THE EFFECTS OF PREBIOTICS ON FAECAL MICROBIOTA IN CRITICAL CARE PATIENTS RECEIVING ENTERAL NUTRITION

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ABSTRACT

The provision of nutrition through enteral nutrition (EN) helps to maintain gut function. However, despite the importance of EN to patients, diarrhoea is a common complication in those receiving EN. Meta-analysis conducted revealed that fibre supplementation in enteral formula reduces diarrhoea incidence in patients receiving EN. However, the positive effect was only seen in non-critically ill patients. The undigested fibre (prebiotics) will be fermented to short chain fatty acid (SCFA) and stimulate the growth of bifidobacteria which helps in minimising diarrhoea. In the current literature, the effect of fibre and prebiotics supplementation on faecal microbiota and SCFA remain disputable. The aim of this study is to evaluate faecal microbiota concentrations (total bacteria, bifidobacteria, lactobacilli, bacteroides, clostridia and *Faecalibacterium prausnitzii*) and the incidence of diarrhoea between critically ill patients who receive enteral formula with and without fibre/prebiotics during EN.

Randomised Controlled Trial (RCT) was conducted on critically ill, adult patients receiving EN as clinically indicated. Patients were randomly allocated to receive EN with fibre-free or fibre/prebiotics-supplemented enteral formula for up to 14 days. Nutritional delivery and diarrhoea scores were recorded daily. Faecal samples were collected at baseline, 1 week and 2 weeks and analysed for the quantification of microbiota using the real time polymerase chain reaction.

Of 702 critically ill patients screened, 68 patients were recruited into the study (35 in the fibre/prebiotics group and 33 in fibre-free group). These two groups of patients were similar in demographics, disease severity, number of antibiotics used, nutritional status and nutritional intake at baseline. The results of the RCT revealed that the provision of 10 g/L of prebiotics containing enteral formula to critically ill patients were not able to increase the faecal bifidobacteria concentration (PP: p= 0.537, ITT: p=

0.974). However, the findings from this study showed that patients in the fibre/prebiotics group had improvements in their faecal outputs as evidence by a trend of lower stool frequency (1.2±0.6) compared to patients receiving the fibre-free enteral formula (1.8±0.9), (PP: p= 0.092, ITT: p= 0.070). However, there were no significant differences in the faecal scores between patients in the fibre/prebiotics group and the fibre-free group (PP: p= 0.613, ITT: p= 0.036).

These data support the view that the provision of prebiotics as the sole source of fibre containing enteral formula may not increase the faecal bifidobacteria concentrations of critically ill patients requiring EN. However, the provision of prebiotics may be useful in alleviating diarrhoea by reducing the stool frequency. It is hoped that the findings from this research will be of use to health care professionals in managing the common complication of EN in this group of patients.

ABSTRAK

Pemberian pemakanan melalui tiub (PT) membantu dalam mengekalkan fungsi sistem penghadaman. Walaupun PT penting kepada pesakit, cirit-birit adalah satu komplikasi yang sering dihadapi oleh pesakit yang menerima EN. Meta-analisis yang dijalankan menunjukkan bahawa suplementasi serat dalam formula enteral mengurangkan kejadian cirit-birit di kalangan pesakit yang menerima PT. Walau bagaimanapun, kesan positif ini hanya dilihat pada pesakit bukan kritikal. Serat yang tidak dapat dihadam (prebiotik) akan difermentasi kepada asid lemak rantaian pendek (SCFA) dan merangsang pertumbuhan bifidobakteria yang boleh membantu dalam mengurangkan cirit-birit. Kajian semasa mendapati kesan suplementasi serat dan prebiotik dalam EN terhadap mikrobiota dan SCFA dalam najis masih dipertikaikan. Kajian ini adalah bertujuan untuk menilai konsentrasi mikrobiota di dalam sampel najis ('total bacteria', bifidobacteria, lactobacilli, bacteroides, clostridia dan *Faecalibacterium prausnitzii*) dan kejadian cirit-birit antara pesakit kritikal yang menerima formula enteral dengan atau tanpa serat/prebiotik semasa PT.

Kajian terkawal secara rawak telah dijalankan ke atas pesakit kritikal dewasa yang menerima PT atas indikasi klinikal. Pesakit di pilih secara rawak untuk menerima EN dengan atau tanpa serat/prebiotik selama 14 hari. Pemberian PT dan skor cirit-birit direkodkan setiap hari. Sampel najis dikumpulkan pada permulaan kajian, 1 minggu dan 2-minggu selepas suplementasi dan dianalisa untuk kuantifikasi mikrobiota menggunakan kaedah tindak balas rantaian polymerase (PCR) masa-nyata.

Dari 702 pesakit kritikal yang di saring, 68 pesakit telah terlibat dalam kajian ini, 35 pesakit menerima PT dengan serat/prebiotik dan 33 pesakit menerima PT tanpa serat. Kedua-dua kumpulan pesakit adalah sama dari segi demografi, tahap penyakit, beberapa antibiotik digunakan, status pemakanan dan pengambilan nutrisi pada awal

kajian. Hasil daripada kajian menunjukkan pemberian 10 g/L prebiotik di dalam enteral formula kepada pesakit kritikal gagal meningkatkan kosentrasi bifidobacteria di dalam najis (PP: p=0.537, ITT: p= 0.974). Walaubagaimanapun, pesakit yang menerima serat/prebiotik di dalam PT berjaya menunjukkan perubahan yang baik dalam kekerapan pengeluaran najis. Ini dibuktikan dengan 'trend' kekerapan membuang najis yang lebih lebih rendah (1.2 ± 0.6) berbanding dengan pesakit yang menerima formula enteral tanpa serat (1.8 ± 0.9), (PP: p=0.092, ITT: p=0.070). Walaubagaimanapun, tiada perubahan signifikan dalam skor pembuangan najis antara pesakit dalam kumpulan serat/prebiotik dan kumpulan tanpa serat (PP: p=0.613, ITT: p= 0.036).

Data daripada kajian ini menyokong pandangan dimana pemberian formula enteral yang mengandungi prebiotik sebagai sumber tunggal serat tidak dapat meningkatkan kosentrasi bifidobacteria di dalam najis. Namun yang demikian, ia mungkin boleh membantu mengurangkan cirit-birit dengan mengurangkan kekerapan najis. Diharap, dapatan dari kajian ini boleh digunakan oleh pengamal perubatan dalam menguruskan komplikasi yang kerap berlaku semasa PT dalam kumpulan pesakit ini.

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LIST OF SYMBOLS AND ABBREVIATIONS

AAD	:	Antibiotic associated diarrhoea
AGE	:	Agarose gel electrophoresis
AMP	:	Antimicrobial peptides
APACHE II	:	Acute Physiology and Chronic Health Evaluation II
BMI	:	Body mass index
CDAD	:	*Clostridium difficile*-Associated Diarrhoea
CI	:	Confidence interval
CRP	:	C-reactive protein
DGGE	:	Denaturing gradient gel electrophoresis
DNA	:	Deoxyribonucleic acid
DP	:	Degree of Polymerization
EN	:	Enteral nutrition
FISH	:	Fluorescent in –situ hybridization
FODMAP	:	Fermentable oligo-, di-, mono-saccharides and polyols
FOS	:	Fructo-oligosaccharides
GALT	:	Gut-associated lymphoid tissues
GIT	:	Gastrointestinal tract
GRADE	:	Grading Of Recommendations Assessment, Development and Evaluation
IBD	:	Irritable bowel diseases
IBS	:	Irritable bowel syndrome
ICU	:	Intensive care unit
OR	:	Odds ratio
PCR	:	Polymerase chain reaction

PHGG	:	Partially hydrolysed guar gum
PIS	:	Patient information sheet
PN	:	Parenteral nutrition
QPCR	:	Quantitative polymerase chain reaction
RBS	:	Random blood sugar
RCT	:	Randomised controlled trials
RNA	:	Ribonucleic acid
RNI	:	Recommended nutrient intakes
RRNA	:	Ribosomal ribonucleic acid
SAPS	:	Simplified acute physiology score
SCFA	:	Short chain fatty acid
SIGN	:	Scottish intercollegiate guidelines network
SOFA	:	Sequential organ failure assessment
TPN	:	Total parenteral nutrition
T-RFLP	:	Terminal-restriction fragment length polymorphism
UMMC	:	University of Malaya Medical Centre

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Overview

Enteral nutrition (EN) is a beneficial support given to patients who are malnourished or at risk of malnutrition, via oral nutritional supplements or tube feeding. Providing nutrition through EN helps to maintain gut function by preventing mucosal atrophy (Alpers, 2002), reducing endotoxin translocation (Luyer et al., 2004) and preserving gut immunity (Sigalet, Mackenzie, & Hameed, 2004). However, despite its importance, diarrhoea remains a common complication, affecting 2% to 95% of patients who consume EN (Whelan, 2007). Higher incidence had been reported in critical care settings (Wiesen, Van Gossum, & Preiser, 2006). Such variations are contributed by the populations studied and how diarrhoea is defined (Majid, Sidek, & Chinna, 2013). Diarrhoea not only causes inconvenience to the patients and their caretakers, but it also contributes to negative clinical consequences.

There are a number of factors involved in the pathogenesis of diarrhoea during EN. This includes enteropathogenic infection, use of antibiotics, and altered physiologic response (Whelan, 2007). Enteral formulas used in EN are rich in nutrients and provide an excellent medium for bacteria proliferation, including pathogens. Poor handling during the preparation and administration of EN can contaminate the feed and cause infection (Levy et al., 1989). Similarly, antibiotic treatment is strongly associated with diarrhoea in patients receiving EN (Guenter, 2010). In fact, antibiotic use alters gut microbiota (Pérez-Cobas et al., 2012), which leads to increased risk of pathogen overgrowth (Rafii, Sutherland, & Cerniglia, 2008). In addition, the EN might also contribute to the occurrence of diarrhoea by altering physiologic responses of the ascending colon where water is secreted into the lumen (Bowling, Raimundo, Grimble, & Silk, 1993).

Traditionally, fibre was not a part of enteral formula ingredients. This is to allow the gut to rest as well as to prevent tube obstruction. However, the fibre was gradually introduced in EN in response to accumulating evidence of its effects in modulating gut function and improving immunity, blood glucose, and serum lipid regulation (Slavin, 2013). Previous meta-analysis had shown that introducing fibre into the enteral formulas was beneficial in reducing the incidence of diarrhoea (Elia, Engfer, Green, & Silk, 2008).

Physiologic effects exerted by the chemical composition of fibre are determined by its properties: viscosity, fermentability and solubility. Fibre also consists of prebiotics that are fermentable, which lead to specific changes in the composition and/or activity of the gut microbiota that benefit upon the well-being and health of the host (Roberfroid et al., 2010). Multiple human studies had shown prebiotics such as fructo-oligosaccharides (FOS), oligofructose and inulin could significantly increase the concentrations of bifidobacteria (Kolida & Gibson, 2007). Similar positive result was also demonstrated in healthy adults when fibre and FOS were added to enteral formulas (Whelan et al., 2005). However, the bifidogenic effect of prebiotics was not clearly observed in patients receiving EN containing prebiotics (Majid, Cole, Emery, & Whelan, 2014; Schneider et al., 2006; Wierdsma et al., 2009). The mechanisms of prebiotics actions to improve human health will be further discussed in the next chapter, literature review.

1.2 Research problem

Provision of five g/day or an optimal dose of ten g/day of prebiotics has been proven to increase bifidobacteria concentrations in healthy human (Bouhnik et al., 1999). However, from the current literature, the minimum or optimal dosage of prebiotics needed to exert the bifidogenic in patients with EN has yet to be established due to the limited studies and conflicting results (Majid et al., 2014; Schneider et al., 2006; Wierdsma et al., 2009). Additionally, the role of prebiotics in preventing diarrhoea is also controversial due to the mixed results (Chittawatanarat, Pokawinpudisnun, & Polbhakdee, 2010; Majid et al., 2014; Schultz, Ashby-Hughes, Taylor, Gillis, & Wilkins, 2000).

As stated previously, there is a wide range of prevalence of diarrhoea reported in the literature. This is contributed by the use of various and inconsistent definitions of diarrhoea in the studies (Majid et al., 2014). There is a need in using a constant definition of diarrhoea which is accepted and validated, especially for research use such as the King's Stool Chart (Whelan, Judd, Preedy, & Taylor, 2008).

1.3 Research questions

This thesis aims to answer this set of questions;

1. Is there any difference in the concentrations of faecal microbiota (total bacteria, bifidobacteria, lactobacilli, bacteroides, clostridia and *Faelibacterium Praunitzii*) between critically ill patients who receive fibre-free and fibre/prebiotics containing enteral formula during EN?
2. Is there any difference in the incidence of diarrhoea between critically ill patients who receive fibre-free and fibre/prebiotics containing enteral formula during EN?

3. What are the recent evidences regarding the effect of dietary fibre and prebiotics supplementation in enteral formula among adult patients requiring EN on diarrhoea, faecal microbiota and SCFAs?

1.4 Research objectives

1.4.1 Primary Objective

To determine the concentrations of faecal bifidobacteria between critically ill patients who receive EN with and without fibre/prebiotics during EN.

1.4.2 Secondary Objectives

1. To evaluate recent evidences regarding the effect of dietary fibre and prebiotics supplementation in enteral formulas in adult patients requiring EN on diarrhoea, faecal microbiota and SCFAs.
2. To determine the concentrations of faecal microbiota (total bacteria, lactobacilli, bacteroides, clostridia and *Faecalibacterium prausnitzii*) between critically ill patients who receive enteral formula with and without fibre/prebiotics during EN.
3. To compare the incidence of diarrhoea in patients receiving enteral formula with and without fibre/prebiotics during EN in critical care setting by using validated King's stool chart.

CHAPTER 2: LITERATURE REVIEW

2.1 Enteral Nutrition

2.1.1 Overview

The European Commission (Directive No. 1999/21/EC) defines EN as the use of "dietary foods for special medical purposes". This definition of EN includes the use of enteral formula through tube feeding via all accessible routes, inclusive of the nasogastric, nasoenteral, orogastric, oroenteral and percutaneous tubes as well as oral nutritional supplements. Evidence-based guideline prepared by the National Institute for Health and Clinical Excellence (2006) recommends that nutrition support be given to individuals who are malnourished. An individual is considered malnourished if they have a body mass index (BMI) of less than 18.5 kg/m^2 or unintentional weight loss greater than 10% within the last three to six months or BMI of less than 20 kg/m^2 with unintentional weight loss greater than 5% within the last three to six months. The guideline also suggests that nutrition support should be considered for individuals who are at risk of malnutrition. This includes individuals with poor oral intake for more than five days, individuals who are expected to have inadequate nutrient intake for the next five days (or longer) and individuals who have impaired absorptive capacity, high nutrient losses or increased nutritional requirements.

The provision of nutrition through EN aids in maintaining gut function by preventing mucosal atrophy (Alpers, 2002), reducing endotoxin translocation (Luyer et al., 2004) and preserving gut immunity (Sigalet et al., 2004). The benefit of EN over parenteral nutrition (PN) is well established in various types of patients (Gramlich et al., 2004; Seres, Valcarcel, & Guillaume, 2013; Windsor et al., 1998). Biopsies taken from the jejunum of patients on total PN revealed a marked reduction in mucosal thickness, villus height, and villus cell count, thus increasing intestinal permeability and susceptibility towards infection (Buchman et al., 1995; Groos, Hunefeld, & Luciano,

1996). The administration of nutrients through the gut helps in preserving gut barrier function as reflected in the lower incidence of septic complications as well as fewer infections compared to trauma patients receiving total PN (Kudsk et al., 1992). It is well established that the provision of nutrients enterally is superior to PN in reducing infection as supported by several meta-analyses conducted in various groups of patients (Al-Omran, Albalawi, Tashkandi, & Al-Ansary, 2010; Braunschweig, Levy, Sheean, & Wang, 2001; Gramlich et al., 2004; Petrov et al., 2008; Yi et al., 2012). In addition, compared to PN, EN has been demonstrated to significantly reduce hospital length of stay and the cost of nutrition support (Heyland, Dhaliwal, Drover, Gramlich, & Dodek, 2003).

2.1.2 Definition and prevalence of diarrhoea during EN

Despite the importance of EN, diarrhoea is a commonly reported complication (Gadewar & Fasano, 2005). There is a considerable variation in the reported incidence of diarrhoea during EN in previous studies, ranging from 2% to 95% (Cataldi-Betcher, Seltzer, Slocum, & Jones, 1983; DeMeo et al., 1998). The lack of uniformity and standardisation in the operational definition of diarrhoea leads to difficulty in interpreting the results and any associations made in the studies. A 2003 review of the literature found 33 different definitions of diarrhoea across a number of studies (Lebak, Bliss, Savik, & Patten-Marsh, 2003). Objective definitions of diarrhoea are preferable in research so that the incidence can be more easily quantified as opposed to subjective definitions that can be used liberally and might cause large inter-individual variability in describing diarrhoea. Lebak et al. (2003) reported that most studies included frequency or consistency as a factor in defining diarrhoea. Additionally, the amount and duration of diarrhoea were identified as elements in the definition of diarrhoea used in some studies. Apart from the research-based definition, it has been found that diarrhoea is interpreted differently by health care professionals and patients. A survey conducted by

Majid, Emery and Whelan (2012) found that nurses and dietitians ranked faecal consistency, frequency and quantity as the most important characteristics of diarrhoea, while patients considered faecal incontinence, frequency and consistency to be the important characteristics.

The challenges in reporting diarrhoea have led to the development of tools to aid its interpretation. A number of stool charts have been developed and tested for research and clinical use comprising words and/or pictures to describe the faecal output. A study by Bliss 2001 highlighted the importance of having reliable tools in producing standardised outcomes (Bliss, Larson, Burr, & Savik, 2001). In their tool, stool consistency is classified into four types, namely, hard and formed, soft and formed, loose and unformed, and liquid. The developed instrument was considered reliable as there was high agreement among inter-raters (nurses, nursing students and laypersons) in the reliability test. The later King's stool chart incorporates faecal consistency, faecal weight and faecal frequency (Whelan, Judd, Preedy, & Taylor, 2004). Its classification of faecal consistency is similar to the previous stool chart, with four classifications. In addition, the King's stool chart has three classifications of stool weight: less than 100 g, between 100–200 g and more than 200 g of stool. The pictorial chart consists of 12 photographs to assist in the accurate characterisation of the faeces. Each photograph has its own unique code with a specific score assigned to it. Cumulative daily faecal scores of 15 or more indicate diarrhoea. The King's stool chart improves the previous tool by incorporating more elements and using clear, coloured photographs. It was validated with a high score in the reliability test.

The availability of validated and comprehensive instruments is vital for researchers and health care professionals in order to standardise the description of faecal output. Such tools facilitate the uniform use of the term "diarrhoea" in regard to patients receiving EN. Standardised use of the definition of diarrhoea allows the true incidence to be measured and also facilitates the management of diarrhoea by the multidisciplinary health care professionals.

2.1.3 Pathophysiology of diarrhoea during EN

Diarrhoea has negative impacts on patients. It causes fluid and electrolyte imbalance, malnutrition, increased susceptibility to infections and increased medical costs (Lima, Guerrant, Kaiser, Germanson, & Farr, 1990; Ringel, Jameson, & Foster, 1995; Wiesen et al., 2006). The pathophysiology of diarrhoea during EN needs to be understood in order to manage this common complication. A number of factors need to be considered when diarrhoea occurs during EN. These include the enteral feeding, medications, infections and the patients' underlying condition and illness.

2.1.3.1 Enteral feeding

Enteral feeding is commonly blamed when diarrhoea occurs during EN. This is because diarrhoea is attributable to the abnormal colonic response when EN is given (Bowling, Raimundo, Grimble, & Silk, 1994). Bowling et al. (2004) investigated water and electrolyte movement in the colon by perfusing two different sites of the human gut, namely, the gastric and duodenum, with solutions containing electrolytes and a non-absorbable marker. The rectal effluent was then retrieved and analysed. The human colonic perfusion conducted in their study revealed the marked secretion of water, sodium and chloride in the ascending colon during intra-gastric infusion when isotonic and hypertonic feeds were given and during intra-duodenal infusion when hypertonic polymeric feeds were given. Additionally, net secretion persisted in the distal colon

when the hypertonic, intra-gastric feeds were perfused. In contrast, net absorption was observed during isotonic, intra-gastric and intra-duodenal infusions. The result from the study revealed that the provision of EN caused the secretion of fluid and electrolytes into the gut which was most notable during hyperosmolar intra-gastric feeds. As such, this abnormal colonic response might play a role in the development of diarrhoea during EN.

It has been proposed that hypertonic feeds cause diarrhoea via an osmotic effect as the presence of a high concentration of non-absorbable carbohydrates in enteral formulas increases the osmotic load in the gut. However, the observation of 50 enterally fed patients showed that the osmolality of enteral formulas did not affect the frequency and duration of diarrhoea (Jack, Coyer, Courtney, & Venkatesh, 2010). The results of a study showed that the introduction of poorly absorbed carbohydrates in enteral formulas to the gut increased the risk of diarrhoea as these carbohydrates are highly osmotic and rapidly fermented by bacteria (Barrett et al., 2010). In that study, the provision of a diet high in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) caused an increase in the total effluent output. These carbohydrates are highly osmotic and rapidly fermented by bacteria in the gut and this can lead to increased gas, distension, bloating, cramping and diarrhoea. The application of high performance liquid chromatography analysis revealed that most commercially-available enteral formulas have high FODMAP content and that the provision of enteral formulas with high FODMAP content increased the risk of developing diarrhoea up to five times compared to patients who were on enteral formulas with low FODMAP content (Halmos et al., 2010). Considering the effect of high FODMAP content on the gut, using enteral formulas with low FODMAP content might result in fewer gastrointestinal symptoms including diarrhoea.

2.1.3.2 Bacterial contamination

The poor handling of EN during preparation and administration by health care professionals could contaminate the feed (Levy et al., 1989). Being nutritionally complete, the enteral formulas used in EN are rich in nutrients. Such formulation is an excellent medium for bacteria proliferation including pathogens. Possible sources of bacterial contamination of EN are the enteral formulas and the feeding delivery system. A prospective observational study conducted in an intensive care unit (ICU) found that 4% of ready-to-use 1-L feeding bottles and 74% of infusion sets were contaminated with more than 10^2 colony-forming units (CFU)/mL of bacteria (Mathus-Vliegen, Binnekade, & de Haan, 2000). The main bacteria isolated in that study were *Enterobacter cloacae*, *Klebsiella oxytoca*, and enterococci. Alarmingly, a third of the cultured bacteria belonged to the Enterobacteriaceae family, which are similar to the bacteria that cause nosocomial infection in ICU patients. Other than poor handling of EN, it is also thought that the continuous regime of enteral feeding might contribute to the bacterial contamination of EN. In continuous feeding, there are only intermittent breaks after long feeding hours (i.e. one hour break after eight hours of feeding). This causes the gastric pH to increase, producing a more ideal environment for pathogens to proliferate (Stroud, Duncan, & Nightingale, 2003). However, bacterial infection of enteral feed is more likely to be contributed by the endogenous contamination especially from the gut through the delivery set compared to the extrinsic factor (Mathus-Vliegen, Bredius, & Binnekade, 2006). For example, the practice of repeated measurement of gastric residual volumes might play a role in the colonisation of bacteria in the feeding delivery system (Beattie, Anderton, & White, 1996). The procedure of aspirating gastric content through nasogastric tubes introduces bacteria in the lumen of the feeding tubes. As such, this highlights the importance of flushing the feeding tubes with water not only to prevent tube obstruction, but also to flush away milk residual and bacteria along the

tubes. Many guidelines have been established to provide guidance on the appropriate measures in handling EN, and such measures are crucial in preventing infection during EN (Bankhead et al., 2009; Best, 2008).

2.1.3.3 Infection

Even though EN is often blamed for the occurrence of diarrhoea, most infection sourcing from the gut will independently cause diarrhoea regardless of whether or not EN is used. Many guidelines recommend microbiological investigation to identify the presence of pathogens when diarrhoea occurs during EN. *Clostridium difficile* is the most common cause of nosocomial diarrhoea accounting for 10% to 20% of the identified cases (Polage, Solnick, & Cohen, 2012). Bliss et al. (1998) compared the incidence of *Clostridium difficile*-associated diarrhoea (CDAD) between patients receiving EN and those who were not on EN. This study revealed that the patients receiving EN had higher incidence (20%) of CDAD as compared to those who were not fed enterally (8%). Apart from poor handling of the tube feeding system by the health care professionals, it was thought that the acquisition of CDAD in the tube-fed patients was caused by the use of fibre-free enteral formulas. The provision of fibre-free EN has been shown to lower the concentration of SCFA and increase the luminal pH, a condition that promotes the proliferation of *C. Difficile* (Sun & O'Riordan, 2013; Bliss et al., 1989). Additionally, Bliss et al. (1998) found that the provision of post-pyloric EN resulted in a higher incidence of CDAD. Such findings highlight the importance of gastric acid in protecting the gut from infection. Although the gastric acid might not be able to kill *C. difficile* spores, the vegetative form of *C. difficile* is sensitive to gastric acid (Rao, Jump, Pultz, Pultz, & Donskey, 2006; Wilson, Sheagren, & Freter, 1985). Considering the higher risk of *C. difficile* infection and no additional benefit from post-pyloric feeding as compared to gastric feeding (Ho, Dobb, & Webb, 2006), the intra-gastric provision of nutrients should be preferred in order to avoid CDAD.

2.1.3.4 Medications

A prospective observational study conducted by Thibault et al. (2013) revealed that EN did not increase the risk of developing diarrhoea. Similarly, a meta-analysis performed by Gramlich also observed that EN did not increase the risk of diarrhoea when compared to critically ill patients receiving PN (Gramlich et al., 2004). Instead, medications are the major contributors to diarrhoea as portrayed by the high number of medications (more than 700) that have diarrhoea as one of the side effects (Polage et al., 2012).

Patients receiving EN are most likely to have medications administered through the enteral feeding tubes. Liquid medications are more preferable when patients are on EN as it causes less tube obstruction compared to solid medications (Klang, McLymont, & Ng, 2013). However, liquid medications usually have added sugars, flavouring agents and excipients to improve tolerability and palatability as they are mainly designed for patients who experience difficulty in swallowing (Dickerson & Melnik, 1988). Sugar alcohols especially sorbitol are widely used in the formulation of liquid medications and, when given undiluted, are responsible for causing osmotic diarrhoea in patients receiving EN (Eherer & Fordtran, 1992). Klang et al. (2013) found that, of the 62 commercially-available liquid medications analysed in their study, 98% of the medications had osmolality higher than 500 mOsm/kg. Surprisingly, 20 of the medications had osmolality higher than 5000 mOsm/kg. Essentially, osmolality of the medications is not an issue as these liquid medications are intended for oral intake in which the medications will be diluted by the saliva, mucous and gastric juices. Unfortunately, the provision of undiluted liquid medications directly to the gut with the osmolality exceeding the threshold may cause osmotic diarrhoea. The stomach has higher toleration to osmolality (<700 mOsm/kg) compared to the jejunum (<300 mOsm/kg). As such, liquid medications need to be diluted in order to improve tolerance

to the liquid medications in patients receiving EN and to minimise diarrhoea. Apart from hypertonic medications, the usage of antibiotics is also an important factor in the development of diarrhoea during EN.

Diarrhoea has been reported to be one of the most commonly occurring side effects of antibiotics. The side effects of antibiotic consumption vary from slight abdominal discomfort to severe diarrhoea and colitis. The development of antibiotic associated diarrhoea (AAD) may be attributable to the direct prokinetic effect of the antibiotics in stimulating the motility of the gut. It is well established that some of the macrolide antibiotics (i.e. erythromycin and azithromycin) act as a motilin receptor agonist in the gut, leading to the stimulation of enteric nerves and smooth muscle (Hawkyard & Koerner, 2007). More importantly, antibiotics have a significant effect in altering gut microbiota composition by suppressing certain groups of bacteria and potentially stimulating the growth of pathogenic or opportunistic bacteria (Pérez-Cobas et al., 2012; Rafii et al., 2008). The alteration of the gut microbiota as a result of antimicrobial therapy plays a significant role in the pathogenesis of diarrhoea by increasing the risk of bacterial translocation into the gut. Evidently, as stated earlier, 10% to 20% of the AAD cases are due to *C. difficile* infection, the most common agent of nosocomial diarrhoea (Polage et al., 2012). As *C. difficile* invades and proliferates in the gut, it releases toxins causing insult to the gut, consequently leading to diarrhoea. It has also been described that the dysbiosis of gut microbiota from antibiotic consumption disrupts the carbohydrate metabolism of the gut (Hogenauer, Hammer, Krejs, & Reisinger, 1998). Reduced SCFA concentration not only deprives the colonocytes from the energy source, but also limits the osmotic effect of SCFA. Consequently, osmotic diarrhoea occurs as a result of the accumulation of organic acids, cations and carbohydrates in the gut.

