, I., Izzuna Mudla, M.G., Nurziyana, I. 2006. Obesity among schoolchildren in Kuala Selangor: a cross-sectional study. *Trop Biomed*.;23:148-54.
Teixeira, Tatiana F. S. et al. 2013. "Higher Level of Fecal SCFA in Women Correlates with Metabolic Syndrome Risk Factors." *The British journal of nutrition* 109(5):914–19.

Tanida M., Shen J., Maeda K., Horii Y., Yamano T., Fukushima Y., et al. 2008. High-fat diet-induced obesity is attenuated by probiotic strain Lactobacillus paracasei ST11 (NCC2461) in rats. *Obes Res Clin Pract*; 2: 159-69.

Tee, E.S., Khor, S.C., Ooi, H.E., Young, S.I., Zakiyah, O., Zulkafli, H. 2002. Regional study of nutritional status of urban primary schoolchildren. 3. Kuala Lumpur, Malaysia. *Food Nutr Bull*.; 23:41-7.

Tiengrim S, Leelaporn A, Manatsathit S, Thamlikitkul V. 2012. Viability of Lactobacillus casei strain Shirota (LcS) from feces of Thai healthy subjects regularly taking milk product containing LcS. *Journal of the Medical Association of Thailand* = Chotmaihet thangphaet.; 95 Suppl 2:S42–7. Epub 2012/05/12.PMID:22574528.

Topping, D., Clifton P. 2001. Short-chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides. *Physiological Reviews*; 81(3)1031-1063.

Torun, B. Energy requirements of children and adolescents. 2005. *Public Health Nutrition*., 8, 968–993

Turnbaugh, P.J., Backhed, F., Fulton, L., Gordon, J.I. 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell. Host Microbe*, 3, 213–223.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., Gordon, J.I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 20;444:1027–31.

Vogt J.A., Wolever T.M.S. 2003. Fecal acetate is inversely related to acetate absorption from the human rectum and distal colon. *J Nutr*.;133(10):3145–3148.

Wang C., Nagata S., Asahara T., Yuki N., Matsuda K., Tsuji H., Takahashi T., Nomoto K., Yamashiro Y. 2015. Intestinal Microbiota Profiles of Healthy Pre-School and School-Age Children and Effects of Probiotic Supplementation. *Ann Nutr Metab*.;67:257-26

Wang, Y. & Lobstein, T. 2006. Worldwide trends in childhood overweight and obesity. *Int. J. Pediatr. Obes.* 1,11–25.

Wong, J., De Souza R., Kendall, C., Emam, A., Jenkins, D. 2006. Colonic Health: Fermentation and Short Chain Fatty Acids. *Journal of Clinical Gastroenterology*; 40(3) 235-243.

Wong, Jyh Eiin and Panam Parikh. 2016. "Physical Activity of Malaysian Primary School Children : Comparison by Sociodemographic Variables and Activity Domains"

Woon, F.C., Chin, Y.S., Kaartina, S., Fara Wahida, R., Hiew, C.C., Mohd Nasir, M.T. 2014. Association between Home Environment, Dietary Practice, and Physical Activity among Primary School Children in Selangor, Malaysia. Malays. *J. Nutr*., 20, 1–14.

World Health Organization 2006 WHO Child Growth Standards. Geneva: WHO. http://www.who.int/childgrowth (accessed July 2017).

World Health Organization 2007 Growth Chart for Children 5–19 years. Geneva: WHO. http://www.who.int/growthref/ en/ (accessed July 2017).

Yakult Malaysia Product. Yakult Ace Light. https://www.yakult.com.my/wp- content/uploads/2016/06/yk-label-msia-ace-light-simple.jpg (accessed November 2017)

Yang, W.Y., Williams, L.T., Collins, C., Chee, W.S.S. 2012. The relationship between dietary patterns and overweight and obesity in children of Asian developing countries: A systematic review. *JBI Database Syst. Rev.Implement. Rep.,* 10, 4568–4599.
Yang, W. Y., Burrows, T., Macdonald-wicks, L., Williams, L. T., Collins, C. E., Siew, W., Colyvas, K. 2017. Body Weight Status and Dietary Intakes of Urban Malay Primary School Children : Evidence from the Family Diet Study, 1–16. http://doi.org/10.3390/children4010005
Zalilah, M.S., Khor, G.L., Mirnalini, K., Sarina, S. 2005. Food neophobia and nutritional outcomes in primary school-children. J. *Commun. Nutr*., 7, 121–129.
Zhang Y-J, Li S., Gan R-Y, Zhou T., Xu D-P, Li H-B. 2015. Impacts of gut bacteria on human health and diseases. *Int J Mol Sci*; 16: 7493-519.
Zhao, G., Nyman, M., & Jönsson, J.A. 2006. Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. *Biomed Chromatogr*.;20(8):674-82.