While antibiotics treatment has been reported to have the strongest association with diarrhoea when patients are receiving EN (Guenter, 2010), the use of prokinetic agents and laxatives may also cause diarrhoea. Prokinetic therapy is often initiated to minimise and improve feeding intolerance especially in critically ill patients. However, some patients suffer from abdominal pain, nausea, vomiting and diarrhoea as the result of using prokinetic agents. Watery diarrhoea is the most established adverse effect of prokinetic therapy, occurring in as many as 25% of critically ill patients requiring EN (Nguyen & Yi Mei, 2011). A prospective observational study conducted on 183 critically ill patients who did not tolerate nasogastric feeds showed that diarrhoea commonly occurred when prokinetic agents were given (Nguyen, Ching, Fraser, Chapman, & Holloway, 2008). Forty percent of patients in that study developed diarrhoea after approximately 10 days of therapy. The study also discovered that the diarrhoea caused by prokinetic therapy was not related to *C. difficile* infection and could be resolved immediately after cessation of the therapy. Similarly, diarrhoea caused by laxatives is often mild and can be controlled by the manipulation of the medication itself (Ferrie & East, 2007).

2.1.3.5 Hypoalbuminaemia

The theory behind the development of diarrhoea as an adverse effect of hypoalbuminaemia is based on the reduction of oncotic pressure when the albumin level is low (Koretz, 1995). The decrease in the oncotic pressure will lead to the gut's mucosal edema which then causes malabsorption. The relationship between low albumin and diarrhoea was initially established by Brinson and Kolts (1987). In their study, all patients with a serum albumin level less than 2.6 g/dl developed diarrhoea, while patients with a serum albumin level of 2.6 g/dl or greater did not develop diarrhoea. Following that study, the researchers attempted to reduce diarrhoea by providing a peptide-based formula compared to a standard formula (Brinson & Kolts,

1988). The intervention was considered successful as the serum albumin concentrations increased and diarrhoea was resolved in patients receiving the peptide-based formula. However, the results must be interpreted with caution due to the methodological limitations of that study. Firstly, the sample size was small (N=12) and only seven patients completed the study. Secondly, out of the seven patients who completed the study, only one patient received the standard formula while the other six received the peptide-based formula. From the successful intervention, it was initially thought that correction of the serum albumin level in patients with hypoalbuminaemia will improve diarrhoea. This was supported by the observational study conducted by Guenter et al. (1991) whereby patients who had diarrhoea were found to have lower serum albumin levels than patients who did not develop diarrhoea (Guenter et al., 1991). Similarly, Hwang in 1994 found that a high proportion (35%) of patients with chronic hypoalbuminaemia developed diarrhoea (Hwang, Lue, Nee, Jan, & Chen, 1994). Nevertheless, other studies were not able to confirm the association between hypoalbuminaemia and diarrhoea (Bittencourt et al., 2012; Eisenberg, 2002; Heimburger, Geels, Bilbrey, Redden, & Keeney, 1997; Ringel et al., 1995). Based on available data, it is now suggested that the low serum albumin level might not have a direct impact on diarrhoea. Instead, hypoalbuminaemia may be a marker of severity of illness which has been shown to be related to the incidence of diarrhoea (Vincent, Dubois, Navickis, & Wilkes, 2003).

2.1.3.6 Severity of illness

Multiple studies have confirmed the association between increased severity of illness and frequency and duration of diarrhoea (Huang, Hsu, Kang, Liu, & Chang, 2012; Jack et al., 2010). An increase in Acute Physiology and Chronic Health Evaluation II (APACHE II) scores has been found to be associated with the frequency and duration of diarrhoea (Jack et al., 2010). During the period of acute illness, critically

ill patients often suffer from hypermetabolic stress response and altered gut function (Ferrie & East, 2007). The abnormal motility patterns and impaired barrier integrity suffered by critically ill patients may eventually lead to the development of diarrhoea (Hill, 2013).

In summary, the aetiology of development of diarrhoea is multifactorial. The mechanism of diarrhoea during EN may involve one or more factors including the enteral feeding itself, the medications received by the patients and the pathological conditions of the patients. The review of the literature indicates that the alteration of gut microbiota plays an important role in the development of diarrhoea. The next section of this chapter discusses the gastrointestinal microbiota in detail in order to understand further the relationship between gut microbiota and faecal output.

2.2 Gastrointestinal Microbiota

2.2.1 Composition of colonic microbiota

The human gut is a complex and dynamic ecosystem consisting of hundreds of bacteria species, referred to as microbiota. The microbial density varies and is not distributed homogenously throughout the gastrointestinal tract (GIT), as presented in Figure 2.1. The highest concentration of microbiota is found in the colon, with the number of bacteria reaching 10^{12} per gram of content (Holzapfel, Haberer, Snel, Schillinger, & Huis in't Veld, 1998; Mowat & Agace, 2014). A metagenomic sequencing conducted on 124 faecal samples of European adults found that the gut microbial genome was at least 150 times more than the human host genome (Qin et al., 2010). That study gathered up to 1150 bacterial species with at least 160 bacterial species from each individual.

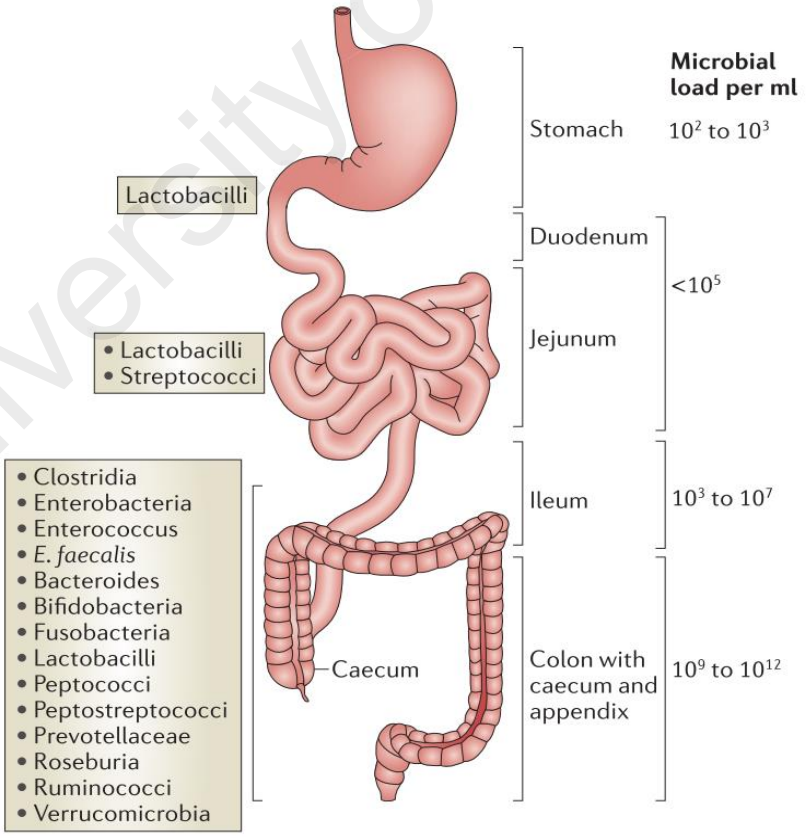

Figure 2.1: Variation of the microbial density throughout the GIT (Mowat & Agace, 2014)

The human gastrointestinal tract is the host for all three domains of life, namely, archae, eukarya and bacteria, although 99% of genes in the gut belong to the bacteria (Qin et al., 2010). Most of the bacterial species found in the human gastrointestinal tract belong to these two phyla; Bacteroidetes and Firmicutes, whereas the minority of the bacteria are classified under the phyla Proteobacteria, Actinobacteria, Fusobacteria, Spirochetes, Verrucomicrobia and Lentisphaera (Rajilić-Stojanović, Smidt, & De Vos, 2007; Zoetendal, Rajilic-Stojanovic, & de Vos, 2008). The majority of gut microbiota are anaerobic bacteria, outnumbering the facultative anaerobic and aerobic bacteria owing to the highly anaerobic environment of the colonic lumen (Flint, Scott, Louis, & Duncan, 2012). Despite being part of the normal commensal bacteria of the gut, anaerobic bacteria can become opportunistic pathogens which may exert pathogenic effects such as infection, diarrhoea, liver damage, carcinogenesis and intestinal putrefaction (Gibson & Roberfroid, 1995). The classification of gut bacteria based on its potential pathogenic or beneficial effect is presented in Figure 2.2.

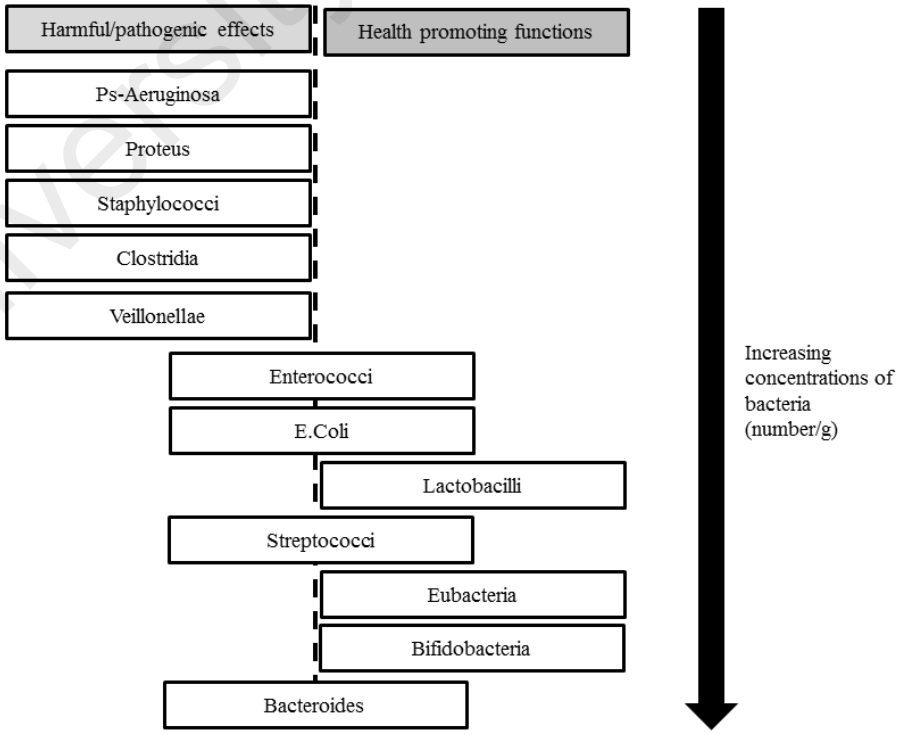

Figure 2.2: Generalised scheme of the composition and health effects of predominant genera of human faecal microbiota (Gibson & Roberfroid, 1995)

2.2.2 Method of studying gut microbiota

Initial works on microbiota studies involved microscopy and culture-based analysis. More recent work has been able to isolate, culture and characterise more than 400 bacteria of the human gut (Rajilić-Stojanović et al., 2007). However, a large fraction of microbiota is uncultivable and this method is time-consuming and laborious. Application of the cultivation-independent techniques in microbiota analysis has revealed the complexity and diversity of gut microbiota, enabling the identification of more than 1000 bacterial species by the detection of more bacteria (Qin et al., 2010). These molecular techniques include quantitative polymerase chain reaction (qPCR), terminal-restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) and fluorescent in-situ hybridisation (FISH). In addition, more recent developments in cultivation-independent techniques, such as Sanger sequencing, 454 pyrosequencing, deoxyribonucleic acid (DNA) micro-array, metagenomics, metabolomics, metaproteomics and metatrasciptomics, allow for more comprehensive analysis of the phylogenetic diversity of gut microbiota (Qin et al., 2010; Zoetendal et al., 2008).

From the various techniques available for microbial analysis, the present study employed qPCR for bacterial quantification in order to meet the study's objectives. The working principle of qPCR is similar to the conventional polymerase chain reaction (PCR); that is, it involves cycles of denaturation, annealing and extension. The amplified target sequence, referred to as the PCR product, can be quantified by measuring the signal from the fluorescence-labelled group- or strain-specific probes or with a non-specific DNA-binding dye (i.e. SYBR® green dye) during the PCR amplification. Signal quantification using SYBR® green dye is illustrated in Figure 2.3. The generation of a standard curve is needed in order to conduct an absolute quantification for qPCR, whereas relative quantification may be employed to measure

the relative abundance of a bacterial species when the number of the target bacteria in samples cannot be obtained by using the calculation of the 2−ΔΔCt method (Livak & Schmittgen, 2001).

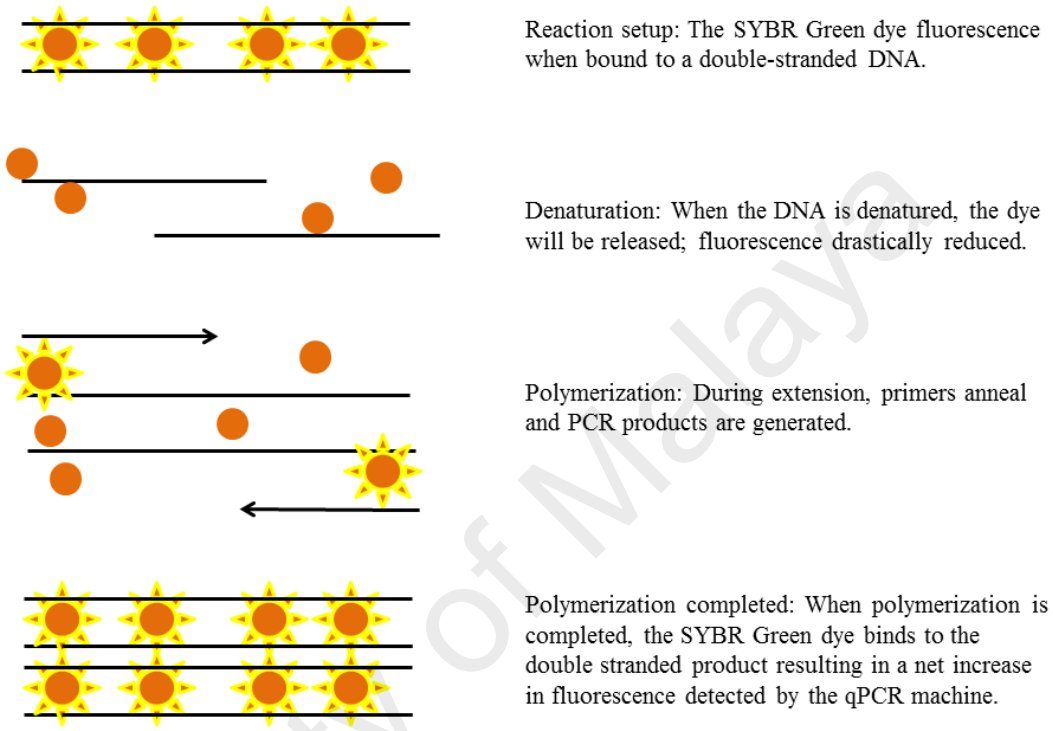

Figure 2.3: Signal detection using SYBR green dye for qPCR

The application of qPCR in the quantification of bacteria voids the need for the time-consuming and laborious procedure of gel electrophoresis, staining and visualisation in the fluorescence setup in conventional PCR. Additionally, the qPCR has higher sensitivity compared to FISH and DNA microarray (Gaj, Eijssen, Mensink, & Evelo, 2008; Haarman & Knol, 2005; Matsuki et al., 2004) due to the use of specific primers that anneal to the single-stranded DNA template. However, the extraction step in qPCR contributes directly to the outcome of this technique. Studies have revealed that different extraction procedures yield different concentrations of bacteria (Li et al., 2003; McOrist, Jackson, & Bird, 2002).

The present study targeted the 16S ribosomal ribonucleic acid (rRNA) gene of the bacteria of interest as it is present in almost all bacteria, remains unchanged over time and has a relatively small size (1500bp) but is sufficient for informatics purposes (Janda & Abbott, 2007). 16S rRNA is widely used as the target gene as it has an appropriate balance of conservation and variability in the identification of bacteria of a specific species and member of larger group/genera. It is currently the gold standard in the analysis of microbiota (Arrieta, Stiemsma, Amenyogbe, Brown, & Finlay, 2014).

2.2.2.1 Faeces as sample material for gut microbiota studies

The collection of faecal samples is commonly used in gut microbiota analysis as it is a convenient and non-invasive method of obtaining material from subjects. Although it serves as a representative of gut microbiota, the bacterial composition found in faeces is never identical to that of the intestine (Fink, Staubach, Kuenzel, Baines, & Roeder, 2013; Holzapfel et al., 1998; Zoetendal et al., 2002). A complex molecular analysis attempted to explore and compare the faecal microbiota and several colonic mucosal sites' microbiota of three healthy human where faecal samples and mucosal tissue samples were taken during colonoscopy sessions (Eckburg et al., 2005). Findings from this study suggested that faecal microbiota is comprised of bacteria originated from the mucous membrane as well as the luminal part of the intestine. While the difference of bacterial diversity of gut and faecal microbiota is acknowledged, sampling of faecal matter also offers a non-invasive sampling technique that is more convenient and acceptable to the study subjects.

2.2.3 Functions of gut microbiota

The advancement of techniques in microbial analysis has led to more sophisticated research, enabling more discoveries on the gut microbiota and their potential functional capacity especially in regard to human health. The protective role of gut microbiota is a result of its interaction with the host (human) which occurs at the lining of the human gut. The two-way interaction between the gut microbiota and the host is crucial in regulating bodily functions and in maintaining a stable environment for the gut microbiota. Several authors have proposed that the metabolic activity of the gut microbiota is equivalent to a virtual organ (Bocci, 1992; O'Hara & Shanahan, 2006). Extensive literature has outlined the three main functions of gut microbiota: metabolic, trophic and protective.

2.2.3.1 Metabolic functions

(a) ***Role of the gut microbiota in carbohydrate metabolism***

Gut microbiota assist in carbohydrate metabolism by assisting in the degradation of undigested carbohydrates. Initially, ingested carbohydrates are hydrolysed by human digestive enzymes, enabling absorption of the nutrient in a simpler form (i.e. monosaccharide and some disaccharides). However, some of the carbohydrates are not able to be broken down by human enzymes and thus enter the colon intact (Gray, 1975). The undigested carbohydrates, which include resistant starch, oligosaccharides and fibre along with unabsorbed mono and disaccharides, are metabolised by the gut microbiota through fermentation. The degradation of the undigested carbohydrates is mainly contributed by the fibrolytic community of anaerobic gut microbes. This community, which includes the *Bacteroides, Roseburia, Ruminococcus* or *Bifidobacterium*, may exert several fibrolytic activities and work collaboratively in degrading a more complex fibre (Chassard & Lacroix, 2013).

The fermentation of carbohydrates by the gut microbiota yields SCFAs (primarily acetate, propionate and butyrate) as well as hydrogen, carbon dioxide and methane (Mortensen & Clausen, 1996). SCFAs are the principal product of fermentation and exert multiple physiologic effects on human metabolism as discussed extensively in the literature (Cook & Sellin, 1998; Macfarlane & Macfarlane, 2012). Acetate, propionate and butyrate are oxidised by the mucosal cell to serve as a source of energy for the colonocyte, contributing up to 10% of the total energy in healthy individuals (McNeil, 1984). Butyrate is the preferred source of fuel, followed by propionate and acetate. The SCFAs also protect the gut in resisting pathogen colonization through the production of hostile acidic environment and its' virulence regulation property (Sun & O'Riordan, 2013). Additionally, SCFAs promote the absorption of sodium and water in the colon (Zaharia et al., 2001). This specific function of SCFA might play a protective role in reducing the fluid volume of faecal output, consequently alleviating the development of diarrhoea (Canani et al., 2004; Binder, 2010; Bowling et al., 1993).

(b) ***Role of gut microbiota in protein metabolism***

A study conducted by MacFarlane, Cummings and Allison (1986) found that a high level of proteolytic activity occurs in the colon which is attributed to the gut microbiota. The initial step of proteolysis involves the breaking down of a long chain of proteins into shorter chains of peptides and amino acids. These proteolytic activities are predominated by the *Bacteroides* spp. and *Propionibacterium* spp. Other members of gut microbiota that have proteolytic activity include the *Streptococcus*, *Clostridium*, *Bacillus* and *Staphylococcus* genera. Protein hydrolysis by these bacteria yields the beneficial SCFAs, carbon dioxide and potentially toxic metabolites such as amines, ammonia, N-nitroso compounds, phenolic compounds and sulphides (Smith & Macfarlane, 1997).

(c) ***Role of gut microbiota in transformation of bile acids***

Bile acids are synthesised from cholesterol by the hepatocyte. A significant amount of bile is secreted into the gut every day, and 95% of it is reabsorbed from the gut (Chiang, 2009). The remaining bile acids in the gut are further metabolised by the gut microbiota into secondary bile acids in the terminal ileum or the colon. Degradation of the C24 N-acyl amide bond of conjugated bile acids is catalysed by bile salt hydrolases. This enzyme has been isolated from several species of *Bacteroides, Lactobacillus* and *Bifidobacterium* (Gérard, 2013).

2.2.3.2 Trophic functions

The involvement of gut microbiota in developing human gut structure begins as early as after birth. Babies are born with a structurally and functionally immature gut. The maturation of the gut requires the gut to have efficient peristaltic motility, sufficient surface area and blood supply. Maturation of the gut's structure is induced by many factors including the host-microbiota interaction (Sommer & Backhed, 2013). The microbiota stimulates substantial changes in gut morphology (Sommer & Backhed, 2013). Multiple animal studies have found evidence of immature gut structure in animals with sterile gut. One animal study observed that germ-free rats had reduced cell generation resulting in smaller villus thickness (Banasaz, Norin, Holma, & Midtvedt, 2002). Another study also observed the abnormal gut structure of germ-free rats whereby the animals had enlarged caecum (Wostmann & Bruckner-Kardoss, 1959). This condition is later associated with reproductive and gut disorders. The introduction of an important member of post-weaning microbiota to the gut of germ-free rats successfully promoted the development of new blood vessels (Stappenbeck, Hooper, & Gordon, 2002). These are a few examples of studies showing the importance of gut microbiota in developing the digestive system.

Gut microbiota not only play an important role in the development of intestinal mucosal but also in the systemic immune system (Sekirov, Russell, Antunes, & Finlay, 2010). Gut microbiota are essential for the development and maturation of gut-associated lymphoid tissues (GALTs). GALTs are the immune structures and their function is to regulate the inflammatory response. GALT maturation only occurs after birth and is dependent on signals from the microbiota (Cerf-Bensussan & Gaboriau-Routhiau, 2010). Germ-free mice have been identified to suffer from poorly-formed spleen and lymph nodes, the hypoplasia of the Peyer's Patches and a reduced number of mature lymphoid follicles (Macpherson & Harris, 2004). Colonisation of germ-free mice with *Clostridium* and a complex microbiota community has been shown to increase the number of regulatory T cells (Atarashi et al., 2011; Geuking et al., 2011). Cross-talk between the host and gut microbiota is crucial in maintaining homeostasis of the gut and in balancing the population of gut microbiota on the gut surface without inducing the immune response.

2.2.3.3 Protective functions

Besides immunomodulation, there are several mechanisms by which the gut microbiota protects the host from intestinal pathogens (Buffie & Pamer, 2013). Gut microbiota provide a physical barrier to prevent pathogens from entering the host by competitive exclusion. The gut microbiota bind to the attachment sites, produce antimicrobial substances and compete for the nutrient source to restrict the pathogen from colonising the gut (Sekirov et al., 2010).

Antimicrobial peptides (AMPs) are part of the innate immune response released by the host. AMPs are able to disrupt the surface structure of pathogens. Gut microbiota are responsible for the regulation of AMPs as the presence of the gut microbiota and its product of metabolism stimulate the production of AMPs (Vaishnava, Behrendt, Ismail,

Eckmann, & Hooper, 2008). Additionally, the gut microbiota is able to prevent colonisation by the production of antimicrobial substances. *Bacilli*, a member of the Firmicutes phylum, has been shown to inhibit the colonisation of *Clostridium difficile* and *Listeria monocytogenes* by the production of bacteriocin, thuricin CD (Rea et al., 2010). Bacteria that belong to the *Lactobacillus* genus are also able to produce lactic acid, producing less ideal conditions for pathogen growth (Alakomi et al., 2000). The acidic environment is also contributed by the fermentation of carbohydrates and proteins by the gut microbiota. Alternatively, the indigenous gut microbiota competes with the pathogenic bacteria for essential nutrients. The commensal *E. coli* competes with enterohaemorrhagic *E. coli* for organic acids, amino acids and other nutrients, causing starvation among the pathogens (Kamada, Chen, Inohara, & Núñez, 2013). Other than that, the colonisation of segmented filamentous bacteria in the distal ileal villi has been proposed to prevent the attachment of *Salmonella enteritidis*, a source of fatal infection (Garland, Lee, & Dickson, 1982).

2.2.4 Factors influencing colonic microbiota

The gut microbiota are immensely diverse and remarkably stable over time with 60% of the bacteria strain still detected over a five year period (Faith et al., 2013). However, the gut microbiota can be altered by endogenous and exogenous factors such as genetics, type of birth, aging process, pathological condition, medication and diet (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012; Schneider et al., 2000; Sekirov et al., 2010).

2.2.4.1 Baseline variations

The gut microbiota composition was found to be varied between individuals (Lozupone et al., 2012). A study conducted on 154 individuals found that there was no one species of bacteria shared by all 154 individuals (Turnbaugh & Gordon, 2009). That

study also found that twins and mother-daughter pairs had a more similar composition of gut microbiota compared to two unrelated persons. This suggests the influence of genetics in the composition of microbiota. However, studies on monozygotic and dizygotic twins have revealed similar variances in gut microbiota composition, indicating that environment might also play a role in determining the microbiota make-up (Dicksved et al., 2008; Turnbaugh & Gordon, 2009). Such results could be due to similar early environmental exposure experienced by the twins as, in one of the studies, most of the twins were still young and living together with their families (Dicksved et al., 2008).

2.2.4.2 Geography

A study of 190 Caucasian and Chinese subjects from Australia and Hong Kong revealed significant differences in microbiota composition among the healthy individuals (Prideaux et al., 2013). A similar finding was also found elsewhere (Yatsunenko et al., 2012). It is hypothesised that differences in social structure and cultural traditions (i.e. food, exposure to pets and livestock) contribute to the variations in the gut microbiota composition.

2.2.4.3 Age

At birth, babies are born with a sterile gut. The aerobic condition of the gut at this time allows only facultative anaerobe bacteria from the *Enterobacteriaceae* family to grow (Matamoros, Gras-Leguen, Le Vacon, Potel, & de La Cochetiere, 2013). Colonization of the babies gut begins immediately after birth by the environmental organism that mainly comes from the mothers. Evidence emerging from studies suggested that there are marked differences in the gut microbiota composition between babies born through vagina and caesarean delivery. Babies born through caesarean delivery have less diversity of intestinal microbiota compared to those who were born

vaginally (Biasucci, Benenati, Morelli, Bessi, & Boehm, 2008). This is characterized by the absence of Bifidobacteria species which is predominant in babies born that are vaginally delivered. As the condition of the gut gradually become anaerobic, allowing strict anaerobic bacteria inclusive of *Bifidobacterium, Clostridium* and *Bacteroides* to flourish. The infant diet of sole milk contributes to the high composition of bifidobacteria. Bifidobacteria is the predominant gut microbiota at this stage as milk (especially breast milk) contains oligosaccharides (Lozupone et al., 2012). In babies' early lives, the predominant gut microbiota consists of *Enterococcae, Streptocioccae, Lactobacillaceae, Clostridiaceae* and *Bifidobacteriaceae*, resembling the maternal skin and vaginal microbiome (Arrieta et al., 2014). Weaning shifts the gut microbiota composition to become more similar to an adult's composition. Consequently, the introduction of food to babies leads to the increase of *Bacteroides*, *Clostridium* and *Ruminococcus* and to the decrease of bifidobacteria (Fallani et al., 2011). In general, children under the age of three have lower diversity in the gut composition as compared to adults (Koenig et al., 2011). The inter-individual variety of gut microbiota is contributed by many factors including the mode of delivery, type of infant feeding, gestational age, hospitalisation and antibiotic therapy (Penders et al., 2006).

The composition of gut microbiota is reported to be stable after the age of three especially during adulthood (Yatsunenko et al., 2012). Temporal changes could be observed, especially with the use of medication (i.e. antibiotics). The aging process alters microbiota significantly (Tiihonen, Ouwehand, & Rautonen, 2010). The changes of gut microbiota as the result of aging are related to the physiological change of the gut, changes in the functionality of the immune system and the modification of lifestyle and nutritional behaviours (Biagi, Candela, Fairweather-Tait, Franceschi, & Brigidi, 2012). A comparison of the gut microbiota of younger adults and the elderly reported lower *Bifidobacterium* and *Lactobacillus* in the elderly (Hopkins, Sharp, & Macfarlane,

2001). However, no significant changes were seen in the composition of the bacteroides, enterococci, enterobacteria and clostridia found in the study. Other than physiological aging, the gut microbiota of the elderly is also impacted by their hospitalisation and the use of antibiotics during illness (Bartosch, Fite, Macfarlane, & McMurdo, 2004).

2.2.4.4 Pathological conditions

Marked alteration of the gut microbiota has been seen in various diseases, especially in gastrointestinal diseases such as irritable bowel syndrome (IBS), irritable bowel disease (IBD) and obesity. Perturbation of the gut microbiota has been reported in people with IBS (Matto et al., 2005). These studies showed an abnormal composition of faecal microbiota, with the most consistent alteration of the *Bifidobacterium* and *Clostridium coccoides–E. rectale* subgroup (Malinen et al., 2005; Si, Yu, Fan, & Chen, 2004). The three subtypes of IBS based on the Rome II criteria are diarrhoea-predominant IBS, constipation-predominant IBS and mixed type of IBS which is characterised by the alternating faeces pattern. The dysbiosis of gut microbiota among the three subtypes of IBS was found to be dissimilar (Malinen et al., 2005).

Similarly, perturbation of the gut microbiota is also seen in patients with IBD. IBD is characterised by the discontinuous inflammation of the colon and the small intestine. A review by Rigottier-Gois in 2013 identified reduced diversity and stability of the gut microbiota among patients with IBD. The dysbiosis was identified by the reduction in the predominant member of the gut microbiota, Firmicutes, along with an increase of Bacteroides and Enterobacteria (Rigottier-Gois, 2013). In contrast, an increment of Firmicutes, along with a reduction of Bacteroidetes was observed in an animal study in relation to the association between obesity and perturbation of the gut microbiota (Ley et al., 2005). There was a marked reduction of diversity in the

composition of the gut microbiota found in obese individuals in comparison to lean individuals (Turnbaugh & Gordon, 2009). Despite the growing body of literature identifying the alteration of the gut microbiota in certain pathological conditions, it has yet to be established whether the dysbiosis of the gut microbiota plays a role in the pathophysiology of the disease or whether it is the result of the pathological conditions (Gerritsen, Smidt, Rijkers, & de Vos, 2011).

2.2.4.5 Medications and disease treatment

Antibiotic therapy is one of the more important factors contributing to the dysbiosis of the gut microbiota. A study conducted in ten healthy volunteers found a reduction in the total anaerobic microbiota when an individual was given one gram of ceftriaxone intramuscularly for five days (Welling et al., 1991). Additionally, antibiotic therapy resulted in the reduction of Bacteroides and the increment of Firmicutes (De La Cochetiere et al., 2005). A study using a multi-omics approach found that some taxa of bacteria (i.e. Streptococcae, Actinobacteria, Betaproteobacteria, Lachnospiraceae, Porporphyromonadaceae and Clostridiales) were not detected even after the cessation of antibiotic treatment (Pérez-Cobas et al., 2012). Antibiotics modified the composition of gut microbiota not only by suppressing certain groups of bacteria but also by potentially stimulating the growth of pathogenic or opportunistic bacteria (Pérez-Cobas et al., 2012; Rafii et al., 2008). It has been noted that the deleterious effect of antibiotic therapy on gut microbiota is dose-dependent: a lower dosage might not reduce the total number of the microbes, but may have an effect on the composition (Sekirov et al., 2008).

2.2.4.6 Diet

From the early stages of life, an individual's diet has significant implications for modulation of the gut microbiota. Breastfed infants were observed to have a significantly higher composition of bifidobacteria than formula-fed infants (Rinne et al., 2005). As babies grow, the consumption of food shifts the microbiota composition so that it more closely resembles the microbiota composition of an adult (Fallani et al., 2011). Over time, the human gut microbiota becomes more stable especially during adulthood (Yatsunenko et al., 2012). A study conducted on six healthy young adults consuming a western diet showed that daily variations in dietary intake did not alter the gut microbiota (Cummings et al., 1978). However, perturbation of the gut microbiota does occur in extreme changes in the dietary intake such as fasting, fibre-free diet and changes to EN and PN. The results of an animal study indicated that fasting reduced the total number of gut microbiota (Sonoyama et al., 2009).

The consumption of fibre, as a substrate for fermentation in the gut, promotes the alteration of the gut microbiota. A study comparing 30 children aged one to six from Burkina Faso and Europe revealed that the children from Burkina Faso had a significantly higher composition of Bacteroidetes and lower concentrations of Firmicutes (De Filippo et al., 2010). The Burkina Faso children consumed high fibre as reflected in their traditional rural African diet, which was predominantly vegetarian. Their diet was high in starch, fibre and plant polysaccharides and low in fat and animal protein. Meanwhile, the European children consumed a typical western diet which was high in animal protein, fat, sugar and starch and low in fibre. The study also found that the children from Burkina Faso had a unique abundance of Prevotella and Xylanibacter, which have the ability to hydrolyse cellulose and xylans. Such genera were completely absent in the European children. Parallel with the high fibre intake, the Burkina Faso children had significantly higher SCFAs than the European children.

Significant changes in diet also cause dysbiosis of the gut microbiota. This is markedly seen in patients receiving nutrients enterally and parenterally (Schneider et al., 2000). The provision of EN has been shown to reduce total faecal bacteria in healthy people (Whelan et al., 2004). A similar observation was made in a study of patients with long-term EN, with a marked reduction of anaerobic bacteria and an increment of aerobic bacteria seen in this group of subjects (Schneider et al., 2000). However, Whelan and colleagues (2009) found no significant changes in the faecal microbiota in patients starting on 14 days of EN. Although dysbiosis was not observed in patients receiving EN, perturbation of the gut microbiota was observed in the patients who developed diarrhoea during the EN in that study. The patients who developed diarrhoea were shown to have higher concentrations of clostridia and lower bifidobacteria concentration than those who did not develop diarrhoea. The perturbation of the gut microbiota seen in patients receiving fibre-free total EN was found to be comparable to the perturbation induced by the use of broad-spectrum antibiotic, ceftriaxone (Welling et al., 1991). Compared to patients receiving EN, patients receiving total PN suffered more extensive alteration of the gut microbiota. During EN, a marked decline of the predominant bacteria (anaerobic bacteria) and an increase in aerobic bacteria were observed when patients were given fibre-free enteral formulas. Meanwhile, patients receiving total PN as the source of nutrients suffered from a reduction of both aerobic and anaerobic bacteria. Such changes might be contributed by the deprivation of substrate such as fibre and other nutrients that are an essential source of substrate for the growth of gut microbiota. Extreme changes in diet have detrimental effects on the gut microbiota. This is supported by the finding of an in-vivo study whereby an elemental diet and total PN were shown to induce bacterial colonisation in the gut (Deitch et al., 1995).

A growing number of studies are being conducted to identify suitable interventions to reverse the perturbation of the gut microbiota during EN. For example, the use of multifibre enteral formula on tube-fed patients increased the number of total bacteria and the concentration of SCFAs (Schneider et al., 2006). It was proposed that the utilisation of undigested carbohydrates and fibre in the enteral formula by the gut microbiota may increase the number of bacteria, especially bifidobacteria. Bifidobacteria is a Gram-positive, nonmotile, anaerobic bacterium that exerts a range of beneficial health effects to the host (human). The next section of the literature review discusses the role of fibre in manipulating the gut microbiota especially during EN.

2.3 Fibre

2.3.1 Definition and classification

Fibre was initially defined as the undigested components of plants, namely, the lignin and polysaccharides. Newer definitions of fibre are based on the analytical method of identifying the chemical components or the physiologic effects of the fibre. Different institutions define fibre differently, as presented in Table 2.1.

Table 2.1: Definitions of fibre

Institution	Definition
American Association of Cereal Chemists, 2001	"Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiologic effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation".
Institute of Medicine, 2005	"Dietary fibre consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fibre consists of isolated, no digestible carbohydrates that have beneficial physiologic effects in humans. Total fibre is the sum of dietary fibre and functional fibre".
Codex Alimentarius Commission, 2009	"Dietary fibre means carbohydrate polymers with ≥10 monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans".

Based on the analytical approach, dietary fibre includes non-starch polysaccharides such as arabinoxylans, cellulose, methyl cellulose and many other plant components such as resistant starch, resistant dextrins, lignin, waxes, chitins, pectins, beta-glucans, fructans, inulin and oligosaccharides.

Traditionally, fibre is classified by its property of solubility in water. Soluble fibre includes acacia gum, partially hydrolysed guar gum (PHGG), inulin, fructooligosaccharides (FOS), pectin, hemicellulose A and oat fibre (Klosterbuer, Roughead, & Slavin, 2011). Soluble fibre increases transit time as it slows the passage of food in the gut by the formation of gel in the presence of water (Meier, Beglinger, Schneider, Rowedder, & Gyr, 1993). Meanwhile, insoluble fibre such as cellulose, soy polysaccharides, resistant starch and hemicellulose B add bulk to the faeces, reducing transit time and thus assisting in the defecation of faeces.

The physiologic effects exerted by the chemical composition of fibres are determined by its properties: viscosity, fermentability and solubility. Generally, soluble fibre has a higher degree of fermentability and viscosity than insoluble fibre (Klosterbuer et al., 2011). However, there are exceptions as some soluble fibres are not viscous (e.g. acacia gum, PHGG) and certain insoluble fibres are highly fermentable as in physically-treated soy polysaccharides. Fermentable fibres include acacia gum, PHGG, inulin, FOS, soy polysaccharides, resistant starch and pectin, whereas examples of non-fermentable fibres include cellulose and outer pea fibre. Viscous fibre may modify the viscosity of the gut content, thus inhibiting the absorption of nutrients especially glucose and cholesterol (Dikeman & Fahey, 2006). Viscous fibres include pectin and guar gum while non-viscous fibres include cellulose, outer pea fibre, soy polysaccharide, resistant starch, PHGG, inulin and FOS (Klosterbuer et al., 2011).

2.3.2 Fibre and gut health

Fibre plays an important role in the gut's health. Both soluble and insoluble fibre play roles in regulating the bowel function. Each fibre has different physical characteristics, hence altering gastrointestinal motility and transit times differently (Harvey, Pomare, & Heaton, 1973; Meier et al., 1993). The consumption of viscous fibre may slow down gastric emptying and increase postprandial intestinal motility (Deitch et al., 1995). On the other hand, insoluble fibre significantly increases stool weight by providing a substrate for microbial growth, contributing to the increase in faecal bacterial mass (Chen, Haack, Janecky, Vollendorf, & Marlett, 1998). The water-retaining property of fibre and the particle size of the fibre also contribute to the weight of the faeces (Brodribb & Groves, 1978; Cummings, Hill, Jenkins, Pearson, & Wiggins, 1976). Fibre with a bigger particle size takes a longer duration for hydrolysis and thus is more likely to be excreted as faeces. The role of fibre in increasing bulk involves several mechanisms including water-holding capacity, stimulation of bacterial proliferation, decreased transit time and increased gas production (Bosaeus, 2004).

The provision of fibre is an effective treatment for constipation (Passmore, Wilson-Davies, Stoker, & Scott, 1993). Fibre acts as a bulking agent. It increases stool bulk and stool frequency when taken with water (Suares & Ford, 2011). A randomised controlled trial compared the efficacy of two different types of fibre (prunes versus psyllium) towards constipation (Attaluri, Donahoe, Valestin, Brown, & Rao, 2011). Psyllium acts as a bulking agent that could result in increased bowel frequency. Meanwhile, dried plums (prunes) contain a combination of fibre, sorbitol and polyphenols. Although the mechanism remained unknown, it was suggested that, apart from the prune's fibre content, sorbitol may act as an osmotic laxative in alleviating constipation. The findings from that study concluded that both types of fibre are safe, well tolerated and efficient in treating constipation. However, prunes were found to be

more effective due to the significant improvement in the number of complete spontaneous bowel movements per week and stool consistency scores compared to the psyllium. Despite the different mechanisms of actions, various types of fibre have been found to help in resolving constipation. Similar positive effects can be seen in other sources of fibre (Cheskin, Kamal, Crowell, Schuster, & Whitehead, 1995; Hull, Greco, & Brooks, 1980; Loening-Baucke, Miele, & Staiano, 2004; Müller-Lissner, 1988).

Fibre may also alleviate the incidence of diarrhoea by several mechanisms. The ability of fibre, especially viscous fibre, to hold water improves the faecal consistency (Eherer, Santa Ana, Porter, & Fordtran, 1993). Moreover, the fermentation of fermentable fibre by gut microbiota yields SCFAs and induces the reabsorption of sodium and water, thus reducing faecal water content (Bowling et al., 1993; Zaharia et al., 2001).

2.3.2.1 Prebiotics

(a) ***Definition***

The definition of prebiotics has evolved over time due to the expansion of research in the area. Roberfroid (2007) updated the definition of prebiotics to "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health". There are a number of nutritional compounds that exert some degree of prebiotic activity (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). However, there are three distinct criteria which a compound needs to meet in order to be classified as a prebiotic; that is, a compound: 1) is resistant to gastric acidity and hydrolysis by mammalian enzymes and gastrointestinal absorption, 2) can be fermented by intestinal microbiota, and 3) can selectively stimulate the growth and/or activity of intestinal bacteria associated with health and well-being (Gibson et al., 2004). Many emerging

compounds meet some of these criteria; however, the only nutritional compounds that meet all the criteria are inulin-type fructans (i.e. FOS, oligofructose, and inulin), lactulose and galactooligosaccharides (Gibson et al., 2004; Tuohy, Rouzaud, Bruck, & Gibson, 2005).

(b) Chemical structure of inulin-type fructans

Inulin-type fructans are members of the fructans, which are polymers of fructose molecules. Fructans can be described based on the degree of polymerisation (DP). Inulin is a naturally-occurring polysaccharide produced by many types of plants. It is present in a range of natural foods such as the chicory root, onion, garlic Jerusalem artichoke, tomato and banana. The compound comprises β (2→1) fructose units which are linked by β(2→1) glycosidic bonds (refer to Figure 2.4). A standard inulin has a DP ranging from 2 to 60. There are two methods to obtain FOS. FOS are the lower molecular weight of inulin-type fructans which have a DP of up to nine. The first method is the hydrolysis of the high molecular weight compound, inulin, with inulase resulting in short chain inulin, called oligofructose. The second method is the enzymatic elongation of sucrose resulting in short chain FOS which has a DP of up to five (Roberfroid et al., 2010).

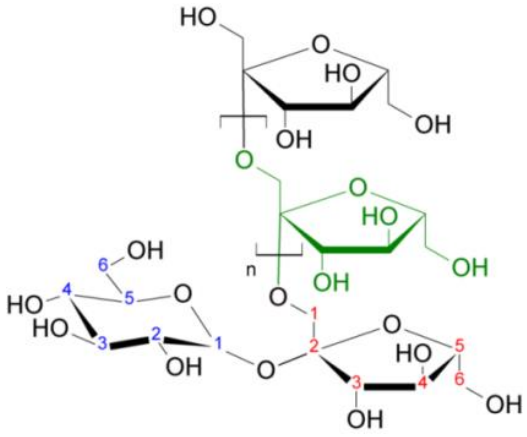

Figure 2.4: Chemical structure of inulin

(c) Safety of prebiotics

Prebiotics is a naturally occurring ingredient in plants. Although the prebiotics content in foods are low, studies conducted had concluded that prebiotics are safe to be consumed and does not exert any adverse effect even at high dosage (Lee & Salminen, 2009). Increased flatulence was observed in a study using 20 g prebiotics per day in healthy human study (Bouhnik et al., 1999).

(d) Health benefits of prebiotics

Prebiotics are fully metabolised by the gut microbiota as evidenced by the absence of traces of inulin and oligofructose in faeces (Alles et al., 1996). The fermentation of prebiotics promotes the growth of beneficial bacteria. Although the definition of prebiotics does not explicitly emphasise any particular group of bacteria, the candidate compound must be able to increase the number and/or activity of bifidobacteria or other lactic acid-producing bacteria for it to be considered a prebiotic. The bifidogenic properties of prebiotics have been confirmed in several human studies (Bouhnik et al., 2004; Hidaka, Eida, Takizawa, Tokunaga, & Tashiro, 1986; Tuohy, Kolida, Lustenberger, & Gibson, 2001). The earliest human study to establish the effects of prebiotics was conducted by Hidaka et al. (1986). The study was conducted on 23 senile adults aged 50 to 90 years old. The daily supplementation of 8 g of FOS increased the number of bifidobacteria by ten times the initial number. The number of lactobacilli increased but the increase was not statistically significant.

An intake as low as 5 g of FOS has been proven to increase the number of bifidobacteria in healthy human subjects (Rao, 2001). The ability of the prebiotics to stimulate the growth of bifidobacteria is dose-dependent. This was initially demonstrated by Bouhnik et al. (1999) whose study randomly assigned 40 healthy volunteers to receive either 0 g, 2.5 g, 5 g, 10 g or 20 g of FOS for 7 days. During that

period, they were allowed to eat a normal diet with the exception of fermented dairy products and products containing high levels of FOS. A significant increase of bifidobacteria was seen in subjects receiving 5 g of FOS or more. There was a significant correlation between the dosage of FOS given and the faecal bifidobacteria counts. The study also found that a high dosage of FOS (20 g per day) led to significantly more frequent and intense flatus. Thus, the study recommended the prebiotic dose of 10 g as the ideal dosage for the prebiotics to exert the bifidogenic effect without excess side effects such as bloating and/or excess flatus.

The selective stimulation of the growth of beneficial bacteria by prebiotics contributes directly and indirectly towards the hosts' well-being as previously discussed in relation to the function of gut microbiota. Figure 2.5 summarises the proposed mechanisms of prebiotic actions in improving human health especially in reducing diarrhoea. A number of reviews have extensively discussed the role of prebiotics in human health (Gibson et al., 2004; Kelly, 2008; Kolida & Gibson, 2007; Macfarlane, Macfarlane, & Cummings, 2006; Meyer & Stasse-Wolthuis, 2009; Roberfroid et al., 2010; Tuohy et al., 2005).

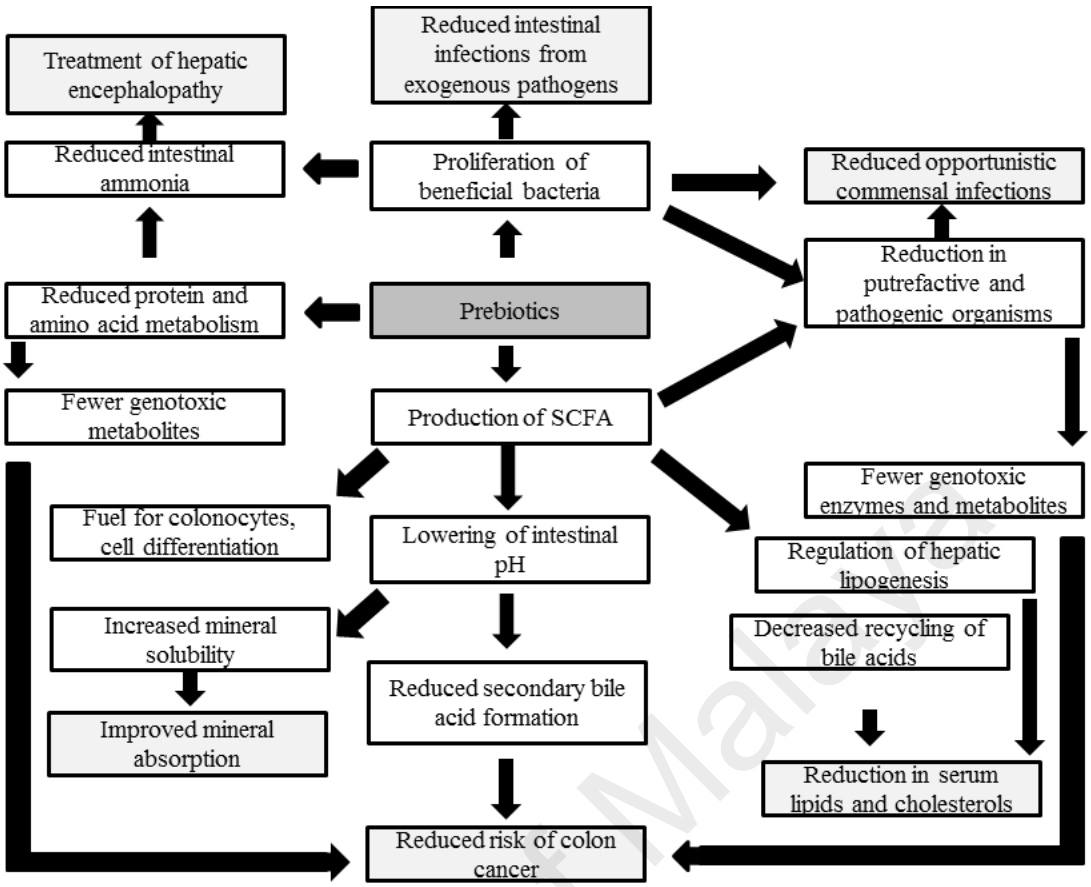

Figure 2.5: Proposed mechanisms of prebiotic actions to improve human health (Tannock, 1999)

2.4 Fibre and prebiotics in EN

Significant changes in diet especially during EN have been shown to cause dysbiosis of the gut microbiota (Schneider et al., 2000). Manipulation of the gut microbiota may therefore be one of the strategies to reduce the incidence of diarrhoea during EN. Inulin type fructans, inclusive of FOS, oligofructose and inulin, exert bifidogenic effects and shift gut microbiota to a "healthier" composition (Kolida & Gibson, 2007). Healthy volunteers have been found to benefit from the fortification of enteral formula with fibre and oligosaccharides as it increases the bifidobacteria concentration (Tuohy et al., 2001). However, studies conducted in patients receiving EN have shown conflicting results.

2.4.1 Fibre and prebiotics in EN in patients

To date, there have been three reviews investigating the effect of fibre during EN (del Olmo et al., 2004; Elia et al., 2008; Yang, Wu, Zhou, & Wang, 2005). These reviews gathered the literature on various types of fibres used in EN and the effects on both healthy individuals and patients. In the most recent review, it was concluded that the addition of fibre in EN reduces the development of diarrhoea in patients receiving EN (Elia et al., 2008). However, the previous reviews did not specifically focus on the role of prebiotics in relation to diarrhoea. Thus, the present study conducted a systematic review with the aim to evaluate recent evidence regarding the effect of dietary fibre and prebiotic supplementation in enteral formula on diarrhoea, faecal microbiota and SCFAs among adult patients requiring EN. The methodology of the systematic review is described extensively in the methodology chapter (Chapter 3).

The literature search identified 538 records. Forty-one studies were retrieved after excluding duplicates and titles that were not relevant to the research questions. A flow diagram describing the selection of studies is shown in Figure 2.6**.** Twenty-two experimental studies and four observational cohort studies that met the inclusion criteria were used for this review. Characteristics of these studies are presented in Table 2.2. Eight studies were conducted on critically ill patients, 16 in mixed wards inclusive of medical, surgical and geriatrics wards, two in outpatient clinics and two included studies did not explicitly mention the departments/units where the patients were hospitalised. Of the 26 studies included in this systematic review, three studies scored 5 on the Jadad Scale indicating high quality RCT, four studies with the score of 4, five studies with the score of 3, five studies with the score of 2, two studies with the score of 1 and seven studies with the score of 0 indicating poor study design of the study or non-experimental study by design. In addition, the SIGN methodological assessment classified eight studies as high quality and the remaining papers were deemed acceptable with the exception of two studies which were classified under unacceptable, which were therefore not used in this review.

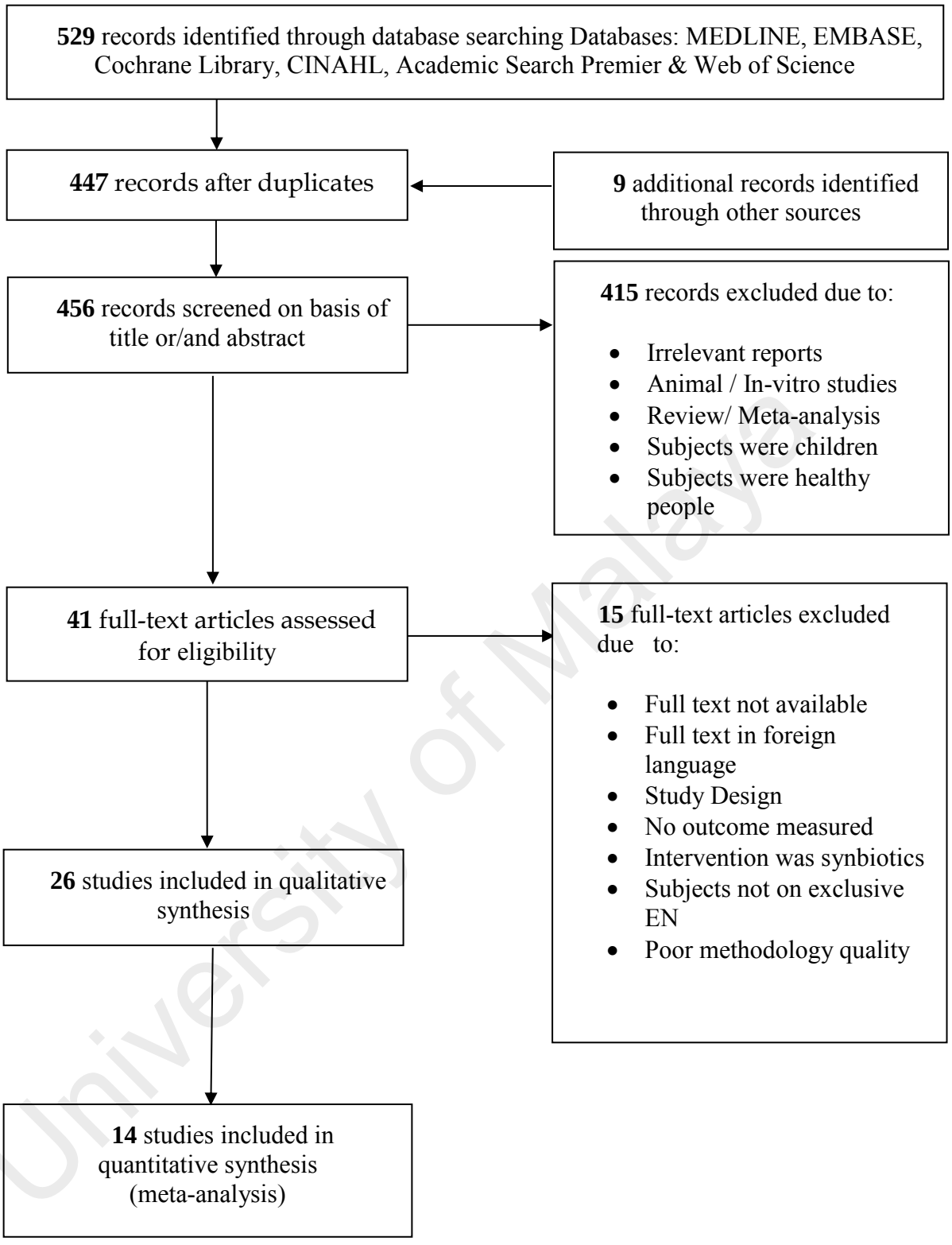

Figure 2.6: Flow diagram of included and excluded studies for the systematic review

Table 2.2: Characteristics of included studies (1990-2014)

Authors	Study design	Study population	Dose & Type of Fibre	Study duration	SIGN category	Jadad Score
Dobb & Towler, 1990	Double-blind, randomised controlled trial	N=91 adult patients in Intensive Care Unit, The Royal Perth Hospital, Australia	Soy polysaccharide,21 g/L	Max of 18 days/ discharge ICU	High Quality	4
Shankardass et al., 1990	Double-blind, cross-over, randomised controlled trial	N=28 long-term EN patients. Multicentre: Chedoke-McMaster Hospitals, Queen Elizabeth Hospitals, Riverdale Hospital, Sunnybrook Medical Centre, University of Toronto, Toronto, Ontario, Canada	Soy polysaccharide, 12.8g/1000kcal	12 weeks	Acceptable	3
Heather, Howell, Montana, Howell, & Hill, 1991	Randomised controlled trial	N=49 mixed wards patients Portland Veterans Affairs Medical Centre, Portland, USA	Psyllium, 15 g/day	6 days	Acceptable	2
Guenter et al., 1991	Non-randomised controlled trial	N=100 ICU patients, Graduate Hospital Pennsylvania, USA	Soy polysaccharide, 14.4g/L	Not mentioned	Acceptable	0
de Kruif & Vos, 1993	Randomised controlled trial	N=60 surgical patients University Hospital, Netherlands	Soy polysaccharide, 20 g/L	5 days	Acceptable	3

Table 2.1 , continued

Authors	Study design	Study population	Dose & Type of Fibre	Study duration	SIGN category	Jadad Score
Collier et al., 1994	Pre-post observational study	N=57 surgical patients, Regional Medical Centre, Memphis Tennessee, USA	Soy polysaccharide, 21 g/L	Not mentioned	NA	0
Homann, Kemen, Fuessenich, Senkal, & Zumtobel, 1994	Double-blind, randomised controlled trial	N=100 surgical & medical patients, Germany	Partially Hydrolysed Guar Gum, 20g/L	10 days	Acceptable	2
Zarling, Edison, Berger, Leya, & DeMeo, 1994	Cross-over, randomised controlled trial	N=10 recovering stroke patients Extended Care facilities, Hines VA Hospital, Illinois, USA	Oat & Soy fibre, 14.4g/L	23 days	Acceptable	2
Reese et al., 1996	Double-blind, randomised controlled trial	N=80 surgical patients (head and neck cancer), University of Iowa Hospital, USA	Soy polysaccharide, 7 or 14 g/L	Until patient change to oral/discharged	High quality	5
Belknap, Davidson, & Smith, 1997	Randomised controlled trial	N= 60 medical-surgical & ICU patients, Department of Veterans Affairs Medical Centre, Oklahoma, USA	Psyllium Hydrophilic Mucilloids (PHM), 14 g/day	7 days	Acceptable	3

Table 2.1, continued

Authors	Study design	Study population	Dose & Type of Fibre	Study duration	SIGN category	Jadad Score
Sobotka et al., 1997	Single-blind, pre-post single group trial	N=9 Charles University, Hradec Krdlove, Czech Republic	Inulin 15 g/L	2 weeks	Acceptable	0
Emery et al., 1997	Randomised controlled trial	N=31 ICU patients Pennsylvania Hospital, USA	Banana flakes, 1.5 g/day	7 days	Acceptable	0
Khalil, Ho, Png, & Ong, 1998	Single-blind, randomised controlled trial	N=16 surgical patients National University Hospital, Singapore	Oat & soy polysaccharides, 14.4 g/L	10 days	Acceptable	2
Cockram et al., 1998	Single-blind, randomised controlled trial	N=79 haemodialysis patients from three outpatients haemodialysis clinics, USA	FOS, 15.4 g/L	3 weeks	Acceptable	2
Schultz et al., 2000	Double blind, 2x2 factorial randomised controlled trial	N=44 critically ill patients Maine Medical Centre, Portland,USA	Mixed fibre[a] and pectin :up to 17g/day, inclusive of 10 g/L FOS	9 days	High Quality	4
Spapen et al., 2001	Double-blind, randomised controlled trial	N=25 critically ill patients Academic Hospital, Vrije, Brussels , Belgium	Partially Hydrolysed Guar Gum, 22 g/L	21 days /withdrawal of EN	High Quality	4

Table 2.1, continued

Authors	Study design	Study population	Dose & Type of Fibre	Study duration	SIGN category	Jadad Score
Nakao et al., 2002	Pre-post single group trial	N=20 geriatrics patients Nagoya University Hospital, Japan	Galactomannan , 7-28 g /day	6 weeks	Acceptable	0
Rushdi, Pichard, & Khater, 2004	Double-blind, randomised controlled trial	N=20 critically ill patients Teaching Hospital, Cairo University, Egypt	Guar gum, 22 g/L	4 days	High Quality	5
Vandewoude, Paridaens, Suy, Boone, & Strobbe, 2005	Randomised controlled trial	N=172 geriatrics patients Universitair Centrum Geriatrie, Belgium	Mixed fibre[b] , 30 g/day inclusive inulin	Not mentioned, measured weekly	Acceptable	1
Schneider et al., 2006	Double-blind, cross-over, randomised controlled trial	N=15 15 long term EN patients University Hospital, Nice, France	Mixed fibre[c], 15 g/L inclusive of 3.45 g/L of FOS	5 weeks	High quality	3
Shimoni et al., 2007	Non-randomised controlled trial	N=148 elderly patients in general internal medicine wards, Gastroenterology Laniado Hospital, Natanyia, Ramat Aviv , Israel	Soy polysaccharides, 13.6 g/1000kcal	5 days	Acceptable	1

Table 2.1, continued

Authors	Study design	Study population	Dose & Type of Fibre	Study duration	SIGN category	Jadad Score
Wierdsma et al., 2009	Double-blind, randomised controlled trial	N=19 patients from Outpatients Clinic of the VU University Medical Centre, Amsterdam, Netherlands	Mixed fibre[a], 17.6 g/L inclusive of 7g of FOS	8weeks	Acceptable	3
Chittawatanarat et al., 2010	Double-blind, randomised, controlled trial	N=34 septic patients in ICU Maharaj Nakorn Chiang Mai Hospital, Thailand	Mixed fibre[d], 15.1 g/L inclusive of 5.3 g of FOS	14 days, at least 5 days	High quality	4
Kato, Nakao, Iwasa, Hasegawa, & Yamada, 2012	Pre-post single group trial	N=15 patients from medical wards of Kameyama Kaisei Hospital, Japan	Psyllium, 5.2 g/day	4 weeks	Acceptable	0
Bittencourt et al., 2012	Sequential and observational study	N=110 adult patients, São Joaquim Hospital of Beneficência Portuguesa, Brazil	Soluble & insoluble fibre, 15g/L	At least 5 days	NA	0
Majid et al., 2014	Double-blind, randomised controlled trial	N=22 critically ill patients, Guy's and St Thomas' NHS Foundation Trust and King's College Hospital NHS Foundation Trust, London, UK	Mixed fibre[c], 15g/L and additional 7g/day oligofructose/inulin	7 - 14days	High quality	5

a Oat, soy polysaccharide, gum arabic and cellulose and FOS
b Cellulose, hemicellulose A, pectin, hemicellulose B, inulin
c Soy polysaccharide, alpha-cellulose, arabic gum, inulin, oligofructose and resistant starch.
d Cellulose, lignin, hemicellulose, pectin and FOS

Most studies that investigated fibre supplementation in EN used soy polysaccharide (n=7), followed by mixed fibre (n=6), partially hydrolysed guar gum (n=3), psyllium (n=3), oat and soy fibre (n=2), FOS (n=1), inulin (n=1), banana flakes (n=1), galactomannan (n=1) and one study did not mention the type of fibre used for the conducted study. Fibre was administered as an integrated component of the enteral formula in 14 studies and added as supplementation in ten studies. Two studies used fibre containing enteral formula with additional fibre supplementation as part of the intervention. Diarrhoea incidence among adult patients receiving EN in this study ranged from 10.5% to 90% (Collier et al., 1994; Majid et al., 2014). Evidently, there was a variation in defining diarrhoea among studies, taking into account partly or all of the stool properties; volume, consistency and frequency. Diarrhoea definitions were based on diarrhoea score, number of liquid stools per day and/or volume, number of loose or watery stools, scale based on consistency and frequency and use of stool chart in interpreting diarrhoea, i.e, Bristol and King's stool chart.

Fourteen experimental studies with data on diarrhoea incidence during EN (Intervention: Fibre containing EN and Comparator: Fibre-free EN) were included for meta-analysis. As shown in Figure 2.7, meta-analytic pooling of the studies under a random effect model confirmed the protective effect of fibre in reducing incidence of diarrhoea among adult patients requiring EN (OR 0.47; 95% CI 0.29-0.77; p = 0.002). Subgroup analysis was conducted due to statistically significant heterogeneity of the data, I^2 = 54%. The analysis revealed homogeneity among studies conducted in non-critically ill patients, I^2 = 28% but studies conducted among critically ill patients were heterogeneous I^2 = 52%. Additionally, positive effect of fibre supplementation during EN on reducing the incidence of diarrhoea was not seen in the critically ill patients (OR 0.89; 95% CI 0.41-1.92; p = 0.77) but clearly observed in the non-critically ill patients (OR 0.31; 95% CI 0.19-0.51; p < 0.001).

Asymmetry presentation of the funnel plot in Figure 2.8 revealed that inter-study heterogeneity existed and this may be an indication of potential publication bias. Of the 26 studies investigating the effect of fibre in EN on the three main outcomes, only eight studies had prebiotics in the intervention (Chittawatanarat et al., 2010; Cockram et al., 1998; Majid et al., 2014; Schneider et al., 2006; Schultz et al., 2000; Sobotka et al., 1997; Vandewoude et al., 2005; Wierdsma et al., 2009). An additional meta-analysis failed to indicate any protective effect of prebiotics supplementation in EN against the incidence of diarrhoea (OR 1.20; 95% CI 0.28- 5.14; p = 0.81) (Figure 2.9).

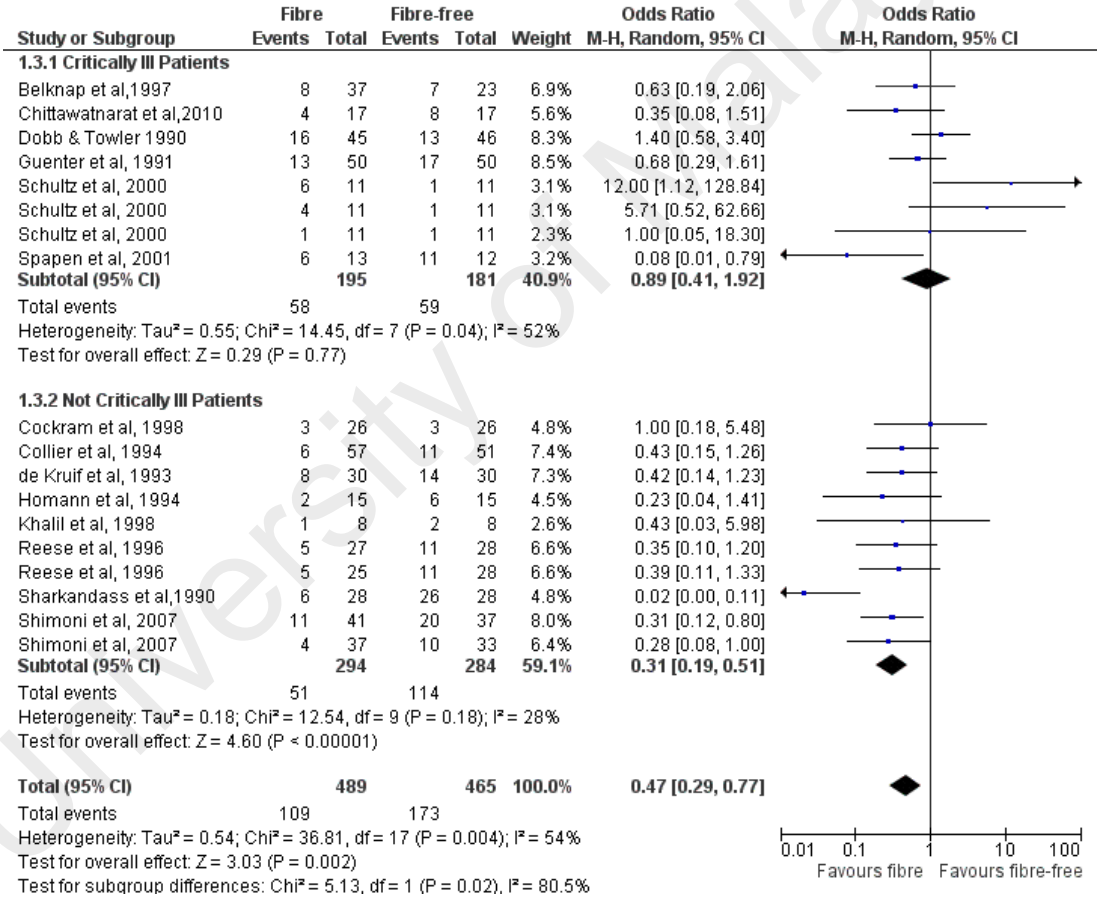

Figure 2.7: Meta-analysis of the effect of fibre supplementation in EN towards incidence of diarrhoea

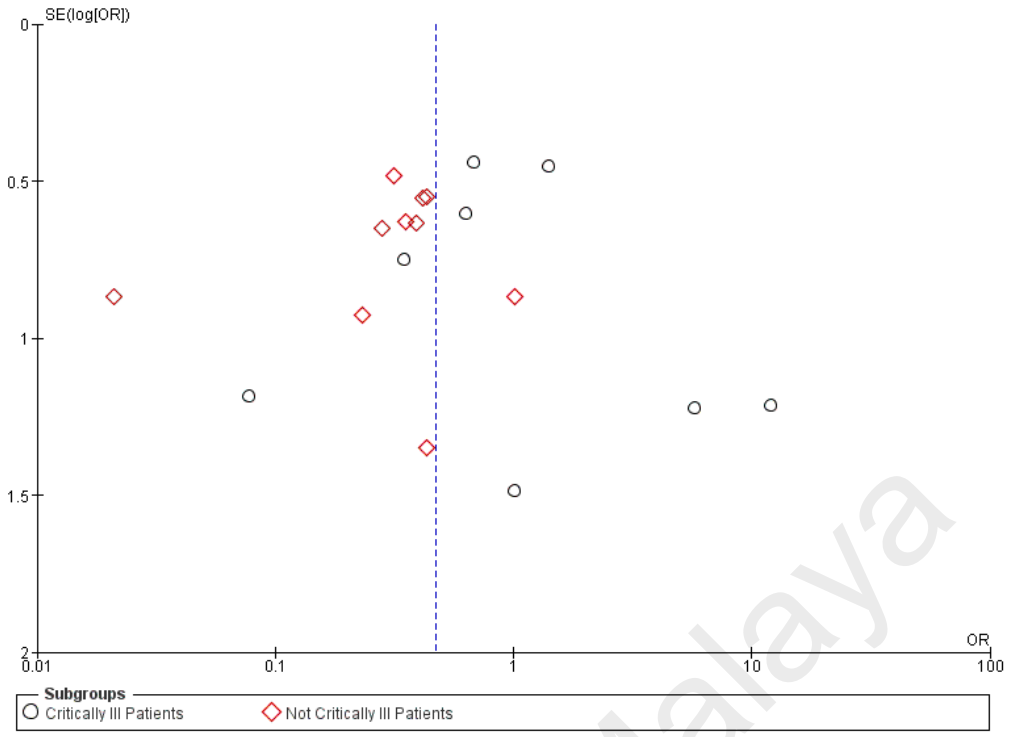

Figure 2.8: Figure 7: Funnel plot for 14 included studies of the effect of fibre supplementation in EN towards incidence of diarrhoea

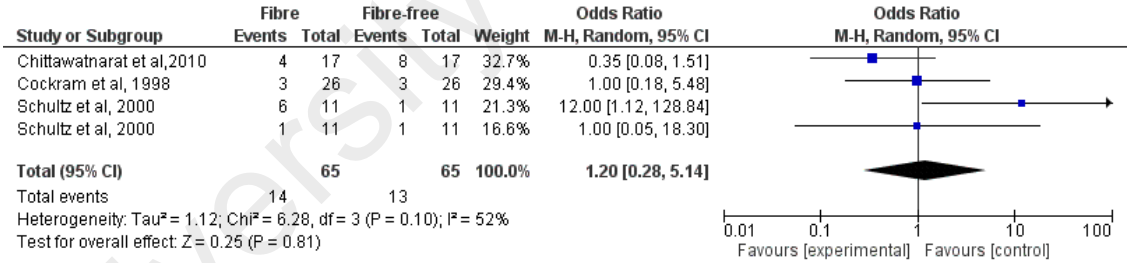

Figure 2.9: Meta-analysis of the effect of prebiotics supplementation in EN towards incidence of diarrhoea

The effect of fibre supplementation in EN towards faecal microbiota was only investigated in four studies (Majid et al., 2014; Nakao et al., 2002; Schneider et al., 2006; Wierdsma et al., 2009) as presented in Table 2.3. Only one study had shown a significant increase in total bacteria when patients were given fibre (mixed fibre with prebiotics) supplemented EN (Schneider et al., 2006). Likewise, the same study found no changes in regard to the composition of the dominant bacteria group (gram positive, gram negative, aerobic and anaerobic). On the contrary, the study by Nakao reported a significant decrease in aerobic bacteria with galactomannan supplementation (Nakao et al., 2002). There were no reports of significant changes in faecal bifidobacteria concentrations found in patients receiving fibre supplemented EN (Majid et al., 2014; Schneider et al., 2006; Wierdsma et al., 2009).

Five studies investigated the effect of fibre supplementation in EN towards SCFA (Kato et al., 2012; Majid et al., 2014; Nakao et al., 2002; Schneider et al., 2006; Sobotka et al., 1997) as summarised in Table 2.4. Fibre supplementation increased total SCFA in two studies (Kato et al., 2012; Schneider et al., 2006) whereas three studies found no changes in the SCFA concentration (Majid et al., 2014; Nakao et al., 2002; Sobotka et al., 1997). In addition, prebiotic supplementation in EN did not increase the concentrations of SCFAs (Majid et al., 2014; Sobotka et al., 1997) with the exception of one positive result (Schneider et al., 2006).

Table 2.3: Studies investigating the effect of fibre supplementation in EN towards faecal microbiota

Study	Total microbiota count	Dominant group	Bifidobacteria	Others
Nakao et al., 2002	No change	↓S of aerobic bacteria	Not measured	Not measured
Schneider et al., 2006	↑S	No change in composition of aerobic and anaerobic, gram positive and gram negative bacteria	No change	↑S in the numbers of enterococci at the end of the fibre-free EN ↑S in the numbers of bacteroides at the end of the mixed fibre EN
Wierdsma et al., 2009	Not measured	Not measured	↓S in patients compared to healthy control Concentration remained stable in the FOS group but ↓S in the non-FOS group during intervention	Not measured
Majid et al., 2014	No change	No change	No change	↓S *Faecalibacterium prausnitzii* and *Bacteroides-Prevotella* in the prebiotics group

↑S : Significantly increased
↓S : Significantly decreased

Table 2.4: Studies investigating the effect of fibre supplementation in EN towards SCFAs

Study	Total SCFA	Acetate	Propionate	Butyrate
Sobotka et al., 1997	No change	No change	No change	No change
Nakao et al., 2002	No change	↑S	↑S	No change
Schneider et al., 2006	↑S	↑S	No change	↑S
Kato et al., 2012	↑S	↑S	No significant amount detected	No significant amount detected
Majid et al., 2014	No change	No change	No change	No change

↑S : Significantly increased
↓S : Significantly decreased

The result of this updated meta-analysis of prospective studies confirms previous evidence showing that fibre supplementation decreases diarrhoea incidence for adult patients requiring EN(Elia et al., 2008). The dosage of fibre used in the included studies ranged from 5.2 g/day to 39 g/day (Kato et al., 2012; Rushdi et al., 2004). In this review, soy polysaccharide emerged as the most extensively studied fibre in EN for patients. Likewise, it is also the most common fibre added in the enteral formula. The mechanism of actions for minimizing diarrhoea incidence include the ability of fibre to hold water (Russell & Bass, 1985), increase bulk (Chen et al., 1998) and improve gut barrier function (Slavin, 2013). However, this effect varies based on the type of patients studied (Elia et al., 2008). Subgroup analysis conducted in this current review shows that the incidence of diarrhoea was only reduced in non-critically ill patients, consistent with previous reviews (Elia et al., 2008; Yang et al., 2005). It is possible that the severity of illness and the antibiotics therapy undertaken by the critically ill patients

counter the beneficial effect if fibre supplementation. The use of antibiotics or antifungal drugs is an independent factor that contributes to higher prevalence of diarrhoea in critically ill patients (Thibault et al., 2013). Moreover, critically ill patients often suffer from gastrointestinal dysfunction with abnormal motility patterns and impaired barrier integrity (Hill, 2013). Although the potential benefits are not clearly observed in the critically ill patients, the main finding of this review has significant implications for health care professionals and advocates the use of fibre-containing over fibre-free enteral formula to other groups of patients.

Prebiotics component of fibre meets three distinct criteria: 1) are resistant to gastric acidity and hydrolysis by mammalian enzymes and gastrointestinal absorption, 2) can by fermented by intestinal microbiota and 3) selectively stimulate the growth and/or activity of intestinal bacteria associated with health and well-being (Gibson et al., 2004). Our results show that prebiotic supplementation in enteral formulas did not minimise the incidence of diarrhoea in adult patients receiving EN. The prebiotic dosage reported by studies included in this meta-analysis ranged from 5.3 to 15.4 g/L of FOS (Chittawatanarat et al., 2010; Cockram et al., 1998). Two of the three studies included in the meta-analysis were conducted in an intensive care unit setting, which may explain the lack of significant benefit from prebiotic supplementation in EN for diarrhoea.

Ingested fibre influences the intestinal microbiota by providing the required substrate for colonic fermentation, and consequently assists in the microbiota proliferation. Over the years, reports revealed that the introduction of prebiotics in healthy humans increases the concentrations of bifidobacteria when EN is given as the sole source of nutrition (Whelan et al., 2005). However, the effect of fibre and prebiotics supplementation on faecal microbiota in adult patients receiving EN could

not be concluded due to the conflicting findings (Majid et al., 2014; Nakao et al., 2002; Schneider et al., 2006; Wierdsma et al., 2009). These inconsistent results observed from previous studies conducted might be due to the heterogeneity of study populations' i.e. stable and critically ill patients. Additionally, concurrent use of antibiotics with EN might alter the colonic microbiota composition. Most antibiotics alter the bacterial composition as reflected by the suppression of anaerobic bacteria of gut microbiota and an increased incidence of *Clostridium difficile* associated diseases (Rafii et al., 2008; Surawicz et al., 1989). However, by controlling the confounding factor, antibiotics therapy remains difficult as it is part of a medical treatment received by patients who are critically ill. The ranges of prebiotics dosages 5.20 g/day to 13.75 g/day may also have contributed to the lacking of a bifidogenic effect. Healthy people require 10g of prebiotics to significantly increase faecal bifidobacteria concentration (Bouhnik et al., 1999), thus patients might require a higher dosage to exert such an effect. Most studies that investigated prebiotic were conducted in patients receiving EN supplemented with a mixture of various types of fibre (inclusive of prebiotics) instead of a single source of fibre; only two studies used FOS as its sole source of fibre in the intervention (Cockram et al., 1998; Sobotka et al., 1997). Due to the limited numbers of RCTs, a meta-analysis investigating the role of fibre, specifically prebiotics on faecal bifidobacteria, other microbiota and SCFAs could not be conducted.

Fermentation of fibre yields SCFAs which are source of nutrients for colonic mucosal cells. Therefore, the luminal acidity produced by the increased concentrations of SCFAs helps maintain an environment with a low pH for the colonic microbiota, subsequently preventing an enteropathogenic infection (Green, 2001). While provision of fermentable fibre increases SCFAs in a healthy humans (Tarini & Wolever, 2010), mixed results emerged in this review for studies investigating the effect of fibre supplementation in EN. Despite two studies displaying no changes in SCFA

concentrations, a significant increase in SCFA concentrations was observed in studies conducted on stable patients; geriatrics, long-term EN and medical patients (Kato et al., 2012; Nakao et al., 2002; Schneider et al., 2006). Similarly, supplementation of fibre in EN given to the critically ill patients did not cause an increase in the SCFA concentration (Majid et al., 2014). According to a recent study, critically ill patients suffered from a low SCFA concentration as compared to healthy individuals possibly due to the reduction in total obligate anaerobes throughout the intensive care unit admission (Yamada et al., 2014). Moreover, fibre fermentation varies depending on the source of fibres used in the studies (Titgemeyer, Bourquin, Fahey, & Garleb, 1991); different fibre yields different concentration of total SCFA.

The main limitation of this review is the heterogeneity of the patients such as the inclusion of patients with varying severity of illness, mainly the critically ill and the non-critically ill patients and the use of antibiotics which might confound the results. However, these factors are inevitable when conducting research in patients especially in critically ill. Secondly, this review also lacks in uniformity with regard to the definition of diarrhoea (Lebak et al., 2003; Majid et al., 2012), for which objective and subjective considerations might influence the results of the studies in terms of the incidence of diarrhoea. As such, the use of a validated tool in defining diarrhoea should be considered in future research. Some studies were also excluded as the definition of diarrhoea was not mentioned explicitly despite indicating a measurable outcome. Lastly, this review only incorporates publications written in English and therefore may introduce language bias to the review.

In conclusion, this systematic review demonstrates that fibre assists in minimizing diarrhoea in adult patients receiving EN particularly those who are not critically ill. However, prebiotics (part of the fibre component) may not provide the same impact as fibre based on current evidence. Such result is contributed by the small number of research conducted studying the effect of prebiotics towards diarrhoea and the gut microbiota. To the best of the investigator's knowledge, there was only one RCT that used prebiotics as sole source of fibre in the study while other studies used multi-fibre mix (Cockram et al., 1998). However, this study was conducted in outpatient setting. Realizing this gap, this project was conducted considering the limited number of studies focusing on this in critical care setting. It is one of the aims of this Master's project to investigate the efficacy of provision of prebiotics (as sole source of fibre) in reducing the incidence of diarrhoea and increasing faecal bifidobacteria concentrations in the critically ill patients by conducting an RCT which will be discussed in the following chapters.

CHAPTER 3: METHODOLOGY

This chapter is divided into two parts; the first part of this chapter will elaborate on the methodology of the systematic review conducted (Secondary objective 1). The second part of the chapter will describe in detail the methodology of the randomised controlled trial conducted with the objective to determine concentrations of faecal microbiota among critically ill patients who receive enteral formula with and without fibre/prebiotics during EN (Primary objective and secondary objective 2 and 3).

3.1 Systematic review

3.1.1 Objective

To evaluate recent evidences regarding the effect of dietary fibre and prebiotics supplementation in enteral formulae among adult patients requiring EN on diarrhoea, faecal microbiota and SCFAs

3.1.2 Literature search

Potential publications describing the effect of EN supplemented with fibre on diarrhoea, faecal microbiota and SCFAs were systematically identified, mainly by searching electronic databases. Studies within the period of January 1990 to May 2014 from the MEDLINE, EMBASE, The Cochrane Library, CINAHL, Academic Search Premier and Web of Science were accessed. Keywords or search terms that focused on enteral nutrition and fibre in combination were used. Additionally, all related keywords, MeSH terms, text words and search terms were identified and used for the literature search. Search terms related to EN that were used in this review includes: artificial nutrition/feeding, nutritional support, enteral alimentation/formula, tube feeding, chemically defined diets, sips feeds, oral nutritional supplements, nutrition therapy and dietary supplements. Next, fibre was searched in both spellings and specific types of fibres were also searched individually: roughage, wheat brans, oligosaccharides,

oligofructose, inulin, fructo-oligosaccharides, non-starch polysaccharides, soy polysaccharides, lignin, resistant starch, pectin, arabic gum, pectin, guar gum acacia gum, cellulose, pea fibre, oat, inulin-type fructans and prebiotics. Lastly, hand searching of local journals, follow up reference lists of key papers and relevant reviews were also conducted to locate additional publications that were not accessible through electronic databases.

3.1.3 Study selection

Inclusion criteria for this review were 1) primary research of randomised controlled trial, non-RCT studies and observational cohort study designs, 2) studies conducted on adult patients receiving enteral nutrition of any health or nutritional status, 3) studies assessing fibre effect in EN on diarrhoea and/or faecal microbiota and /or SCFA, 4) studies conducted from January 1990 to May 2014. In contrast, exclusion criteria included studies which 1) did not use enteral formula as the sole or main source of nutrients either orally or through tube, 2) involved supplementation of synbiotics (prebiotics and probiotics) in the enteral formula, 3) considered animal, in-vitro studies and publications, case control and cross-sectional studies, review articles and dissertations. This review also was limited to published and available full articles in the English language.

3.1.4 Data extraction & outcome measures

Following initial search, reference list was imported to reference manager software, EndNote version 7.1, Thomson Reuters, Philadelphia, USA. Two reviewers independently assessed potentially relevant articles for eligibility after eliminating duplications. Selection of articles underwent three stages; selection based on titles, followed by abstract consideration and finally by assessing the full text. Disagreements were resolved through discussion prior to consensus made.

Outcomes of interest were diarrhoea incidences, faecal microbiota and SCFAs concentrations of patients receiving EN. Data were extracted by two reviewers for each finalised study that were included for this systematic review. The data extraction forms were used to gather information on population and its' setting (i.e. population description, location, inclusion and exclusion criteria, method of recruitment and consent), methodology of the study (e.g. aim, design, study duration and ethical approval), risk of bias assessment, participants (i.e. number of randomised, withdrawals, exclusions, characteristics of the study participants), interventions (i.e. timing and delivery of EN, enteral formula used, fibre dosage and type) and outcomes (i.e. diarrhoea incidence, faecal microbiota and SCFA changes).

3.1.5 Quality assessment

The assessment of methodological quality of included studies was undertaken independently by two reviewers and disagreements were discussed among the reviewers. Tools used for study quality includes the Jadad Scale for Reporting Randomised Controlled Trials and Scottish Intercollegiate Guidelines Network (SIGN) Critical Appraisal of The Medical Literature. The Jadad Scale considers criteria relating randomisation, blinding, withdrawals and dropouts (Jadad et al., 1996). Scores ranging from 0-5 were given based on fulfilment of criteria addressed with higher scores representing studies of better quality (Appendix A). The second tool, the SIGN Critical Appraisal of The Medical Literature was another method of assessing methodology of a study which implements Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach within its guideline development (SIGN, 2014). The quality assessment of controlled trials in SIGN incorporated ten items: focused research question, randomisation, adequate concealment, blinding of subjects and investigators, similar group characteristics', methodology of measuring relevant outcomes, study drop out, intention to treat analysis and comparable results for multicentre research

(Appendix B). The study was assigned to either three groups: high quality, acceptable or unacceptable based on the methodological quality to minimise bias.

3.1.6 Statistical analyses

Binary outcomes were combined using the Mantel-Haenzel with results presented as odds ratio (OR) using a 95% confidence interval (CI). The value of OR>1 indicates that fibre supplementation in enteral formula is associated with higher odds of outcome i.e. diarrhoea. Next, statistical heterogeneity was evaluated using I^2 statistic, an estimation of variation in the effect of treatment beyond chance. An I^2 value of more than 50% might be regarded as substantial heterogeneity. In addition, the fixed effect model was used in the absence of heterogeneity and the random effect model with subgroup analysis was used vice versa. A visual appraisal of Funnel plot was used to indicate possibility of publication bias in the study.

3.2 Randomised controlled trial

3.2.1 Aim

The aims of this study were to evaluate faecal microbiota concentrations and incidence of diarrhoea in critically ill patients who receive either enteral formula with or without fibre/prebiotics during EN.

3.2.2 Objectives

3.2.2.1 Primary objective

To determine concentrations of faecal bifidobacteria in critically ill patients who receive either enteral formula with or without fibre/prebiotics during EN.

3.2.2.2 Secondary objectives

1. To determine the concentrations of faecal microbiota (total bacteria, lactobacilli, bacteroides, clostridia and *Faecalibacterium praunitzii*) in critically ill patients who receive either enteral formula with or without fibre/prebiotics during EN.
2. To determine the incidence of diarrhoea in critically ill patients who receive either enteral formula with or without fibre/prebiotics during EN by using validated King's stool chart.

3.2.3 Hypothesis

3.2.3.1 Null hypothesis

There is no difference in the concentrations of faecal bifidobacteria in critically ill patients who receive either enteral formula with or without fibre/prebiotics during EN

3.2.3.2 Alternative hypothesis

There is a difference in the concentrations of faecal bifidobacteria in critically ill patients who receive either enteral formula with or without fibre/prebiotics during EN.

3.2.4 Study design

Randomised Controlled Trial (RCT) was conducted on the critically ill, adult patients receiving EN as clinically indicated with the aim to compare concentrations of faecal microbiota of the critically ill patients who receive either enteral formula with or without fibre/prebiotics during EN.

3.2.5 Subject selection

Patients were recruited from the Intensive Care Unit (ICU) of a teaching hospital in Kuala Lumpur, University of Malaya Medical Centre (UMMC). Critically ill patient is defined as physiologically unstable patients requiring medical intervention to prevent death or significant morbidity (Frost & Wise, 2007; Robertson & Al-Haddad, 2013). The general ICU of UMMC (mixed case of surgical and medical cases) was selected as the setting of the study as it is the largest ICU in UMMC, comprising of 26 beds for critically ill patients. In UMMC, critically ill patients, mostly in need for mechanical ventilator supports are centralised at the general ICU. Enteral nutrition is usually commenced in the ICU. Thus the highest chance of recruiting critically ill patients who are starting EN is best in the general ICU of UMMC. Stable patients will be transferred out to Neuro ICU, Coronary Care Unit, or other wards depending on the primary medical intervention needed.

The sampling strategy employed by this RCT was consecutive sampling. All admissions to the ICU were screened for eligibility. Eligible critically ill patients who met the inclusion and exclusion criteria of the study were recruited into the study.

3.2.5.1 Inclusion criteria

The lists below are patients that were included in the study;

1. Patients receiving exclusive EN, nasogastric, continuous infusion.
2. Adult patients, 18 years old and above.
3. Critically ill patients.
4. Expected to require EN more than five days (Bleichner, Blehaut, Mentec, & Moyse, 1997).

3.2.5.2 Exclusion criteria

The lists below are patients that were excluded from the study;

1. Patients with gastrointestinal disease (Sokol et al., 2006) or GIT surgery (Scheppach, Sachs, Bartram, & Kasper, 1989) or GIT radiation therapy (Kapkac et al., 2003) as the condition alters gut microbiota of SCFA.
2. Patients receiving lactulose as it is a prebiotics (Bouhnik et al., 2004).
3. Currently under chemotherapy treatment (Ratnaike & Jones, 1998).
4. Patients who had received prebiotics and probiotics supplements within the previous one month.
5. Patients who have diarrhoea, 3 consecutive days (Majid et al., 2014).

3.2.6 Sample size calculation and statistical power

The sample size was calculated to compare the increase between pre-intervention and post intervention of 1.0 $\log_{10}$ bifidobacteria cells/g faeces in patients receiving EN in the group receiving EN supplemented and fibre/prebiotics with the control group. The formula was calculated using the formula for statistical superiority (Zhong, 2009):

$$N = 2 \times \left(\frac{z_{1-\alpha/2} + z_{1-\beta}}{\delta}\right)^2 \times s^2$$

where;

N= No of sample per group

δ= Clinically meaningful difference

s = Standard deviation of both comparison groups

$z_{1-\alpha/2}$ = Corresponds to two-tailed significance level (1.96 for α=0.05)

$z_{1-\beta}$ = Corresponds to power (1.28 for 90% power)

The value 1.0 for δ was used as difference in concentration of bifidobacteria in the faeces of 1.0 $\log_{10}$ /g weight would be considered clinically significant. Meanwhile, from value of 1.0 for s was taken from previous study conducted (H. A. Majid, Emery, & Whelan, 2011).

Thus,

$$N = 2 \times \left(\frac{1.96 + 1.28}{1.0}\right)^2 \times 1.0^2$$

$$= 21$$

Sample size calculation yielded 21 subjects needed for each group. Thus, subjects required for both arms were 42. With the consideration of 37% lost to follow up from a previous study (Majid et al., 2014), a total of 58 patients was needed to be enrolled in the study to detect a significant difference in bifidobacteria concentrations.

3.2.7 Ethical considerations and clinical trial registration

Written approval of the study was obtained from University of Malaya Medical Centre (UMMC) Ethics Committee prior to the commencement of the study. The reference number of the RCT is 989.31. Ethical approval letter received is attached in Appendix C. In addition, the Ethics Committees were informed of any protocol changes made whereby application for protocol amendment was submitted to the Ethics Committees for approval prior to its implementation to the study.

The protocol of the RCT was registered online through Clinical Investigation Centre, UM at the ClinicalTrials.gov with identifier number of NCT02144168 (refer to Appendix D).

3.2.8 Recruitment and consent of participants

Only consented patients were enrolled into the study. Consents for patients who met inclusion and exclusion criteria of the study were obtained from patients or their legal representatives in view that most critically ill patients were sedated and unconscious. Patients or their legal representatives were briefed regarding the objectives and the design of the study. Consent form (refer to Appendix E) was filled by the patients or their legal representatives and a copy of Patient Information Sheet (PIS) containing important information regarding the study was given to them (refer to Appendix F).

3.2.9 Randomisation

A randomisation list was generated using the website Randomization.com (http://www.randomization.com). The randomisation list is attached in Appendix G. Referring to the randomisation list, patients were randomly assigned to receive either enteral formula with or without fibre/prebiotics once a baseline faecal sample was

collected from the recruited patients after EN were initiated. The ICU consultants and dietitian were informed regarding the randomisation of the recruited patients.

3.2.10 Enteral formulas

Critically ill patients recruited for this study were exclusively on EN as clinically indicated. The volume of enteral formula prescribed was based on each patient's total energy requirement, which was calculated by the attending dietitian (not the principal investigator). Estimation of energy and nutrient requirements was based on the Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill (McClave et al., 2009). EN prescribed by the attending dietitian met the vitamin and minerals requirement as proposed in Recommended Nutrient Intakes (RNI) of Malaysia (National Coordinating Committee on Food and Nutrition 2005). Enteral formula was prepared and delivered through a Ryles tube by the ICU staff nurses according to dietitian's order and the UMMC's Enteral Feeding Protocol (Appendix H). Upon randomisation, patients were given either Osmolite 1 Cal for patients in the control arm and Ensure FOS for patients in the intervention arm. These products are used UMMC as standard care. The nutritional content of both products is listed in Table 3.1.

Table 3.1: Macronutrients of the standard and fibre/prebiotics enriched enteral formulas

Nutrients/ Enteral formula	Osmolite 1 Cal	Ensure FOS
Energy (kcal/L)	1060	1000
Energy (kcal/ml)	1.06	1.0
Protein (g/L)	44.3	37.2
Fat (g/L)	34.7	32.7
Carbohydrate (g/L)	143.9	134.2
Fibre (g/L)	0	10.0
Fructooligosaccharides/Inulin(g/L)	0	10.0
Osmolality (mOsm/kg H2O)	300	460

3.2.11 Faecal samples and data collection

Three faecal samples were required for analysis from each patient. The samples were collected at three different time point: at baseline, post one week and two weeks. Faecal samples from the recruited patients were collected for analysis at baseline, which was the first stool output after initiation of EN. Once baseline sample (Day 0) was collected, patients were randomly assigned to receive fibre-free or fibre/prebiotics supplemented enteral formulas. In view of the absence of habitual or meal cues to stool output, samples were collected from patients during 3 days periods at Day 6–8 for second faecal sample and Day 12–14 for the third faecal sample. The timeline of faecal sample collection is presented in Figure 3.1.

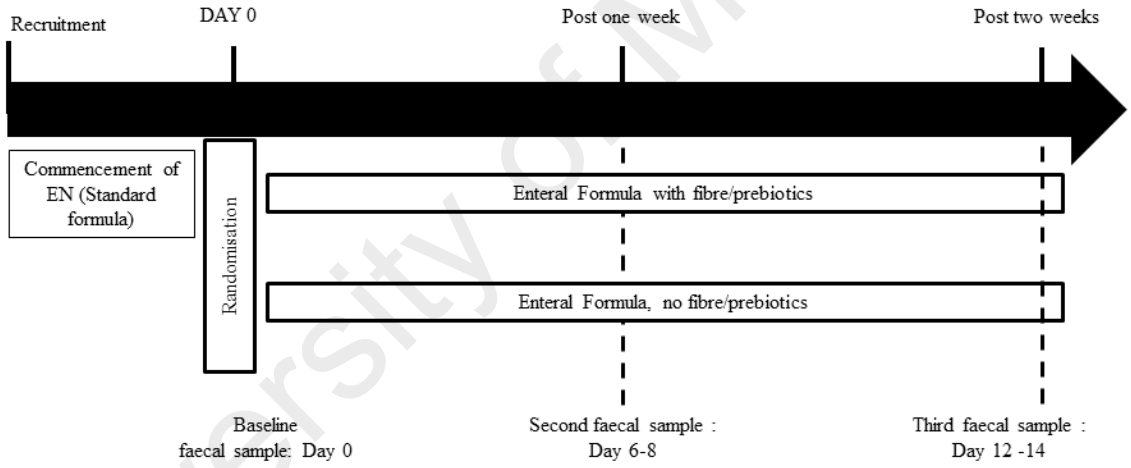

Figure 3.1: Timeline of faecal sample collection

Faecal output was recorded using King's Stool Chart, a stool chart validated for the use in patients requiring EN (Refer to Appendix I). The chart aids in the characterization of faecal frequency, consistency and weight, which were then summarised into a daily stool score. A score of 15 or more was used to indicate diarrhoea (Whelan et al., 2008). Staff nurses were required to make observation and record the stool output based on the code in the chart. Each code has its own unique score and the daily faecal score was obtained by summing the faecal score for the day.

Table 3.2: Faecal score based on the characteristics of the faeces, adopted from (Whelan et al., 2008)

	Faecal weight		
Faecal consistency	<100 g	100-200 g	>200 g
Hard and formed	1	2	3
Soft and formed	2	3	4
Loose and unformed	4	6	8
Liquid	8	10	12

Data collection progressed for 14 days from passage of the first faecal sample after EN initiation or until the patient was transferred out from the ICU. Patients were visited daily during weekdays and contacted by telephone during weekends for data collection. Demographic data such as age, sex, race, biochemical data such as total protein, albumin, white blood cell, random blood sugar, C-reactive protein and Clinical data such as Simplified Acute Physiology Score II (SAPS II), Sequential Organ Failure Assessment (SOFA) score, diagnosis and antibiotics received were gathered at baseline. Details of EN regimen; the formula used, amount, frequency, route and mode of delivery were recorded progressively and clinical details such as antibiotic, medication prescription and patients' progression were extracted from the patients' medical notes daily. The flow of the study is presented in Figure 3.2.

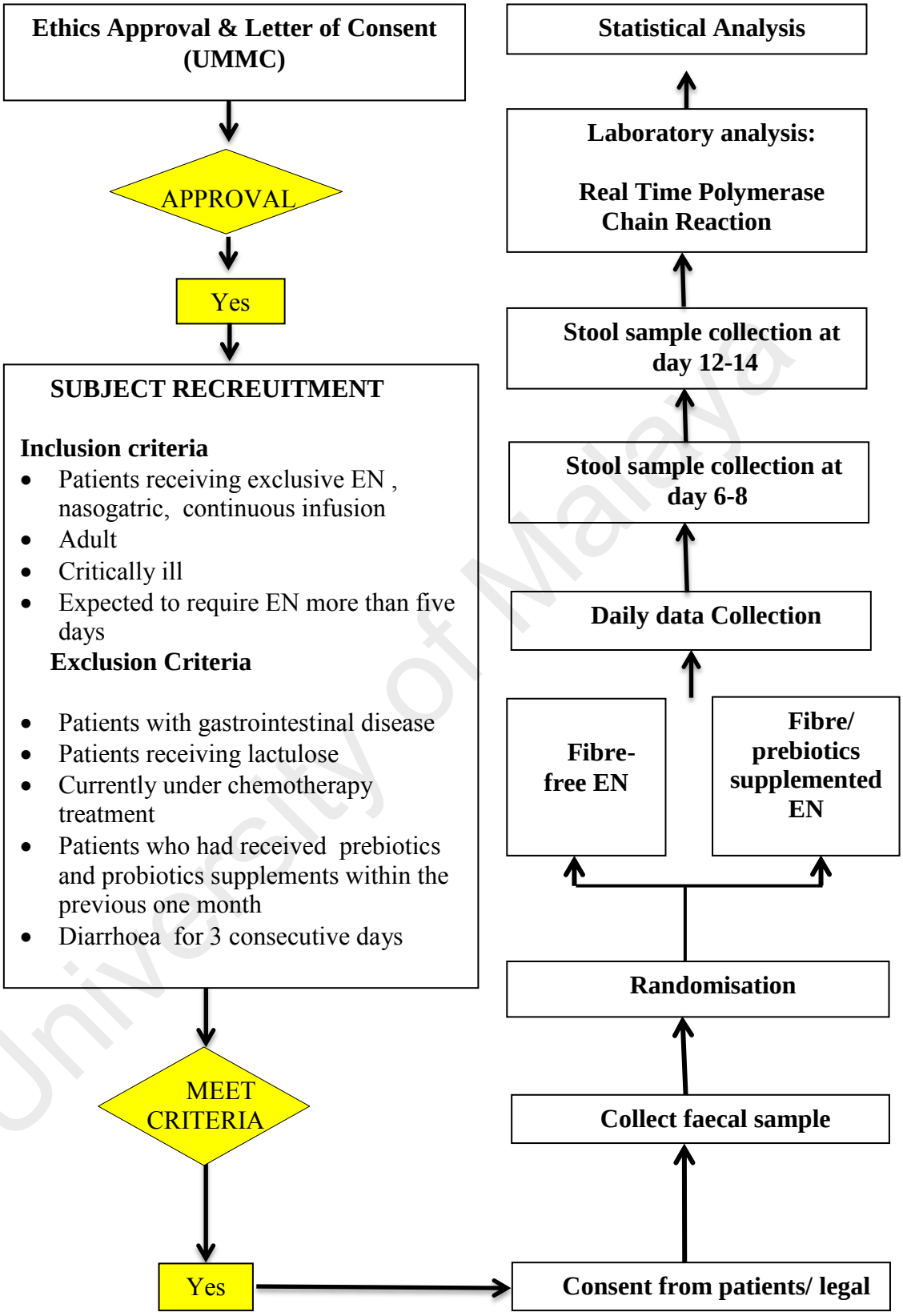

Figure 3.2: Flow of the study

3.2.12 Blinding

Although the study was an open-label study, the researcher was blinded for the analysis of the faecal sample for the quantification of faecal microbiota. Blinding was done prior to the bacterial quantification. An independent blinder, who was not involved in the clinical study, manipulated the label of the PCR tubes containing the extracted DNA from faecal samples.

3.2.13 Microbiological assessment for bacteria quantification

The laboratory works performed in this study complied with the general safety and laboratory practices. DNA extraction, traditional PCR and qPCR were performed at the Department of Parasitology and Medical Biotechnology Laboratory, University of Malaya.

3.2.13.1 Sample collection and storage

Faecal samples obtained from the recruited patients were stored in a deep freezer in Animal Experimental Unit, Faculty of Medicine, University of Malaya. To guarantee accurate measurement of microbiota, only fresh faecal samples were analysed. Faecal samples were collected within 1 hour of voiding and immediately transported to the storage facility (Majid et al., 2014). Faecal samples were frozen at -80°C until DNA is extracted.

3.2.13.2 DNA extraction from faecal samples

Bacterial DNA was isolated from faecal samples using QIAamp Fast DNA Stool Mini Kit (QIAgen, Cat No: 51604, Hilden, Germany). It is a commercial DNA extraction kit that has been used extensively in recent gut microbiota studies. Briefly, the procedure involved the lysis of bacterial cell, adsorption of DNA inhibitors, DNA-damaging substances and impurities with InhibitEX buffer and DNA purification on spin columns.

Bacterial DNA from 200 mg aliquot of faecal sample was extracted according to the manufacturer's protocol (QIAamp Fast DNA Stool Mini Handbook) with one modification made, where the initial incubation temperature was raised to 95˚C instead of 70˚C to make the cell lysis more efficient (Appendix J). Extracted DNAs were stored at -20˚C.

3.2.13.3 Measuring the DNA purity and concentration

The purity and concentration of the extracted DNA samples were measured using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., USA) at 260 nm and 280 nm. Elution buffer in the DNA extraction kit, Buffer ATE was used as control. The accepted value of the ratio of absorbance at 260 nm and absorbance at 280 nm (A260/A280) for the purity of extracted DNA is 1.8- 2.0 (Boesenberg-Smith, Pessarakli, & Wolk, 2012).

3.2.13.4 Pre-amplification

A total of six PCR assays targeting the small subunit ribosomal RNA genes of selected intestinal bacterial groups or species were chosen from literature. The primer sequences and the annealing temperature are listed in Table 3.3. The oligonucleotides of the target sequences were synthesised commercially by Intergrated DNA Technologies, USA, supplied by First BASE Laboratories Sdn Bhd, Malaysia.

Table 3.3: Group- or species-specific 16S-targeted primers and optimised conditions for real-time PCR

PCR assay (amplicon size)	Oligonucleotide sequence (5'→3')	Annealing temperature (^{0}C)	Reference
Bacteroides–Prevotella–Porphyromonas (140 bp)	F: 5'- GGT GTC GGC TTA AGT GCC AT-3' R: 5'-CGG A(C/T)G TAA GGG CCG TGC-3'	60	Rinttila, Kassinen, Malinen, Krogius, & Palva, 2004
Bifidobacterium spp. (243 bp)	F: 5'-TCG CGT C(C/T)G GTG TGA AAG-3' R: 5'-CCA CAT CCA GC(A/G) TCC AC-3'	58	Rinttila et al., 2004
Clostridium coccoides–Eubacterium rectale group (429 bp)	F: 5'-CGG TAC CTG ACT AAG AAG C-3' R: 5'-AGT TT(C/T) ATT CTT GCG AAC G-3'	55	Rinttila et al., 2004
Faecalibacterium prausnitzii (140 bp)	F: 5'- AGA TGG CCT CGC GTC CGA-3' R: 5'- CCG AAG ACC TTC TTC CTC C -3'	55	Wang, Cao, & Cerniglia, 1996
Lactobacillus group (341 bp)	F: 5'-AGC AGT AGG GAA TCT TCC A-3' R: 5'-CAC CGC TAC ACA TGG AG-3'	58	Walter et al., 2001 Heilig et al., 2002
Total bacteria (466 bp)	F: 5'-TCC TAC GGG AGG CAG CAG T-3' F:5'-GGA CTA CCA GGG TAT CTA ATC CTG TT-3'	60	Nadkarni, Martin, Jacques, & Hunter, 2002

3.2.13.5 Traditional PCR

Preliminary optimizations of primers were performed by performing a series of gradient PCR assays using conventional PCR using MyCycler Thermal Cycler (Bio-Rad, Hercules, USA). Some of the extracted DNAs were used to validate the PCR methods before the whole sample set were analysed with qPCR.

For traditional PCR, the amplifications reactions were performed with 20 µl reaction mixture consisting Ultra-Pure Taq PCR Master Mix with Dye (GENET BIO, South Korea), one Molar of forward and reverse primers, water (DNAse/RNAse free water (Sigma-Aldrich, Cat No. W4502, St Louis, MO) and 2 µL of DNA template. The reaction mixture for one PCR reaction is represented in Table 3.4.

Table 3.4: Reaction mixture for one PCR reaction

Reagent	Volume (µl)
PCR Master mix	10
Forward primer	2
Reverse primer	2
Aqua	4
DNA template	2
Total	20

Briefly, the amplification steps included an initial denaturation step where the sample was heated at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 15 s, primer (gradient) annealing at 50–70° C for 20 s and primer extension at 72°C for 45 s, with final extension at 72°C for 5 min. Figure 3.3 illustrates the processes involved in PCR;

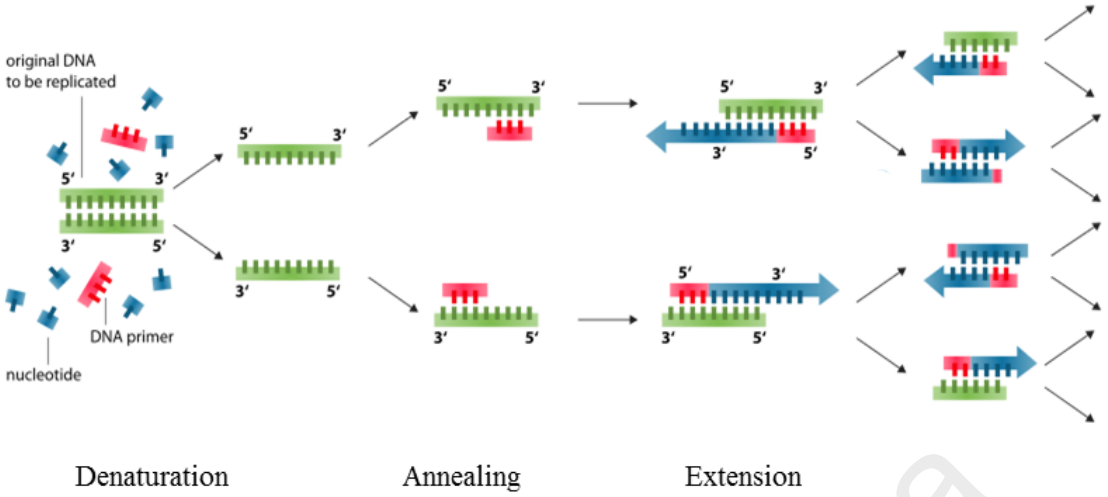

Figure 3.3: Basic steps of PCR

The amplified PCR products were subjected to Agarose Gel Electrophoresis (AGE) to confirm that a single band with expected size was obtained for each PCR assays. Gel electrophoresis of extracted PCR products was performed with 1.5 % agarose gel. 10 µl of each PCR product was loaded into the gel wells. The agarose gel was constituted of agarose powder, 1x TAE buffer and SYBR® Safe DNA Stain (Invitrogen, Cat. No. S33102, Canada). 100 bp DNA ladder was used as standards (Invitrogen, USA). The PCR products were separated via electrophoresis at 40 mA / ~100 V for 30 min. Finally, the gel was visualised under UV transluminator. The expected PCR product's sizes were listed in Table 3.3.

3.2.13.6 Sequencing PCR

PCR products were purified using QIAquick Gel Extraction Kit (QIAgen, cat. No. 28104, Hilden, Germany). The DNA concentrations were then measured with NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., USA). DNA sequencing of the PCR fragments obtained from the purified PCR band on AGE was carried out by MyTagc Bioscience Enterprise (SOLGENT Co. LTD, Korea). PCR purification was done by the company. Forty ml of amplified PCR product was supplied to the company for the sequencing process. The sequences were searched from

GenBank DNA database using the Basic Local Alignment Search Tool (BLAST, National Centre for Biotechnology Information, available at http://blast.ncbi.nlm.nih.gov).

3.2.13.7 Generation of standard curves

Standard curves were constructed from a series of dilution of purified PCR products of the previously performed traditional PCR. Gel extraction kit used for purification of PCR products was NucleoSpin® Gel and PCR Clean-up (Appendix K). Ten-fold dilutions were made and the number of bacteria in each dilution was determined by calculation based on the DNA concentration. The DNA concentration of the DNA pool was measured with NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., USA) at 260 nm. Water (DNAse/RNAse free water (Sigma-Aldrich, Cat No. W4502, St Louis, MO) was used as a negative control. The concentration of DNA fragments in the pool could be calculated based on the DNA concentration with the assumption that the molecular weight of one DNA base pair is 660 g/mol,

Fragment size (bp) x 660g/ mol = molecular weight of one fragment (g/mol)

$$\frac{\text{DNA concentration (ng/µl)}}{\text{Molecular weight of one fragment (g/mol)}} = \text{fragment concentration (nmol/ µl)}$$

When the fragment concentration (mol/µl) was multiplied with Avogadro's number (6.0221415×10^{23}), the number of fragments per one µl was gained.

Generation of standard curves for each PCR assays was made by plotting Ct values corresponding to the ten-fold dilution of PCR products. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). The slopes of the standard curves were calculated by performing a linear regression analysis with the StepOne™ Software

v2.2.2, StepOnePlus Real-Time PCR System (Applied Biosystem, USA). A mixture of all PCR reagents without any DNA was used as a negative control. Amplification efficiency (E) was estimated by using the slope of the standard curve and the formula $E= (10^{-1slope})^{-1}$. A reaction with 100% efficiency will generate a slope of -3.32. The standard curves were used for determining the detection limit for each of the assays, enumeration of all targeted bacteria in the faecal samples and a measure of PCR efficiency.

3.2.13.8 qPCR amplification

Quantitative PCR was performed with Applied Biosystem StepOnePlus Real-Time PCR System (Applied Biosystem, USA). The amplifications reactions were performed with 18 µl reaction mixture consisting SYBR® Select Master Mix (Applied Biosystem, Cat. No.4472908, USA), lM of forward and reverse primers, water (DNAse/RNAse free water (Sigma-Aldrich, Cat No. W4502, St Louis, MO). The reaction mixture for one PCR reaction is represented in Table 3.5. Two µL of DNA template was added to each reaction mixture, thus a total of 20 µl reaction mixture for one qPCR reaction was added to each well of a MicroAmp® Fast 8-Tube Strip (Applied Biosystem, Cat. No. 4358293, USA). Sample strip was quickly centrifuged with Mini centrifuge (Bio-Rad, Hercules, USA) before inserting the strips to the machine and starting the program. Quantitative PCR amplification was done for all six PCR assays to quantify all targets based on qPCR condition set as in Table 3.6 on the machine (Appendix L). Each sample was run in triplicate.

Table 3.5: Reaction mixture for one qPCR reaction

Reagent	Volume (µl)
PCR Master mix	10
Forward primer	2
Reverse primer	2
Aqua	4
DNA template	2
Total	20

3.2.13.9 Sensitivity and specificity of the assays

Specificity of PCR amplification was verified by melting curve analysis and AGE. Melt curve analysis was conducted after amplification in real time PCR by gradual cooling from 95°C to 60°C with fluorescence collection. On the other hand, analysis of the standard curve allowed verification of PCR efficiency and sensitivity as well as detection of quantification limit.

Table 3.6: Optimised qPCR conditions for group- or species specific 16S-targeted primers

PCR ASSAY	UDG ACTIVATION		DNA POLYMERASE ACTIVATION		DENATURATION		ANNEALING		EXTENSION	
	Temperature (°C)	Time	Temperature (°C)	Time	Temperature (°C)	Time	Temperature (°C)	Time	Temperature (°C)	Time
Bacteroides–Prevotella–Porphyromonas	50	2 min	95	2min	95	15 s	60	15 S	72	1 min
Bifidobacterium spp.	50	2 min	95	2min	95	15 s	58	15 S	72	1 min
Clostridium coccoides–Eubacterium rectale group	50	2 min	95	2min	95	15 s	55	15 S	72	1 min
Faecalibacterium prausnitzii	50	2 min	95	2min	95	20 s	55	15 S	72	1 min
Lactobacillus group	50	2min	95	2min	95	15 s	58	15 S	72	1 min
Total bacteria	50	2 min	95	2min	95	15 s	60	15 S	72	1 min

3.2.14 Statistical analysis

All data were analysed by using SPSS for Windows (Version 18.0, Chicago, IL, US). The normality of the distribution was tested using Kolmogorov-Smirnoff for baseline data and Shapiro-Wilk for data set less than 50. Data was presented as means (standard deviation) or medians (Interquartile range) as indicated based on its normality. A p-value of <0.05 was taken to be considered statistically significant. Effect size calculated using Cohen's *d*. Effect size is considered small with Cohen's *d* of 0.2, medium at 0.5 and large at 0.8 and above (McGough & Faraone, 2009). Bacterial numbers were transformed to Log. Comparison between two groups was done using unpaired t-test if the distribution was normal or using Mann- Whitney test if it was not normally distributed. Categorical data were compared between groups using the Chi-square or Fisher's exact test as needed. Paired t-test was used to compare pre- and post-intervention measurements. Alternatively, the paired-sample Wilcoxon Signed Rank Test was used in view of data not normally distributed. Univariate Analysis of Covariance (ANCOVA) was used to compare faecal microbiota's concentration at post-intervention while controlling for the co-variates. Additionally, Pearson correlation coefficient was used to find correlation between variables that are normally distributed; otherwise Spearman's rank correlation coefficient was employed.

Sensitivity analyses conducted in this study incorporated per protocol (PP) and intention to treat (ITT). While PP analysis was performed by analysing dataset from subjects who successfully completed the 2 week intervention with no or minimal protocol deviation, ITT analysis was performed by analysing all patients randomized into the study. Missing data was imputed according to the last observation carried forward (LOCF) method for the ITT analysis.

CHAPTER 4: RESULTS

The RCT was conducted in the ICU, University of Malaya Medical Centre from December 2013 up to September 2014. During the 10 months study period, sixty- eight critically ill patients, 44 males and 24 females were recruited into the study. Patients recruited were mainly from the respiratory, renal, cardiac, neurology, oncology, medical and trauma units. From 702 critically ill patients screened, seventy-five percent (524 patients) were not recruited into the study for not meeting the eligibility criteria. The main reasons for the patients not meeting the inclusion and exclusion criteria were; expected to start on oral diet, on long term EN or TPN, have gastrointestinal problems, was given lactulose, started on products with prebiotics or expected to be discharged from the ICU soon. The remaining 15% of the screened patients where eligible, but some were denied from being recruited into the study due to their participation in other research trials or objection from clinician mostly due to the poor prognosis of the patients or unable to obtain faecal sample. The recruitment and randomisation process of the study is presented in the flow diagram of the study, Figure 4.1.

Of 68 critically ill patients (33 patients in control, 35 patients in intervention) who underwent randomisation, only 54 % of the patients (19 patients in control, 18 patients in intervention) completed the study for at least one week and only 22% of the patients (7 patients in control, 8 patients) on intervention completed the study. The main reasons for lost-to-follow up were 1) patients were transferred out of ICU, or 2) cessation of EN as they were able to consume diet orally, or 3) switched to another EN formula or to parenteral nutrition for longer periods (i.e. more than five days) or 4) patients passed away.

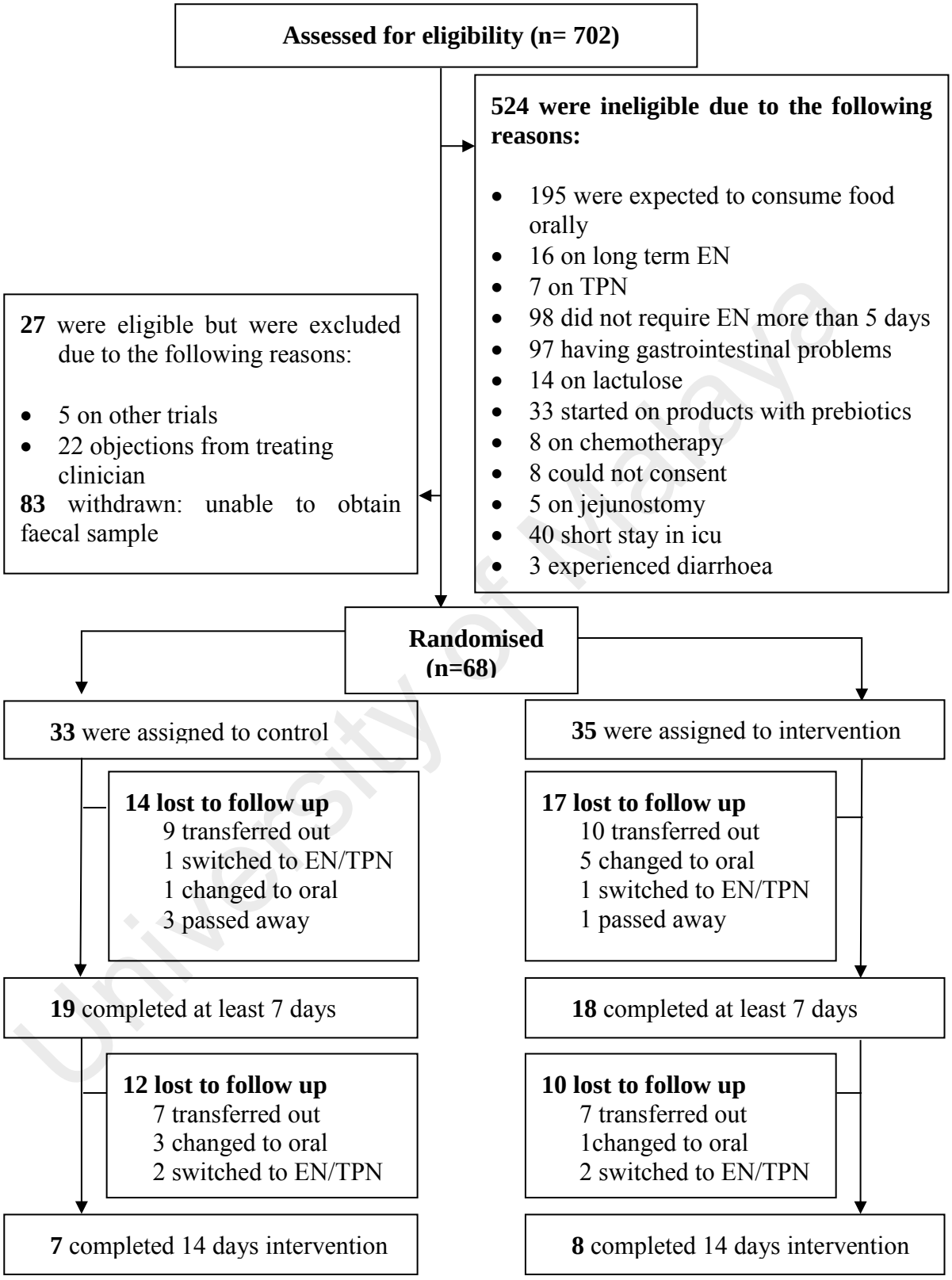

Figure 4.1: Flow diagram of the study

Upon randomisation, 33 patients in the control arm received fibre-free enteral formula, Osmolite 1 Cal while 35 patients in the intervention arm received fibre/prebiotics containing enteral formula, Ensure FOS. The baseline characteristics between the two groups were comparable (refer to Table 4.1). There was no significance different in age, BMI, total protein, albumin, white blood cell, C-reactive protein (CRP) and random blood sugar (RBS). The severities of the illness in both groups were also similar as translated in the SAPS II and SOFA scores. Nutritional intake (energy and protein) was also comparable except for fibre and FOS intake as the intervention group received 10 g of fibre/prebiotics per litre of enteral feed while enteral formula used in control group was fibre-free. Both groups received EN on the first day of ICU admission. On average, baseline faecal samples for both groups were received after four days EN started.

There were temporary switch of enteral formula in some patients due to fluid restriction implied to the patients. One patient from the control arm was switched temporarily to receive Novasource renal (Nestle), a fibre-free, high energy enteral formula (2kcal/ml). Two patients from intervention arm were switched to receive Nepro (Abbott), an enteral formula which provides 2 kcal/ ml energy and contain 3.7 g of FOS per eight ounce as sole source of fibre in the formula. The temporary transient of enteral formula lasted between two to four days. Data from the study were analysed per protocol and ITT.

Table 4.1: Baseline patient's characteristics and nutritional intake

	Fibre-free (n= 33)	Fibre/prebiotics (n= 35)	P-value
Age(year) [b]	54.5 (28)	56.0 (33)	0.905
Gender, n (%) [c]			
Male	23 (69.7)	21(60.0)	0.403
Female	10 (30.3)	14 (40.0)	
Race, n (%)			
Malay	8 (24.2)	12 (34.3)	
Chinese	10 (30.3)	11(31.4)	
Indian	14 (42.4)	9 (25.7)	
Others	1 (3.0)	3 (8.6)	
Disease category, n (%)			
Respiratory	13 (39.4)	11 (31.4)	
Renal	0 (0.0)	2 (5.7)	
Cardiac	0 (0.0)	1 (2.9)	
Neurology	0 (0.0)	1 (2.9)	
Oncology	1 (3.0)	0 (0.0)	
Trauma	6 (18.2)	4 (11.4)	
Medical	13 (39.4)	16 (45.7)	
Body Mass Index (kg/m^2) [b]	24.3 (10.39)	23.9 (10.8)	0.408
Total Protein (g/L) [a]	55.7 (7.0)	57.5 (8.4)	0.337
Albumin (g/L) [a]	24.2 (8.0)	26.8 (6.4)	0.118
White blood Cell (g/L) [a]	12.9 (5.9)	14.1 (7.4)	0.345
C Reactive Protein(g/L) [b]	8.4 (11.4)	9.6 (6.2)	0.876
SAPS2 score [a]	45.3 (16.2)	37.8 (16.4)	0.109
SOFA score [b]	9.0 (4.5)	8.0 (6.5)	0.614
Random blood sugar (g/L) [b]	8.2 (2.3)	7.7 (1.5)	0.231
Nutritional intake at baseline			
Energy (kcal/day) [a]	1423.4 (532.5)	1377.6 (478.8)	0.718
Protein (g/day) [a]	58.21 (22.1)	52.7 (18.1)	0.140
Nutritional intake			
Energy (kcal/day) [a]	1501.7 (396.7)	1465.2 (337.4)	0.683
Protein (g/day) [a]	60.1 (14.4)	55.6 (12.7)	0.182
Fibre (g/day) [b]	0.0 (0.0)	14.8 (4.8)	0.001
FOS (g/day) [b]	0.0 (0.0)	14.8 (4.8)	0.001
Day start EN after admission to ICU [b]	0 (1.5)	0 (1.0)	0.719
Number of days from starting EN to the day baseline faecal sample collected [b]	4.0 (3.0)	3.0 (4.0)	0.346

[a] Data between the two groups were analysed using Student's t-test and reported in mean (SD)
[b] Data between the two groups were analysed using Mann-Whitney Test and reported in median (IQR)
[c] Data between the groups were analysed using Chi-Square test

The antibiotic therapy received by both groups of patients is listed in Table 4.2. The median of the number of antibiotics received by the recruited patients throughout the study was two. There was a wide range of antibiotics used as antimicrobial therapy to fight infection in the critically ill patients. Only 10% of the recruited patient did not receive any form of antibiotic therapy prior to the study.

Table 4.2: Antibiotics therapy received by patients in the study

	Fibre-free (n= 33)	Fibre/prebiotics (n= 35)	P value
Number of antibiotics prescribed [a]	2.0 (2.0)	2.0 (3.0)	0.910
Previously not on antibiotics, n (%)[b]	4 (12.1)	3 (8.6)	0.705
Type of antibiotics used, n			
Piperacillin	22 (66.7)	21 (60.0)	
Imipenem	9 (27.3)	10 (28.6)	
Amoxicillin	5 (15.2)	9 (25.7)	
Ceftriaxone	6 (18.2)	8 (22.9)	
Azithromycin	4 (12.1)	5 (14.3)	
Metronidazole	3 (9.1)	5 (14.3)	
Meropenem	4 (12.1)	4 (11.4)	
Vancomycin	5 (15.2)	2 (5.7)	
Ceftazidime	6 (18.2)	1 (2.9)	
Cloxacillin	2 (6.1)	4 (11.4)	
Colistin	4 (12.1)	2 (5.7)	
Cefuroxime	2 (6.1)	3 (8.6)	
Bactrim	3 (9.1)	2 (5.7)	
Ampicillin	3 (9.1)	1 (2.9)	
Clindamycin	3 (9.1)	0 (0.0)	
Gentamycin	2 (6.1)	0 (0.0)	
Levofloxacin	1 (3.0)	1 (2.9)	
Penincillin	0 (0.0)	1 (2.9)	
Cefepime	0 (0.0)	1 (2.9)	
Doxycycline	0 (0.0)	1 (2.9)	

[a] Data between the two groups were analysed using Mann-Whitney Test and reported in median (IQR)
[b] Data between the two groups were analysed using Fisher's Exact test

4.1 Quantification of faecal microbiota using qPCR

4.1.1 Specificity of the PCR assays

Six PCR assays were used in this study to quantify specific group and species of bacteria;total bacteria, lactobacilli, bifidobacteria, bacteroides, clostridia and *Faecalibacterium prausnitzii* in the faecal samples of the critically ill patients. The specificity of PCR amplification for all 6 assays were confirmed by the production of single peak in the melt curve analysis and one band in the gel electrophoresis for each assay as displayed in Figure 4.2 ,4.3 and Appendix M and N.

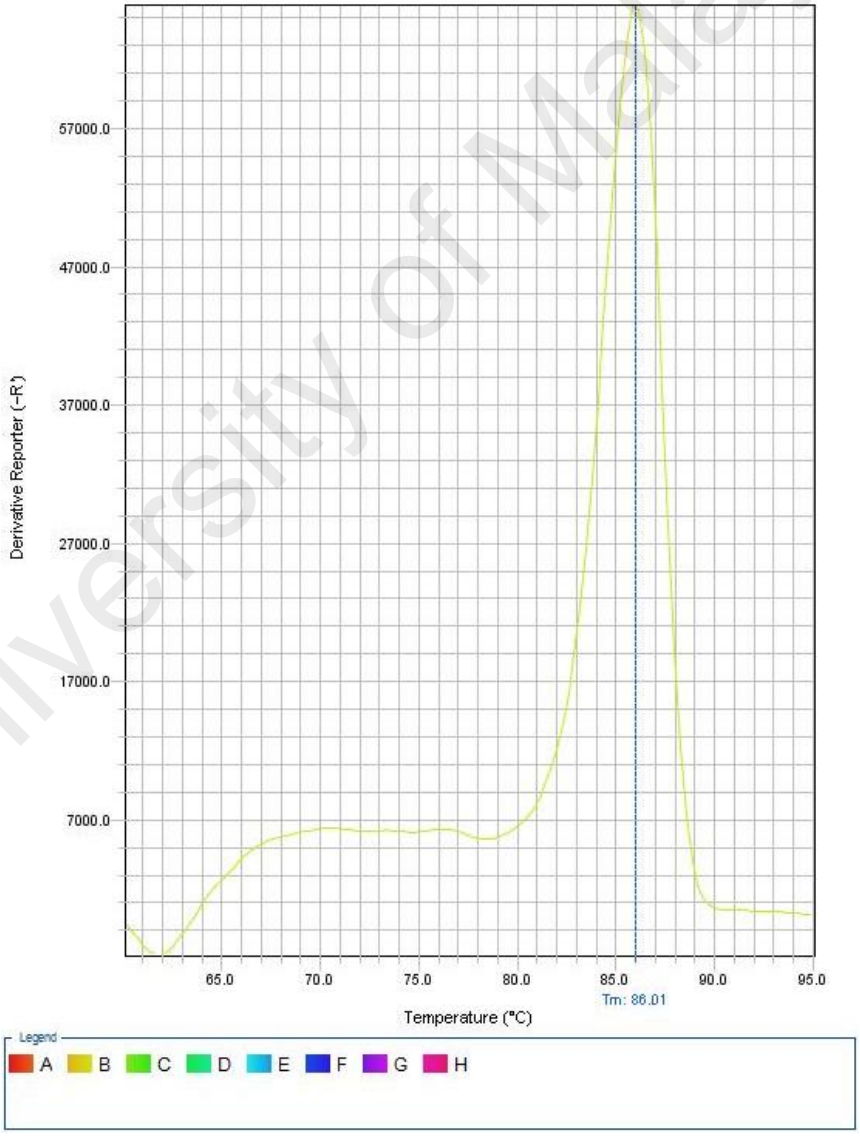

Figure 4.2: Melt curve analysis for *Bacteroides–Prevotella–Porphyromonas* assay.

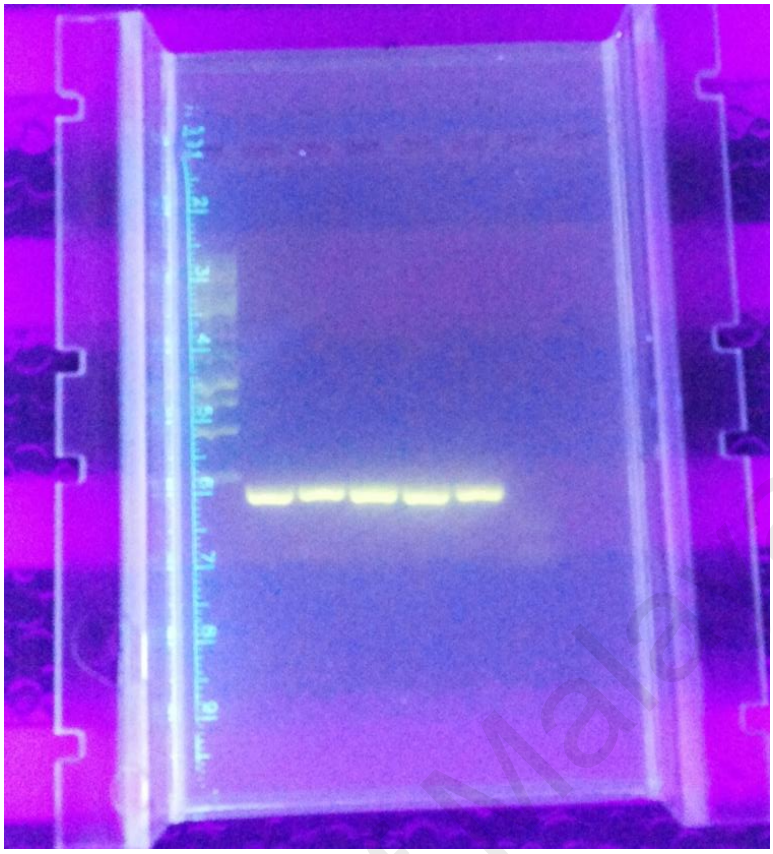

Figure 4.3: Agarose gel electrophoresis for Bacteroides–*Prevotella*–*Porphyromonas* assay, presentation of one band for 5 samples tested

4.1.2 Linearity and quantification limit of PCR assays

The linearity of the standard curves was determined by using 10-fold serial dilutions of the DNA obtained from the extraction of PCR products. Standard curve was generated by plotting Ct (cycle threshold) values from each dilution. The slope of the standard curve was used to determine the PCR efficiencies by computing the slope value in the formula $E= [10^{(-1/slope)} – 1]$ (Figure 4.4 and Appendix O). The efficiencies of the 6 PCR assays ranged from 83.2% to 110% (Table 4.3). The precision of all data fitting to the standard curve is represented by the correlation coefficient, r^2 value. The correlation coefficient values for all 6 assays were between 0.951 and 0.998 (Table 4.3). The quantification limit ranged from 10 to 1000 copy number per PCR reaction (Table 4.3).

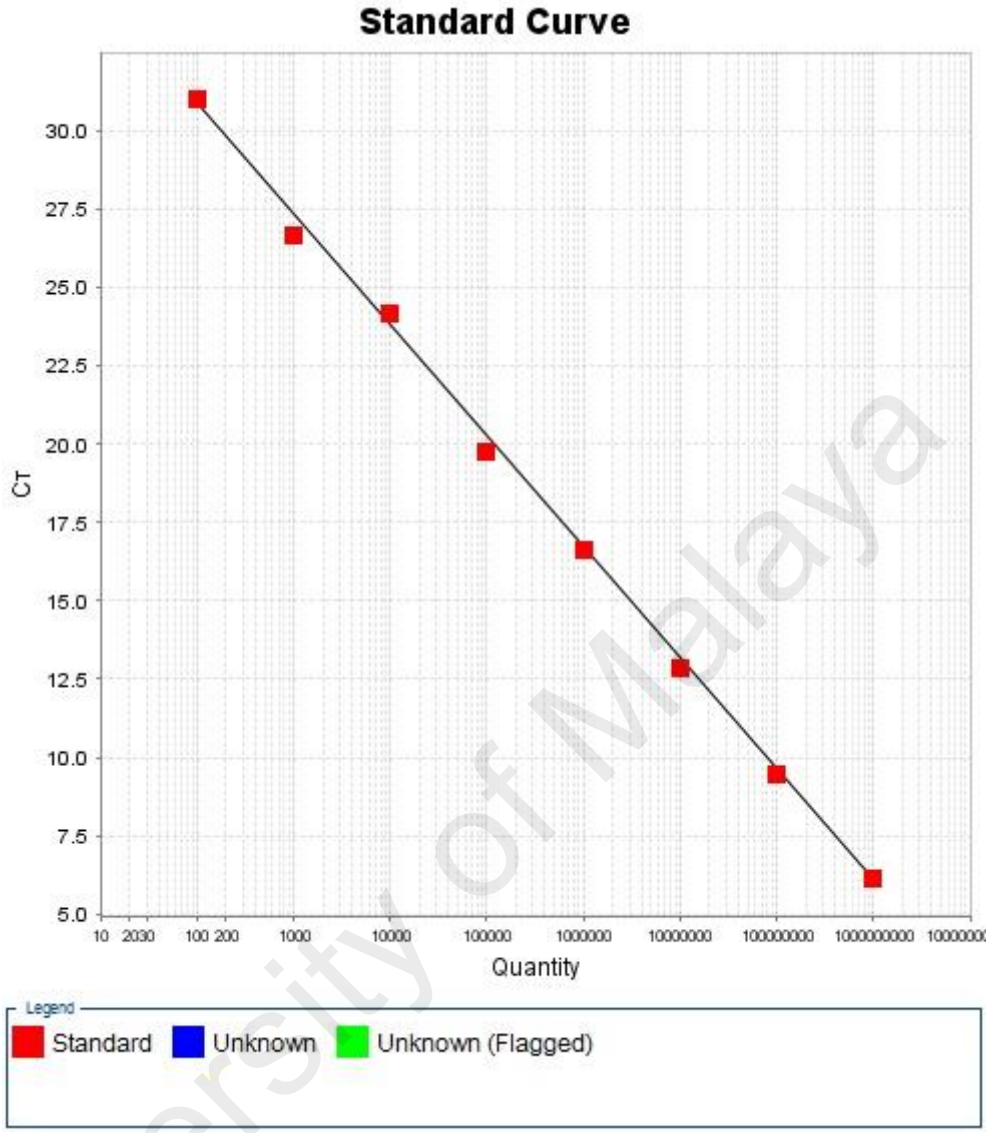

Figure 4.4: Standard curve for *Bacteroides–Prevotella–Porphyromonas* assay

Table 4.3: Amplification slopes, efficiencies, correlation coefficients, quantification limit and equation for copy number calculation for each qPCR assays

PCR Assay	Slope	PCR efficiencies (%)	Correlation coefficient (r^2)	Quantification limit per PCR reaction (copy number)	Quantification limit per gram faecal weight (copy number)
Bacteroides–Prevotella–Porphyromonas	-3.531	92.0	0.998	100	2.5×10^4
Bifidobacterium spp.	-3.271	102.9	0.993	10	2.5×10^3
Clostridium coccoides Eubacterium rectale group	-3.804	83.19	0.998	1000	2.5×10^5
Faecalibacterium prausnitzii	-3.232	103.9	0.974	10	2.5×10^3
Lactobacillus group	-3.651	87.9	0.951	10	2.5×10^3
Total bacteria	-3.103	110.0	0.995	10	2.5×10^3

4.1.3 Quantification of faecal microbiota from faecal samples of critically ill patients

A total of 120 faecal samples (68 baseline samples, 37 post 1-week samples and 15 post 2-weeks samples) were extracted and later subjected to qPCR for bacterial quantification. Each sample was tested for six PCR assays. Copy number calculation was made by real time PCR by computing the Ct value into the standard curve generated.

There were no significant differences in all faecal microbiota concentrations except for the lactobacillus concentration at baseline between patients receiving fibre free and fibre/prebiotics supplemented EN (Table 4.4 and 4.5). The control group had higher baseline concentration of lactobacillus compared to the intervention group (PP: p= 0.01, ITT: p= 0.11. After 2 weeks of intervention, there was no significant difference of faecal microbiota concentrations between the two groups of patients, except for *Faecalibacterium Praunitzii*'s concentrations , (PP: p=0.029, ITT: p= 0.236). The difference in *Faecalibacterium Praunitzii*'s concentrations after 2 weeks intervention between the two groups remained significant when baseline values were controlled (covariate).The *Faecalibacterium Praunitzii*'s concentrations were significantly higher in patients receiving fibre-free enteral formula compared to patients receiving fibre/prebiotics supplemented EN(PP: p=0.033, ITT: p= 0.133).

As presented in Figure 4.5 and 4.6, each individual had different concentrations of faecal bifidobacteria at baseline, ranging from 3.32 to 9.19 $\log_{10}$ copy number/g faeces. The faecal bifidobacteria concentrations were altered during provision of EN using fibre-free or fibre/prebiotics supplemented enteral formula for 2-weeks. Some had large increments, some had stable and some had large reductions of faecal bifidobacteria over time. Overall, there was no significant change in bifidobacteria

concentration after two weeks of intervention in both groups of patients receiving either fibre-free or fibre/prebiotics containing enteral formulas (PP: p= 0.113 versus p= 0.537, ITT: p= 0.430 versus p= 0.974). The difference in bifidobacteria concentration post two weeks intervention between the two groups remained insignificant, (PP: p=0.066, ITT: p=0.201) when baseline values were controlled (covariate).

A non-significant trend of increased concentrations of total bacteria, bacteroides, bifidobacteria and *Faecalibacterium Praunitzii* was observed in patients receiving fibre-free EN while an opposite trend was found in patients receiving fibre/prebiotics supplemented EN (Figure 4.7 and 4.8). As compared to the baseline, the lactobacillus concentration's trend was found to be lower at post -intervention in the control group (PP: p=0.072, ITT: 0.342) while there was no significant change of lactobacillus concentration in the intervention group, (PP: p=0.617, ITT: p= 0.062). The concentration of clostridium remained the same at post 2-weeks intervention in both, intervention and control group, (PP: p= 1.00 versus p=0.464, ITT: p=0.18 versus p=0.464).

Additionally, correlation test was performed to investigate the relationship between the amount of FOS delivered in the enteral formula and faecal microbiota concentrations. There was no significant correlation found between the amount of FOS given throughout the study and the concentration of faecal microbiota (data were not shown). Based on the Spearman's correlation test, the bacteria of interest, bifidobacteria concentrations in the faecal samples did not correlate with the amount of FOS delivered through EN in patients receiving fibre/prebiotics supplemented EN during the period of the study (correlation coefficient = -0.40 , p=0.320).

Table 4.4: Per protocol analysis for faecal microbiota concentrations at baseline and post-intervention with EN supplemented with fibre/prebiotics or control

Mean (SD) log_{10} copy number/g faeces	Baseline for patients who completed the intervention	Post-Intervention 2-weeks	P value Post 2-weeks intervention control vs fibre/prebiotics[1]	P value Baseline vs Post 2-weeks intervention [2]	P value ANCOVA[3]
Control	n=7	n=7			
Fibre	n=8	n=8			
Total bacteria					
Control	9.17 (0.90)	9.47 (1.10)	0.360	0.527	0.379
Fibre/prebiotics	9.18 (1.39)	8.86 (1.36)		0.691	
Bacteroides–Prevotella					
Control	8.69 (0.55)	9.00 (0.62)	0146	0.332	0.130
Fibre/prebiotics	8.09 (1.65)	7.74 (2.10)		0.747	
Bifidobacterium spp.					
Control	6.54 (1.95)	8.15 (1.85)	0.066	0.113	0.066
Fibre/prebiotics	6.63 (2.14)	5.70 (2.73)		0.537	
Lactobacillus group					
Control	7.17 (1.60)	4.82 (1.47)	0.905	0.072	0.509
Fibre/prebiotics	4.29 (1.09)	4.70 (2.16)		0.617	
F.prausnitzii					
Control	6.02 (0.98)	6.88 (1.65)	0.029	0.173	0.033
Fibre/prebiotics	6.05 (1.91)	4.49 (2.05)		0.142	
Clostridium coccoides-E. rectale group					
Control	5.70 (0.39)	5.46 (0.19)	0.151	0.464	0.227
Fibre/prebiotics	5.36 (1.03)	5.36 (0.05)		1.000	

1 Data were analysed using Student's t-test to compare post-Interventions faecal microbiota concentrations between patients receiving standard EN and EN supplemented with fibre/prebiotics
2 Data were analysed using paired t-test to compare baseline and post-interventions faecal microbiota concentrations
3 Data were analysed using Univariate Analysis of Covariance, post-intervention value as the outcome variable and baseline value as covariate

Table 4.5: Intention-to-treat analysis for faecal microbiota concentrations at baseline and post-intervention with EN supplemented with fibre/prebiotics or control

Mean (SD) log_{10} copy number/g faeces		Baseline for all patients recruited	Post-Intervention 2-weeks	P value Post 2-weeks intervention control vs fibre/prebiotics[1]	P value Baseline vs Post 2-weeks intervention [2]	P value ANCOVA[3]
	Control	n=33	n=33			
	Fibre	n=35	n=35			
Total bacteria						
	Control	9.05 (0.92)	8.85 (1.82)	0.619	0.560	0.098
	Fibre/prebiotics	9.01 (1.18)	9.04 (1.14)		0.878	
Bacteroides–Prevotella						
	Control	7.68 (1.48)	7.86 (1.72)	0.772	0.419	0.304
	Fibre/prebiotics	7.77 (1.53)	7.98 (1.55)		0.470	
Bifidobacterium spp.						
	Control	6.38 (1.92)	6.64 (2.15)	0.891	0.430	0.201
	Fibre/prebiotics	6.58 (1.88)	6.57 (2.48)		0.974	
Lactobacillus group						
	Control	5.78 (2.12)	5.35 (2.25)	0.582	0.342	0.163
	Fibre/prebiotics	5.06 (1.53)	5.63 (1.90)		0.062	
F.prausnitzii						
	Control	5.79 (1.38)	5.83 (1.72)	0.236	0.899	0.133
	Fibre/prebiotics	5.55 (1.69)	5.32 (1.82)		0.483	
Clostridium coccoides-E. rectale group						
	Control	5.45 (0.61)	5.32 (0.33)	0.700	0.128	0.824
	Fibre/prebiotics	5.30 (0.67)	5.27 (0.65)		0.464	

1 Data were analysed using Student's t-test to compare post-Interventions faecal microbiota concentrations between patients receiving standard EN and EN supplemented with fibre/prebiotics
2 Data were analysed using paired t-test to compare baseline and post-interventions faecal microbiota concentrations
3 Data were analysed using Univariate Analysis of Covariance, post-intervention value as the outcome variable and baseline value as covariate

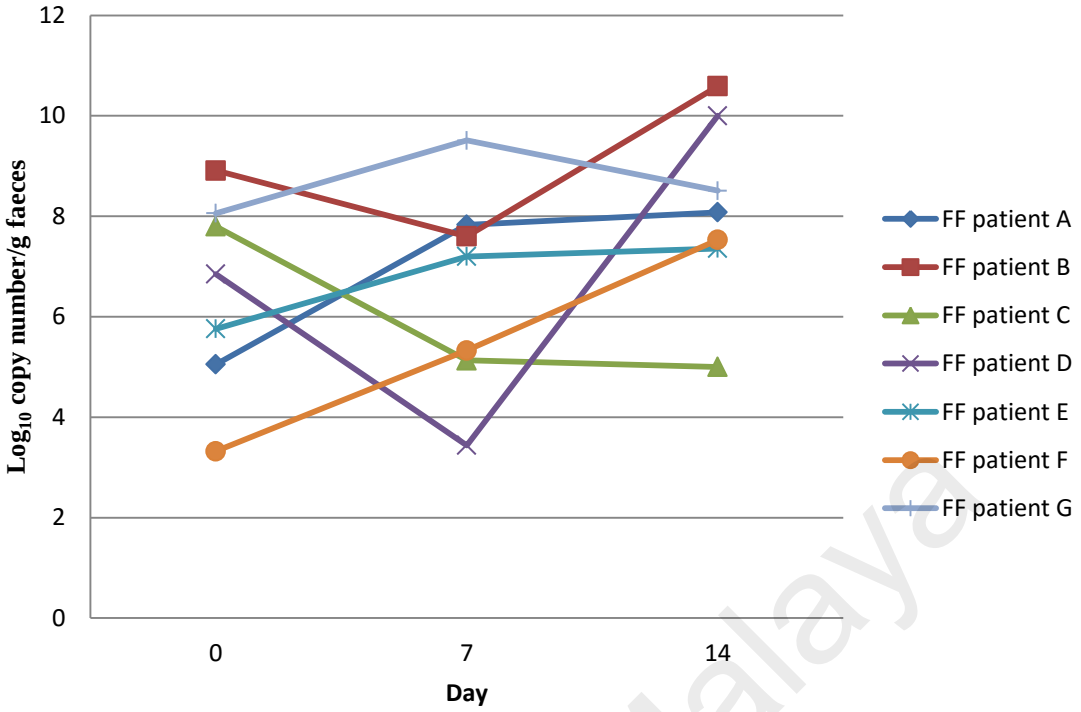

Figure 4.5: Individual faecal bifidobacteria concentrations at baseline, post 1-week and post 2-weeks intervention for patients receiving fibre-free EN (FF).

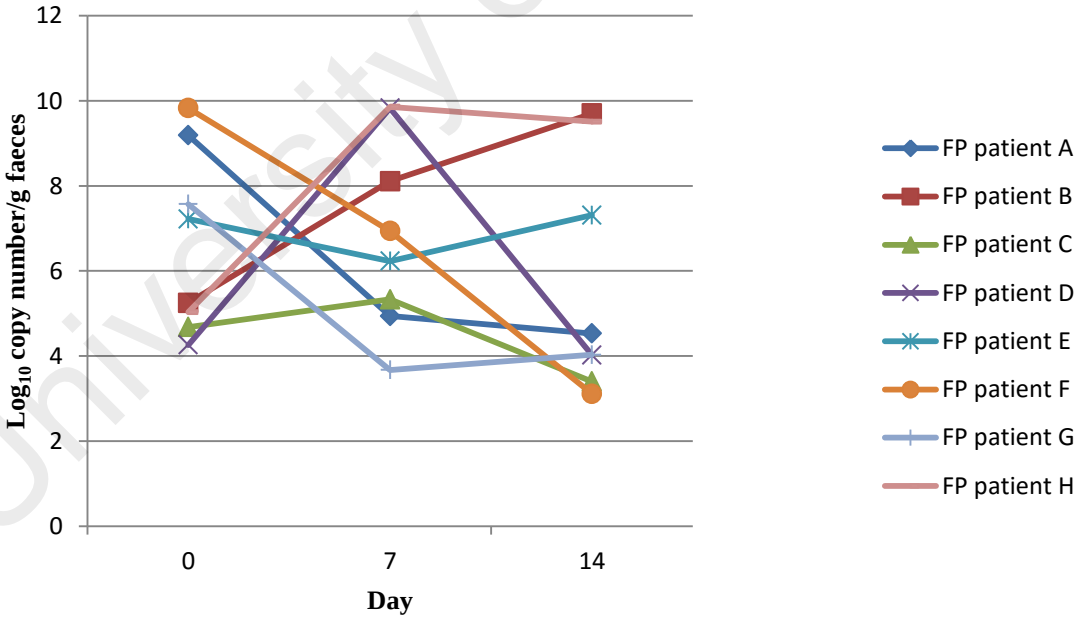

Figure 4.6: Individual faecal bifidobacteria concentrations at baseline, post 1-week and post 2-weeks intervention for patients receiving fibre/prebiotics supplemented (FP) EN

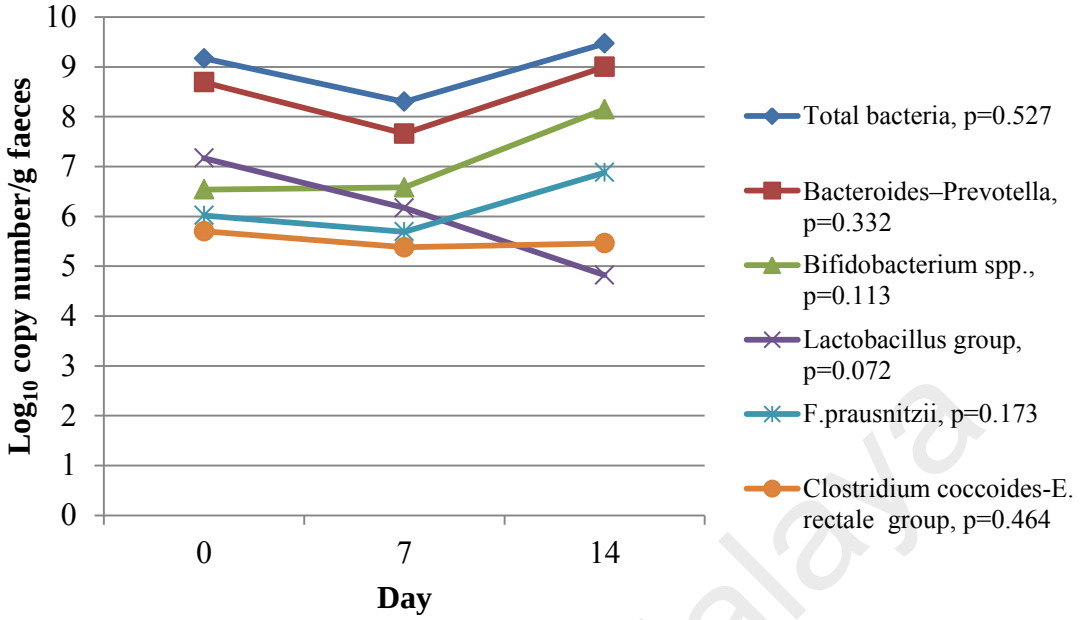

Figure 4.7: Faecal microbiota concentrations at baseline , post 1-week, post 2-weeks for patients receiving fibre-free EN (n= 7)

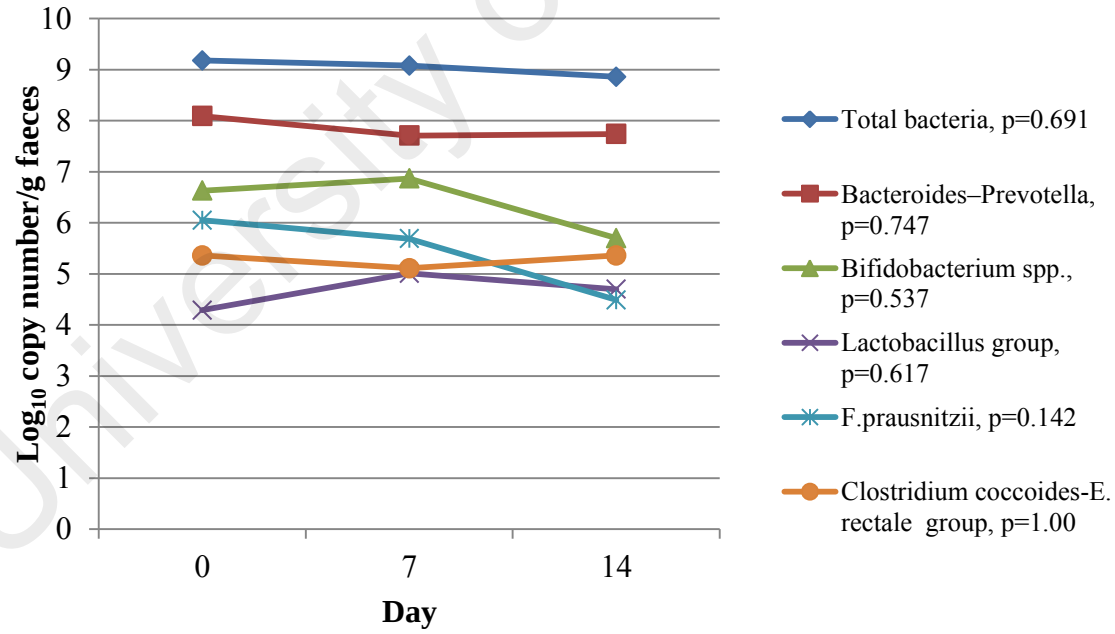

Figure 4.8: Faecal microbiota concentrations at baseline , post 1-week, post 2-weeks for patients receiving fibre/prebiotics supplemented EN (n=8)

4.2 Faecal output

Data analysis was conducted for patients who completed two weeks of intervention as presented in Table 4.6 and 4.7. The prevalence of diarrhoea in the critically ill patients conducted in this study was 73% with no significant difference in diarrhoea incidence between the control, 71% and the intervention group 75% , (PP: p =0.662, ITT: p=0.494). Diarrhoea was defined as daily faecal score of ≥ 15, referring to a validated stool chart used in this study (Whelan et al., 2008). Both groups had comparable number of days of diarrhoea. It was found that patients in the fibre/prebiotics group had a trend of lower stool frequency (mean±SD, 1.2±0.6) compared to patients receiving fibre-free enteral formula (mean±SD, 1.8±0.9), (PP: p=0.092, ITT: p=0.070). Patients in the fibre/prebiotics group also had lower faecal scores (median±IQR, 4.8±10.3) than the fibre-free group (median±IQR, 9.2±4.2). However, the difference in the faecal score was not statistically significant, (PP: p=0.613, ITT: p= 0.036). Faecal scores of fibre/prebiotics group was consistently lower than the fibre-free group at baseline, 1 week and 2 weeks, but the differences were not significant (Figure 4.9).

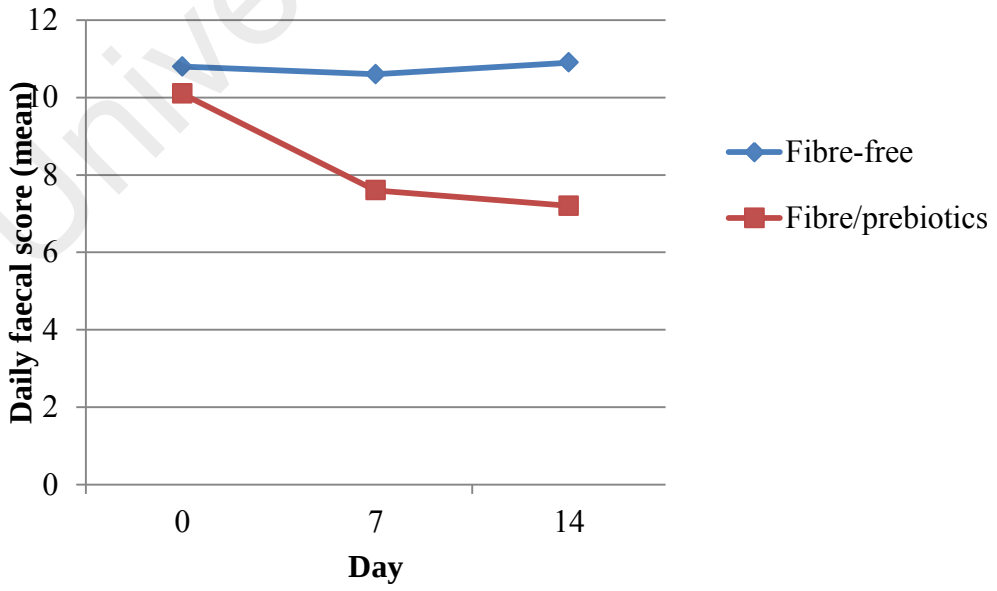

Figure 4.9: Daily faecal score at baseline , post 1-week, post 2-weeks for patients receiving fibre-free formula(n=7) or fibre/prebiotics supplemented EN (n=8)

Table 4.6: Faecal output of patients during EN with fibre/prebiotics or control (Per protocol analysis).

	Control (n= 7)	Fibre/prebiotics (n= 8)	P value
Stool frequency per day [a]	1.8 (0.9)	1.2 (0.6)	0.092
Daily faecal score [b d]	9.2 (4.2)	4.8 (10.3)	0.613
Prevalence of diarrhoea			
No of patients with at least one day of diarrhoea, n (%) [c]	5 (71)	6 (75)	0.662
No of diarrhoea days [b]	2.0 (4.0)	2.0 (4.0)	0.833
Total days of intervention [b]	14.0 (2.0)	14.0 (1.0)	0.613
Daily faecal score [d]			
at baseline (day 1-3) [a]	10.8 (7.8)	10.1 (7.2)	0.872
at 1 week (day 6-8) [a]	10.6 (7.6)	7.6 (8.9)	0.506
at 2 weeks (day 12-14) [a]	10.9 (12.2)	7.2 (5.8)	0.450

a Data between the two groups were analysed using Student's t-test and reported in mean (SD)
b Data between the two groups were analysed using Mann-Whitney U test and reported in median (IQR)
c Data between the groups were analysed using Fisher's Exact test
d Faecal scores were calculated using King's Stool Chart, score of ≥ 15 was used to define diarrhoea

Table 4.7: Faecal output of patients during EN with fibre/prebiotics or control (Intention-to-treat analysis).

	Control (n= 33)	Fibre/prebiotics (n= 35)	P value
Stool frequency per day [a]	1.68 (0.79)	1.34 (0.72)	0.070
Daily faecal score [b d]	9.2 (9.3)	5.6 (8.19)	0.036
Prevalence of diarrhoea			
No of patients with at least one day of diarrhoea, n (%) [c]	18 (51)	18 (55)	0.494
No of diarrhoea days [b]	1 (2)	1 (3)	0.417
Total days of intervention [b]	7 (10)	7 (9)	0.931
Daily faecal score [d]			
at baseline (day 1-3) [b]	10 (8.8)	6.7 (8.0)	0.063
at 1 week (day 6-8) [b]	9.3 (13.7)	5.0 (9.7)	0.059
at 2 weeks (day 12-14) [b]	8.0 (11.8)	5.5 (9.7)	0.123

a Data between the two groups were analysed using Student's t-test and reported in mean (SD)
b Data between the two groups were analysed using Mann-Whitney U test and reported in median (IQR)
c Data between the groups were analysed using Fisher's Exact test
d Faecal scores were calculated using King's Stool Chart, score of ≥ 15 was used to define diarrhoea

Correlation test was performed to investigate the relationship between the daily faecal score and important factors influencing diarrhoea highlighted in previous studies. Based on the Spearman's correlation test conducted, there was no significant correlation between the faecal score and the age, fibre and FOS intake, bifidobacteria concentrations, and the number of antibiotics used by the patients in the study (data was not shown). However, positive correlations were found between the SOFA score (correlation coefficient =0.690, p= 0.013), SAPS II score (correlation coefficient =0.544, p= 0.044) and stool frequency (correlation coefficient =0.725, p= 0.002) and the faecal scores. This indicates increases in severity of illness (as reflected by higher SOFA and SAPS II scores) and stool frequencies were positively correlated with increases in faecal scores.

CHAPTER 5: DISCUSSION

The bifidogenic effect of FOS had been proven in healthy human studies with dosages as low as 5 g found to stimulate the growth of bifidobacteria (Bouhnik et al., 1999; Rao,2001). However, studies conducted on patients requiring EN have exhibited conflicting results (Majid et al., 2014; Schneider et al., 2006; Wierdsma et al., 2009). The RCT conducted in the present study was designed to investigate the bifidogenic effect of an enteral formula commonly used in hospitals in Malaysia that contains FOS as a sole source of fibre compared to another widely-used fibre-free enteral formula. The findings from this study showed that supplementation of 10 g/L of FOS per day for two weeks did not increase the faecal bifidobacteria concentration of the critically ill patients, (PP: p=0.537, ITT: p= 0.974) (large effect size of 1.051).

A number of reasons can be identified to explain why bifidobacteria concentrations fail to increase after FOS supplementation. Firstly, a possible explanation is antibiotic therapy. It has been established that antimicrobial therapy alters the composition of the gut microbiota by suppressing certain groups of bacteria and potentially stimulating the growth of pathogenic or opportunistic bacteria (Rafii et al., 2008). Patients who were subjected to antibiotic treatments have been shown to have an abnormal faecal microbiota composition with a significant reduction of bifidobacteria concentration (Bartosch et al., 2004). Usually, studies evaluating the efficacy of fibre in stimulating growth of bifidobacteria have excluded individuals who were on antibiotics. This is to prevent the antagonist effect of antibiotics towards gut microbiota. For example, the only study that showed an increase in bifidobacteria concentration (not significant) upon the provision of FOS in patients requiring EN (N=19) omitted patients who were on antibiotics or had been on antibiotics for the past one month (Wierdsma et al., 2009). Similar to the finding in the present study, a more recent multicentre, double-

blind RCT which recruited patients requiring EN on antibiotic therapy was also not able to demonstrate the bifidogenic effect of FOS (Majid et al., 2014). In that study, patients were provided with 13.75 g of prebiotics through multi-fibre enteral formula and additional supplementation of prebiotics. Despite the administration of a high amount of prebiotics to the critically ill patients in the study, the concentrations of bifidobacteria remained unchanged after the intervention.

The role of antibiotics in inhibiting the bifidogenic effect of the FOS needs to be considered. In the RCT conducted in the present study, all the patients had received at least one type of antibiotic throughout their stay in the ICU. There were 20 different types of antibiotics considered in this study: ampicillin, amoxicillin, azithromycin, Bactrim, cefepime, ceftazidime, ceftriaxone, cefuroxime, clindamycin, cloxacillin, colistin, doxycycline, gentamycin, imipenem, levofloxacin, meropenem, metronidazole, penincillin, tazocin and vancomycin. The main antibiotics given to patients in both groups were piperacillin that was prescribed by clinicians to 63% of the patients in the trial. Piperacillin is a broad-spectrum antibiotic covering most Gram-positive and Gram-negative aerobic bacteria and anaerobic bacteria. It has been shown to suppress the concentration of enterobacteria, enterococci, and anaerobic bacteria including bifidobacteria, eubacteria, lactobacilli, clostridia and Gram-positive cocci of the gut microbiota (Nord & Lahnborg, 1994).

The median number of antibiotics received by both groups of patients was two, but the types of antibiotics used were not identical. In total, the patients in the fibre/prebiotics group were prescribed with 18 different types of antibiotics and the fibre-free group was prescribed with 17 types of antibiotics. Other than piperacillin, the main three antibiotics used in the control arm were imipenem (27.3%), ceftriaxone (18.2%) and ceftazidime (18.2%). Meanwhile, imipenem (28.6%), amoxicillin (25.7%)

and ceftriaxone (22.9%) were the most commonly used antibiotics in the fibre/prebiotics arm. Broad-spectrum antibiotics were used in the study as empiric treatment upon suspicion of infections inflicting the patients. The choice of antibiotic to prescribe was based on numerous factors and guided by the Ministry of Health Antibiotic Guideline (Ministry of Health, 2008). Introduction of empiric antibiotics has been shown to result in the undesirable reduction of bifidobacteria diversity and colonisation in the gut (Hussey et al., 2011).

An in-vitro study investigated the sensitivity of numerous strains of *Bifidobacterium* spp. against 30 types of antibiotics (Moubareck, Gavini, Vaugien, Butel, & Doucet-Populaire, 2005). The results from that study showed that bifidobacteria was sensitive to most antibiotics including penicillins (such as penicillin G, amoxicillin, piperacillin, ticarcillin, imipenem as well as anti-Gram-positive antibiotics), macrolides, clindamycin, pristinamycin, vancomycin and teicoplanin. Another in-vitro study observed that the provision of FOS on faecal culture treated with the antibiotic clindamycin did not stimulate the growth of bifidobacteria and reduced the colonisation resistance as evidenced by the increased colonisation of *Clostridium difficile* (Hopkins & Macfarlane, 2003). It is noted that in the RCT conducted in the present study, none of the patients receiving fibre/prebiotic-supplemented EN were treated with clindamycin. However, these patients were subjected to many other broad-spectrum antibiotics with the capacity to suppress anaerobic bacteria including bifidobacteria.

In contrast to the earlier findings, a double-blind RCT conducted by Lewis, Burmeister and Brazier (2005) found that bifidobacteria flourished when FOS was given to patients treated with antibiotics. In that study, hospitalised patients with CDAD were randomised to receive either 12 g FOS per day or a placebo while receiving

metronidazole as the first-line treatment for CDAD. Vancomycin was also used for patients who were not tolerating or improving well with metronidazole. It was discovered that the patients receiving the FOS treatment in the study were also less likely to have a relapse of diarrhoea. Similar implications were not found in the RCT in the present study. Possible explanations for this might be the difference in antibiotic therapy and severity of illness of the patients between the two studies. The participants recruited in the present study were critically ill patients with complex pathological conditions on various types of antibiotics.

Secondly, the severity of illness might impede the bifidogenic effect of the FOS. Studies have demonstrated that the gut microbiota composition was altered during the period of critical illness (Marshall, 1999; Shimizu et al., 2006; Zaborin et al., 2014). In the RCT in the present study, the severity of illness of the recruited patients was different from one individual to another as reflected by the SOFA and SAPS II scores. The SOFA score of the recruited patients ranged from three to 16. The SOFA is a scoring system with a maximum score of 24, designed to describe and quantify the organ function of patients. While it was not developed to predict outcomes, studies have shown the relationship between organ failure and mortality (Moreno et al., 1999; Regel, Grotz, Weltner, Sturm, & Tscherne, 1996). The SAPS II scores of the patients also varied (ranging from 13 to 78) but there was no significant difference in both scores between the two groups. The SAPS II score describes the severity of illness and can also predict mortality among critically ill patients. According to Shimizu et al. (2006), patients with systemic inflammatory response syndrome had significantly lower bifidobacteria, lactobacillus and total anaerobic counts and higher counts of pathogenic group bacteria than healthy people. Moreover, the marked pathological colonisation of bacteria during critical illness was similar to the species that predominate in nosocomial infections (Marshall, 1999).

In terms of the absolute quantification of bacteria in this study, the comparison of the findings in this study's RCT with other studies was limited due to the difference in molecular analysis used in the studies (i.e. culture, PCR, FISH). This study employed the quantification of 16S rRNA gene copy numbers using qPCR. The 16S rRNA copy number cannot be directly converted into cell counts, as the gene copy number of each bacterium varies among species (Lee, Bussema, & Schmidt, 2009). A comparison of findings in studies with a similar methodology and unit of outcome (copy number) found that the bifidobacteria concentrations of the patients in this study's RCT was lower than the concentrations in healthy, young adults (6.4 vs 10.4 $\log_{10}$ copy number/g faeces) (Ohashi et al., 2012). When compared with IBS patients, the bifidobacteria concentrations of the critically ill patients in this RCT were still lower than the concentrations in healthy IBS patients (6.40 vs 9.35 $\log_{10}$ copy number/g faeces). Interestingly, the bifidobacteria concentrations found in this study is similar to the results in a study by Wierdsma et al. (2009) (6.4 vs 6.3 $\log_{10}$ copy number/g faeces). That study was conducted on older adult patients who required EN for at least eight weeks (n=16). An additional comparison of the concentration of bifidobacteria in patients after two weeks consumption of fibre-free EN and healthy people recruited in the study revealed that the bifidobacteria concentrations in the group consuming the fibre-free EN were lower (2.1 x 10^6copy number/g faeces; equivalent to 6.3 $\log^{10}$ copy number/g faeces) than in the healthy people (n=16) (2.1 x 10^7copy number/g faeces; 7.3 $\log^{10}$ copy number/g faeces).

Based on the comparison made, it is important to highlight that younger adults (Malinen et al., 2005; Ohashi et al., 2012) had higher faecal bifidobacteria concentrations as compared to older adults (e.g. the present study and Wierdsma et al. (2009)). In the former case, the ages of the participants recruited by Malinen et al. (2005) and Ohashi et al. (2012) were 45 and 21–24. In the latter case, the ages of the

participants recruited by Wierdsma et al. (2009) were 61.4 in the control group and 55.5 in the intervention group. In the RCT in the present study, the patients were older adults with the median age of 54.5 in the control arm and 56 in the intervention arm. It is noted that other factors were also not constant across all the compared studies. This includes the participants' pathological conditions, geographical locations and dietary intakes. Thus, direct comparison is not possible. However, various studies have shown that age plays a role in influencing gut microbiota composition and concentration (Arrieta et al., 2014; Bartosch et al., 2004; Biagi et al., 2012; Tiihonen et al., 2010). Bifidobacteria concentrations have been found to be lower during old age compared to adulthood (Mitsuoka, 1996). Thus, the change in bifidobacteria concentrations in older adults upon provision of prebiotics may be limited despite the presence of the substrate for the bacteria.

Fourthly, since the effect of FOS towards the bifidobacteria concentration is dose-dependent, the dosage of FOS given in the study is important to consider when studying the impact of its supplementation on the faecal bifidobacteria concentration of the critically ill patients. As demonstrated in this study, the provision of 10 g/L FOS as a sole source of fibre in EN did not increase the bifidobacteria concentration in the critically ill patients. A previous study on prebiotics showed that dosages as low as 5 g of FOS per day stimulated the growth of bifidobacteria concentration but the optimal dosage was 10 g/day (Bouhnik et al., 1999). This amount of prebiotics was consumed by healthy humans, in addition to a daily normal dietary intake that yielded approximately 5 g/day (Moshfegh, Friday, Goldman, & Ahuja, 1999). This brings the FOS consumption needed to establish the exertion of the optimum bifidogenic effect to a total of 15 g/day. In the RCT conducted in the present study, considering the patients were to receive 1800 kcal energy of isocaloric feeds from the FOS-supplemented EN, the patients were expected to receive 18 g of FOS per day, which is a sufficient dosage

of FOS to exert the bifidogenic effect. However, the average amount of energy received by the patients in the intervention arm was 1465 kcal/day which yielded 14.8 g of FOS received through EN. Despite the optimisation of calories planned by the attending dietitian, it was unavoidable for patients to receive suboptimal rates and volumes of feeds. An observational study conducted in the same institution found that approximately 79% of patients experienced feeding interruptions (Yip, Rai, & Wong, 2014). The most prevalent reason for interruption was the carrying-out of procedures followed by high gastric residual volume, diarrhoea, difficulty in the positioning of the nasogastric tubes and vomiting. While the patients in this study received a dosage of FOS which exceeded the minimum amount of FOS needed to stimulate bifidobacteria growth, the intervention was proven unsuccessful. Since the prebiotic dosage of 10 g/day was established only for healthy people, it might appear that critically ill patients would require a higher dose of prebiotics for any increase in bifidobacteria concentration to be detected.

Fifthly, another justification for why bifidobacteria concentrations fail to increase after FOS supplementation is that lactobacilli compete against bifidobacteria for the source of substrate. An in-vitro study in which the faecal samples of four healthy adults were cultured on an anaerobic culture medium containing FOS found a reduced concentration of bifidobacteria and increased concentration of lactobacilli (Sghir, Chow, & Mackie, 1998). In that study, molecular analysis targeting the 16S rRNA showed that the bifidobacteria levels were maintained at 10% to 20% of the total 16s rRNA measured during the first six days of the observation and drastically reduced when the lactate concentration was at a maximum. Meanwhile, the lactobacilli concentrations were found to be low at the initial stage of the study, increasing until Day 9 and remaining at a high level until the end of the study on Day 21. The study suggested that lactobacilli were able to utilise FOS and compete with bifidobacteria for the source of

substrate. In the RCT conducted in the present study, the concentration of lactobacilli in the intervention arm increased (non-significant, PP: p=0.617, ITT: p= 0.062) after two weeks of FOS supplementation while the lactobacilli concentrations in the control arm showed a lowering trend after two weeks (PP: p=0.072, ITT: p= 0.342). In relation to the in-vitro study, it could be postulated that FOS failed to stimulate bifidobacteria due to the competition for substrate with lactobacilli.

Other than bifidobacteria, studies have demonstrated that prebiotics also stimulate the growth of *F. prausnitzii* when given to healthy individuals in addition to their normal dietary intake (Dewulf et al., 2013; Ramirez-Farias et al., 2009). *Faecalibacterium prausnitzii* is a dominant member of butyrate-producing bacteria of the gut microbiota, consisting of 5% of total faecal microbiota in healthy individuals. It plays an important role in maintaining the physiological function and homeostasis of the gut (Miquel et al., 2013). However, the provision of prebiotics during EN failed to stimulate the growth of *F. prausnitzii* (Benus et al., 2010). Benus et al. (2010) conducted a prospective, double-blind, randomised, cross-over study investigating the effect of fibre supplementation in EN on gut microbiota and SCFA of healthy people. They found that the concentration of *F. prausnitzii* was reduced during EN regardless of whether or not fibre was present in the feeds. The amount of fibre used in the study was 14 g/L of feeds, comprising 8.9 g/L of pea fibre and 5.1 g/L of FOS. It was speculated that the inability of fibre-supplemented EN to proliferate *F. prausnitzii* in the study was due to the higher content of insoluble fibre instead of soluble fibre (FOS) which is the preferred substrate for the bacteria.

In this study's RCT, despite the higher dose of FOS used (10 g/L), there were no significant changes in *F. prausnitzii* concentrations for pre- and post-interventions in both groups of patients (PP: p=0.142 vs. p= 0.173, ITT: p=0.899 vs.p= 0.483). However, at the end of the intervention, *F. prausnitzii* concentrations were significantly lower in the patients receiving fibre/prebiotic-supplemented EN (PP: p=0.033 ITT: p=0.133). Similar findings were also observed in critically ill patients receiving up to 13.75 g of prebiotics per day from prebiotic supplementation and fibre-supplemented EN (Majid et al., 2014). It is possible that antibiotic therapy hindered the stimulating effect of the FOS. Bartosch et al. (2004) found that patients on antibiotics had significantly lower *F. prausnitzii* compared to healthy people and patients who were on antibiotic therapy. It appears that the growth of *F. prausnitzii* could not be stimulated despite the higher dose of prebiotics due to the reduction of the concentration of *F. prausnitzii* upon administration of antibiotics.

In addition to the ability of fibre to 'normalise' the gut microbiota composition, fibre has also been used clinically to alleviate diarrhoea (Elia et al., 2008; Green, 2001). It provides a substrate for bacterial fermentation, allowing the proliferation of bifidobacteria and thus preventing the colonisation of pathogen (Tuohy et al., 2001). Additionally, the provision of fibre was proven to increase the faecal SCFA concentration (Schneider et al., 2006). Earlier studies have found that SCFA has the ability to promote the absorption of sodium and water in the colon, and consequently reduces the risk of developing diarrhoea (Bowling et al., 1993; Zaharia et al., 2001). In the RCT in the present study, the patients receiving supplementation of 10 g/L prebiotics in enteral formula for two weeks had a trend of lower stool frequency compared to the patients receiving fibre-free enteral formula (PP: p=0.092 ITT: p=0.070). The effect size calculated by Cohen's *d* indicated that the effect size was large (Cohen's *d*=0.926). As stool frequency is part of the definition, a reduction in stool

frequency influences the faecal score. This is supported by another finding from this RCT whereby increases in stool frequency were found to be positively correlated with faecal score (correlation coefficient=0.725, p=0.002). However, despite the reduction in stool frequency (non-significant), the 10 g of prebiotics used in this study did not reduce the incidence of diarrhoea and the faecal score in the critically ill patients.

At the time of writing, a study by Chittawatanarat et al. (2010) is the only study able to demonstrate the efficacy of prebiotics in reducing the diarrhoea score of critically ill patients. Despite the improvement in the diarrhoea score in patients receiving fibre/prebiotic-supplemented EN (similar to the RCT in the present study), the incidence of diarrhoea remained the same (Chittawatanarat et al., 2010). The finding of that study was also in agreement with the finding by Majid et al. (2014) that the supplementation of additional prebiotics in EN failed to improve the diarrhoea incidence in critically ill patients receiving fibre-supplemented EN.

One of the main possible reasons for the failure of the supplementation of prebiotics to reduce diarrhoea is the antibiotic therapy received by the patients. Patients in this study's RCT received various regimens of antibiotic therapy (up to seven types of antibiotics) depending on their medical conditions throughout their stay in the ICU. Antibiotic therapy has been shown to play a role in the development of diarrhoea (Bergogne-Bérézin, 2000). The pathogenesis of antibiotics-associated diarrhoea involves the dysbiosis of the gut microbiota, thus altering the physiologic function of the gut microbiota in serving as a barrier against the pathogen, also known as colonisation resistance (Sekirov et al., 2010). Additionally, the antibiotic therapy may impact the gut directly by causing enteropathy with malabsorption and the prokinetic effects on the motility of the gut (Beaugerie & Petit, 2004). Inclusive of the current study, prebiotics supplementation during EN has not been found to lower the incidence

of diarrhoea in critically ill patients receiving antibiotic therapy (Chittawatanarat et al., 2010; Majid et al., 2014).

In addition to antibiotic therapy, it has been proposed that the severity of illness is related to diarrhoea (Wiesen et al., 2006). An observational study of 278 ICU patients found that patients with diarrhoea had more severe disease as reflected by high APACHE II and SAPS II scores at admission. In agreement with the observation, the results of the RCT in the present study demonstrated that patients with higher SAPS II and SOFA scores during admission had higher faecal scores. Although gut function is not directly measured in either of these scores, critically ill patients often suffer from gastrointestinal dysfunction as part of multiple organ failure. The critical illness may cause abnormal motility patterns and impaired barrier integrity in both epithelial cells and gut microbiota, leading to diarrhoea (Hill, 2013; Mittal & Coopersmith, 2014).

In contrast to the findings in the present study's RCT, positive outcomes from fibre supplementation during EN towards diarrhoea were demonstrated in a study of critically ill patients treated with antibiotics (Spapen et al., 2001). The study introduced a higher dose soluble and fermentable type of fibre (Partially hydrolysed guar gum, 22 g/L) to mechanically-ventilated and septic patients and found significant reductions in the frequency and days of diarrhoea. Despite the insignificant outcomes in the current RCT, observations in this study found a trend of lower diarrhoea scores and stool frequencies in patients who received EN containing 10 g/L of prebiotics. Thus, it can be postulated that the introduction of higher dosages of FOS/inulin in EN or different compositions of mixed soluble fibre formulation might reduce the incidence of diarrhoea. However, increasing the dosage of prebiotics must be done with caution as the provision of 20 g of FOS per day was not well-tolerated by healthy people (Bouhnik et al., 1999).

Another possible reason for the failure of FOS to reduce the incidence of diarrhoea is the high osmolarity of the fermentable fibre, FOS/inulin. There is a theory that Fermentable, Oligo-, Di-, Mono-saccharides and Polyols (FODMAP) content in enteral formula is associated with the incidence of diarrhoea in patients receiving EN (Halmos et al., 2010). FODMAPs are carbohydrates classified based on the length of the carbohydrate chains and consist of fructose, lactose, fructans (FOS), galactans and polyols. These carbohydrates share similar functional properties as these carbohydrates are poorly absorbed in the small intestine, highly osmotic and rapidly fermented by bacteria in the gut. One study concluded that patients who were on EN with high FODMAP-enteral formula were five times more at risk of developing diarrhoea compared to those on formulas with lower FODMAP content (Halmos et al., 2010). However, that study was a retrospective study and more research is needed to confirm this theory.

5.1 Strengths and limitations

The main strength of this study is the study design. This study employed a prospective randomised controlled trial. This study design has high internal validity from the power of randomisation (Booth & Tannock, 2014). Randomisation was done with the objective to ensure patients in both arms were comparable and the only difference between both groups was the intervention received (FOS supplementation during EN). Thus, any result from the study can reflect the efficacy of the intervention given.

Another strength of the RCT in this study is the method of bacterial quantification used in the study. The application of qPCR in the quantification of bacteria is cultivation-independent technique which facilitates the detection of more bacteria compared to culture-related techniques. Additionally, this technique is sensitive as it has a low detection limit attributed by the use of specific primers to identify bacteria of interest. This enables accurate quantification of the bacteria of interest.

The power of the study also lies in the reliability of the definition used to describe diarrhoea. This RCT defined diarrhoea using the faecal score of the King's stool chart. A score of 15 or more was used to indicate diarrhoea (Whelan et al., 2008). This stool chart has been validated for use in patients requiring EN. This enabled a valid and standard definition of diarrhoea to be used throughout the study and also avoided bias and subjective interpretations of diarrhoea by the health care professionals. However, this scoring system should only be used to define diarrhoea, and should not be extended to measure the severity of diarrhoea.

The major limitation of this study is that the calculated sample size to complete the study was not met. Based on the sample size with 90% power of study calculation (α=0.05), 21 samples were required for each arm. With consideration of the dropout rate in previous study, 58 patients needed to be recruited. Although this study was able to recruit 68 patients, only fifteen patients completed the two weeks of intervention. Despite the intensive daily screening, 524 patients were excluded for not meeting the criteria of the study. The numbers of eligible patients were further reduced if patients were involved in another trial or were viewed as unsuitable candidates for the study by the attending clinicians. A portion of patients was also not able to join the study due to failure to obtain baseline faecal samples before the patients were transferred out of the ICU. Additionally, due to the high turnover rate for the ICU admission and short stay in the ICU, most of the recruited patients were not able to complete this study. Such a situation is not rare and is unavoidable, especially in studies with long periods of intervention. Considering the high lost to follow up subjects than anticipated, future study may consider to continue data collection at the ward level despite any protocol deviation due to change of feeding or treatment to gather more data. This would be a good strategy to reduce missing data and will be useful for the ITT analysis.

As the study was conducted in the general ICU, heterogeneity of patients' recruited need to be addressed. While patients admitted into the general ICU are multidisciplinary, attempts have been made to ensure homogeneity of patients recruited in the RCT. This was made possible by excluding patients with compromised gut functions or any possible conditions/ illnesses that might be affecting the gut microbiota from being recruited. Additionally, the randomization process ensured the baseline characteristics of both groups were comparable in term of severity of illness as reflected by the comparable SAPSII and SOFA scores.

Another limitation of the study was blinding was not done during the intervention period. Double-blinding was not conducted in the current RCT mainly due to the different forms of enteral formula. One of the enteral formula used was in solid form while the other was in liquid form. As such, blinding of the enteral formula cannot be done. Limited resources and lack of research staff nurse had made suggestion of appointing unblinded nurse to prepare for the feeding of recruited patients cannot be implemented. However, while the investigator was not blinded during the intervention, it was highly unlikely that the investigator in the study would be able to interfere with the intervention. This is because the main investigator was not involved in treating the patients. Clinical decisions were made by the attending physician and feedings (rate and volume) were prescribed by the attending dietician according to the patients' requirements. Blinding was successfully done prior to molecular analysis to prevent bias in analysing the faecal stool sample.

In this study's RCT, patients were required to be in the intervention for two weeks. Thus, the patients recruited in this study were patients who had to stay a relatively long period in the ICU. This reflected the complexity and/or severity of the illness of the critically ill patients recruited in the study. The majority of the patients in this study were older adults and were subjected to major antibiotic therapy. The patients recruited in this study might not be a good representative of ICU patients; rather, they may reflect a subpopulation of critically ill patients. Thus, the interpretation of the findings in this RCT must be made cautiously while considering the characteristics of the patients included in this study.

In some patients (4%), the enteral formula was changed due to their medical condition and the clinical judgement by the attending physicians and dietitian. Changes were made when patients were on fluid restrictions and in need of energy-dense feeds. In this RCT, the patients in the control arm received Novasource renal (Nestle), a fibre-free, high energy enteral formula (2 kcal/ml) and patients in the intervention arm were switched to receive Nepro (Abbott), an enteral formula which provides 2 kcal/ml energy. This enteral formula contains 15.6 g/L of FOS as a sole source of fibre in the formula. The temporary switch lasted for 2–4 days before the patients resumed their original feeds. The deviations from protocol were minimal and still maintained the provision of fibre-free formula to the control arm and FOS-containing EN in the intervention arm.

Additionally, comprehensive laboratory analyses such as analysis of SCFA, faecal pH and water content were not done in this study. This was not done due to the resource constraints of the Master's project. Such analysis would provide more information on the mechanism of prebiotic actions towards alleviating diarrhoea. These analyses could be conducted in the future using the preserved stool samples.

CHAPTER 6: CONCLUSION & RECOMMENDATIONS

In conclusion, the administration of enteral formula containing 10 g/L of FOS for two weeks to the critically ill patients did not increase the bifidobacteria concentrations. There was no significant difference in bifidobacteria concentrations after two weeks of intervention between patients receiving fibre/prebiotic-supplemented and fibre-free enteral formulas. Low concentrations of bifidobacteria were identified in patients receiving EN in this study. Interestingly, the RCT demonstrated a trend of reduced stool frequency in patients receiving FOS-supplemented EN. However, the provision of fibre/prebiotics in EN did not improve diarrhoea. Thus, the provision of 10 g/L of single source fibre may not be the best approach to reduce diarrhoea in the critically ill. This RCT adds valuable input to the limited pool of data in this area. Based on the meta-analysis conducted and the findings in this RCT, there is lack of evidence to support the use of prebiotic-supplemented EN in critically ill patients to reduce the incidence of diarrhoea or to stimulate bifidobacteria. More studies are warranted to probe the dosage and also the composition of fibre (e.g. the use of multifibre) that could exert effects in reducing diarrhoea as well as stimulating bifidobacteria in critically ill patients. Alternatively, probiotic and synbiotic supplementation during EN should be explored as a possible strategy to alleviate diarrhoea and to promote the growth of beneficial bacteria in the gut in order to improve gut function in critically ill patients.

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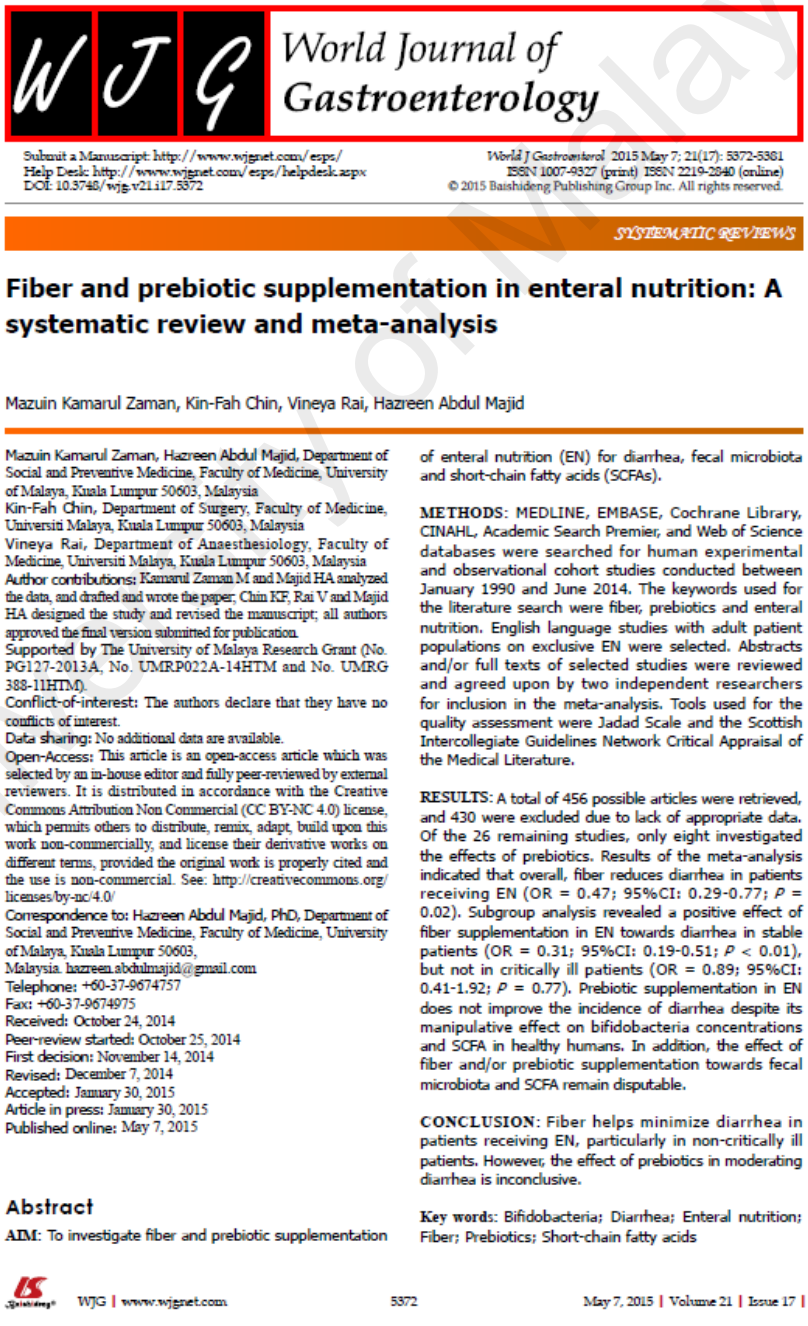
LIST OF PUBLICATIONS AND PAPERS PRESENTED

ACADEMIC JOURNAL:

Kamarul Zaman, M., Chin, K.-F., Rai, V., & Majid, H. A. (2015). Fiber and prebiotic supplementation in enteral nutrition: A systematic review and meta-analysis. World J Gastroenterol, 21(17), 5372-5381. doi: 10.3748/wjg.v21.i17.5372 *(ISI Cited Publication)* **Q2**



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SYSTEMATIC REVIEWS

Fiber and prebiotic supplementation in enteral nutrition: A systematic review and meta-analysis

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Abstract

AIM: To investigate fiber and prebiotic supplementation of enteral nutrition (EN) for diarrhea, fecal microbiota and short-chain fatty acids (SCFAs).

METHODS: MEDLINE, EMBASE, Cochrane Library, CINAHL, Academic Search Premier, and Web of Science databases were searched for human experimental and observational cohort studies conducted between January 1990 and June 2014. The keywords used for the literature search were fiber, prebiotics and enteral nutrition. English language studies with adult patient populations on exclusive EN were selected. Abstracts and/or full texts of selected studies were reviewed and agreed upon by two independent researchers for inclusion in the meta-analysis. Tools used for the quality assessment were Jadad Scale and the Scottish Intercollegiate Guidelines Network Critical Appraisal of the Medical Literature.

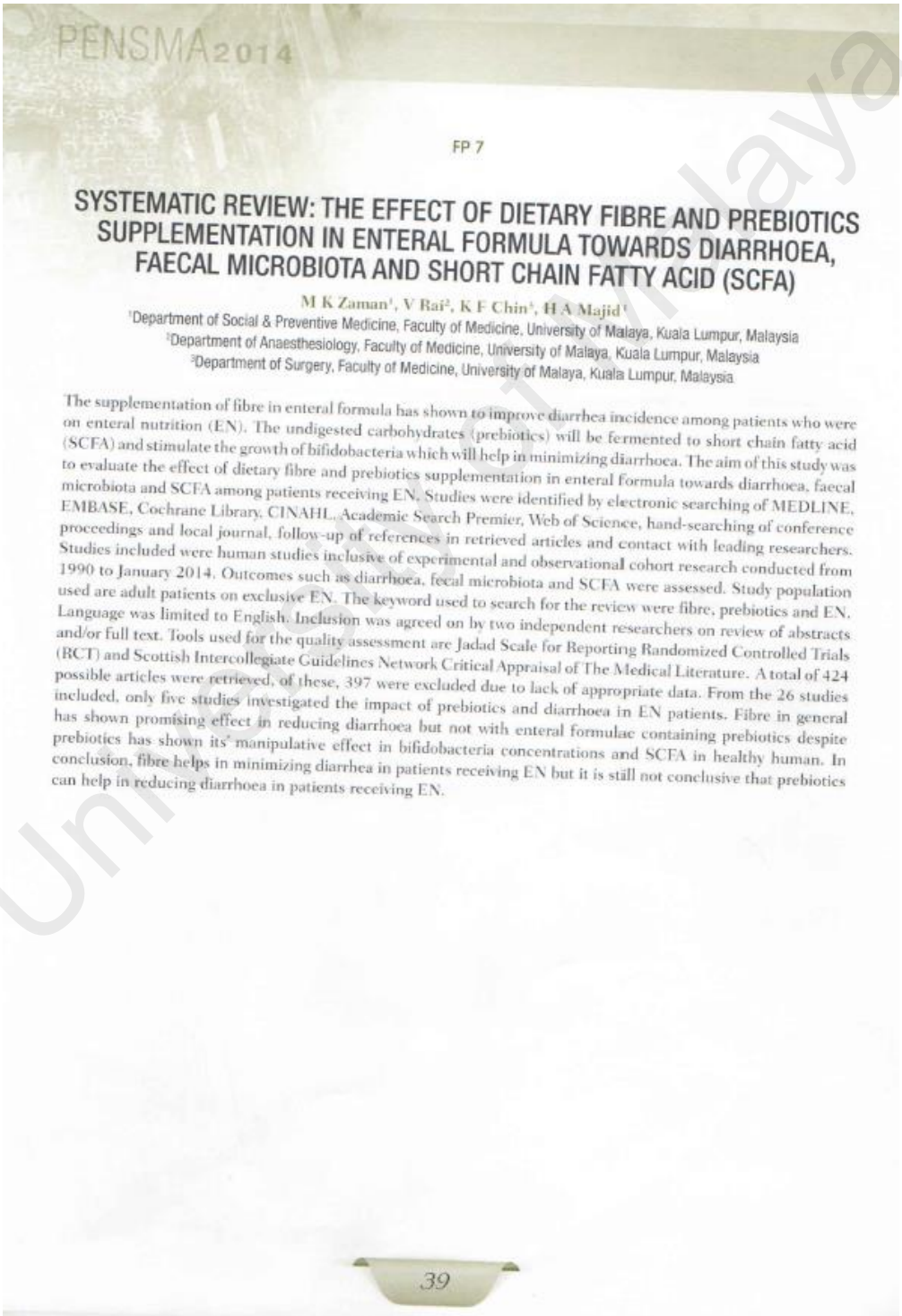
RESULTS: A total of 456 possible articles were retrieved, and 430 were excluded due to lack of appropriate data. Of the 26 remaining studies, only eight investigated the effects of prebiotics. Results of the meta-analysis indicated that overall, fiber reduces diarrhea in patients receiving EN (OR = 0.47; 95%CI: 0.29-0.77; P = 0.02). Subgroup analysis revealed a positive effect of fiber supplementation in EN towards diarrhea in stable patients (OR = 0.31; 95%CI: 0.19-0.51; $P < 0.01$), but not in critically ill patients (OR = 0.89; 95%CI: 0.41-1.92; P = 0.77). Prebiotic supplementation in EN does not improve the incidence of diarrhea despite its manipulative effect on bifidobacteria concentrations and SCFA in healthy humans. In addition, the effect of fiber and/or prebiotic supplementation towards fecal microbiota and SCFA remain disputable.

CONCLUSION: Fiber helps minimize diarrhea in patients receiving EN, particularly in non-critically ill patients. However, the effect of prebiotics in moderating diarrhea is inconclusive.

Key words: Bifidobacteria; Diarrhea; Enteral nutrition; Fiber; Prebiotics; Short-chain fatty acids

PAPER PRESENTED:

M K Zaman, V Rai K F Chin, H A Majid.Systematic review: The effect of dietary fibre and prebiotics supplementation in enteral formula towards diarrhoea, faecal microbiota and short chain fatty acid (SCFA). Parenteral and Enteral Nutrition Society of Malaysia (PENSMA) Congress, Malacca, Malaysia. 20-22 June 2014. **(Oral)**



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SYSTEMATIC REVIEW: THE EFFECT OF DIETARY FIBRE AND PREBIOTICS SUPPLEMENTATION IN ENTERAL FORMULA TOWARDS DIARRHOEA, FAECAL MICROBIOTA AND SHORT CHAIN FATTY ACID (SCFA)

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The supplementation of fibre in enteral formula has shown to improve diarrhea incidence among patients who were on enteral nutrition (EN). The undigested carbohydrates (prebiotics) will be fermented to short chain fatty acid (SCFA) and stimulate the growth of bifidobacteria which will help in minimizing diarrhoea. The aim of this study was to evaluate the effect of dietary fibre and prebiotics supplementation in enteral formula towards diarrhoea, faecal microbiota and SCFA among patients receiving EN. Studies were identified by electronic searching of MEDLINE, EMBASE, Cochrane Library, CINAHL, Academic Search Premier, Web of Science, hand-searching of conference proceedings and local journal, follow-up of references in retrieved articles and contact with leading researchers. Studies included were human studies inclusive of experimental and observational cohort research conducted from 1990 to January 2014. Outcomes such as diarrhoea, fecal microbiota and SCFA were assessed. Study population used are adult patients on exclusive EN. The keyword used to search for the review were fibre, prebiotics and EN. Language was limited to English. Inclusion was agreed on by two independent researchers on review of abstracts and/or full text. Tools used for the quality assessment are Jadad Scale for Reporting Randomized Controlled Trials (RCT) and Scottish Intercollegiate Guidelines Network Critical Appraisal of The Medical Literature. A total of 424 possible articles were retrieved, of these, 397 were excluded due to lack of appropriate data. From the 26 studies included, only five studies investigated the impact of prebiotics and diarrhoea in EN patients. Fibre in general has shown promising effect in reducing diarrhoea but not with enteral formulae containing prebiotics despite prebiotics has shown its' manipulative effect in bifidobacteria concentrations and SCFA in healthy human. In conclusion, fibre helps in minimizing diarrhea in patients receiving EN but it is still not conclusive that prebiotics can help in reducing diarrhoea in patients receiving EN.

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