

# Effectiveness of probiotic *Bifidobacterium animalis* DN-173010 in the management of constipation-predominant irritable bowel syndrome in black South African women

**MY Rammbwa**  
**22062904**

Mini-Dissertation submitted in *partial* fulfillment of the requirements for the degree *Magister Scientiae* in Dietetics at the Potchefstroom Campus of the North-West University

Supervisor: Prof E Wentzel-Viljoen  
Co-Supervisor: Prof JC Jerling  
Co-Supervisor: Prof R Blaauw

May 2014

## **ACKNOWLEDGEMENTS**

First and foremost, thank you Lord for your unfailing love and greatness. Words will never articulate my love and desire for you.

The completion of this dissertation would not have been possible without the help, support and patience of my Supervisors:

To my supervisor, Prof Edelweiss Wentzel-Viljoen, I would like to express my very great appreciation for your trust in me and your valuable and constructive suggestions throughout the research process. Your guidance and enthusiastic encouragement have been invaluable. I feel very honoured to have learnt under your leadership.

My deep gratitude goes to my co-supervisor, Prof Johann Jerling, who has continually and persuasively conveyed a spirit of adventure with regard to this research. It was always a pleasure to observe your way of thinking; without your supervision and constant help this dissertation would not have been possible.

I would also like to acknowledge with much appreciation the role of Prof Renée Blaauw, whose suggestions, telephonic conversations and encouragement helped me to coordinate my project.

I also take this opportunity to express my profound gratitude to Mr Stiaan Bester for making this project possible.

Ms Anneke Coetzee, librarian at NWU, your thoughts of me when you came across literature related to my topic and all your assistance are greatly appreciated. Many thanks, to Prof Suria Ellis for her statistical analysis assistance.

A great deal of appreciation goes to Prof Douglas Drossman for sending me literature related to my project which I did not even request, and for approval to use their validated IBS 34 questionnaire.

Special thanks to all the medical doctors who diagnosed and referred all the study participants: Dr B.A Mulaudzi, Dr M. Tun, Dr B.L Khulu, Dr A. Khayinza, Dr S. Essa; without your support this study would not have been a reality.

I wish to acknowledge the help given by my research assistance, Ms Basetsana Motene; thank you for always paying attention to your work. A special thanks to Mrs Ronel Benson, for her willingness to offer me help.

I express my sincere thanks and appreciation to my friend and sister, Dr Doreen Ross, for constantly encouraging me to continue studying even when the demands of my work and studies seemed too much to handle.

I would like to recall the affection of my mum and dad. Without their blessing and inspiration, I don't know what would have become of me. My brothers, Gilbert and Rendani, and sister, Olga, and the rest of my family members, many thanks for your endless support and encouragement. Thabelo Mafhuwa, your assistance has been massive.

Last but not least, thank you to my sweet daughter, Shaina, for being able to put up with me when spending long hours doing my academic work.

## **ABSTRACT**

### **Background**

Irritable bowel syndrome (IBS) is a poorly understood functional gastrointestinal disorder and is a major cause of abdominal discomfort and gut dysfunction. IBS symptoms encompass abdominal pain, bloating, flatulence and irregular bowel movements such as constipation, diarrhoea and alternating bowels, bloating, flatulence and irregular bowel movements. Physiological studies have shown that manipulation of the intestinal microbiota by antibiotics, prebiotics or probiotics can affect intestinal functions in the pathogenesis of IBS. The probiotic concept suggests that supplementation of the intestinal microbiota with the right type and number of live microorganisms can improve gut microbiota composition and promote health in IBS sufferers.

### **Aim**

The aim of the main clinical trial is to determine whether ingestion of fermented milk containing *Bifidobacterium animalis* DN-173010 is associated with improved defecation frequency, stool consistency and quality of life in black South African females with constipation-predominant IBS (IBS-C).

### **Methods**

A pilot and process evaluation approach was employed during the current study to examine and understand the feasibility of implementing the study and to explore the facilitating implementation of the main clinical trial. Twenty black female participants, aged 18-60, with IBS-C were recruited from the practices of gastroenterologists, specialist physicians and medical doctors in Soweto. Participants fulfilling the Rome III criteria for IBS-C and inclusion criteria were randomized into two groups to participate in a 4-week, double blind, placebo controlled study. The placebo group received unflavoured sweetened, white base yoghurt and the intervention group received similar yoghurt with the probiotic, *Bifidobacterium animalis* DN-173010 [ $>3,4 \times 10^7$  CFU/g]. Participants were required to record their bowel movements daily and IBS symptoms weekly in questionnaires during the four-week study period. Quality of life was assessed at baseline and at the end of the treatment period. Participants visited the study unit weekly to collect the placebo or probiotic study products and return the completed questionnaires during the study period.

### **Results**

Seventeen participants completed the study (eight intervention and nine placebo). There were not significant differences in IBS symptoms between the two groups, but differences were observed overtime within groups. The severity of abdominal pain score within both groups was statistically significant ( $p=0.004$ ), and the number of days with pain was also statistically significant ( $p=0.00001$ ). The frequency of

normal stools reported was statistically significant different compared to all the other stool types (constipation and loose stools) throughout the four-week study period in both the intervention and placebo group. There was no significant difference in the quality of life between the intervention group compared to the placebo group.

## **Conclusion**

Process evaluation allows for the monitoring of a programme and corrections of problems as they occur. The intervention is feasible to implement, acceptable and safe to participants. The study indicates that consumption of the probiotic *Bifidobacterium animalis* DN-173010 for four weeks is not superior to the placebo in relieving IBS symptoms.

## **Keywords**

Irritable bowel syndrome (IBS), constipation, probiotics, *Bifidobacterium animalis*.

## **OPSOMMING**

### **Agtergrond**

Prikkelbarederm-sindroom is 'n funksionele maagdermkwaal wat nie goed verstaan word nie en is 'n belangrike oorsaak van abdominale ongemak en dermwanfunsie. Die simptome daarvan is abdominale pyn, buikopsetting, winderigheid en 'n onreëlmatige stoelgangpatroon soos hardlywigheid, diarree en afwisselende samestelling van die stoelgang. Fisiologiese studies het getoon dat manipulasie van die dermbiotika deur antibiotika, prebiotika of probiotika die dermfunksies in die patogenese van prikkelderm-sindroom kan beïnvloed. Die probiotiese konsep dui daarop dat aanvulling van die dermmikrobiotika met die regte tipe en hoeveelheid lewende mikro-organismes die kenmerke van die mikrobiotika mag verbeter en lei tot verbeterde gesondheid in lyers aan prikkelderm-sindroom.

### **Doel**

Die doel van die hoof- kliniese proef is om vas te stel of die inname van gegiste melk wat *Bifidobacterium animalis* DN-173010 bevat, geassosieer word met verbeterde frekwensie van stoelgange, stoelgangdigtheid en lewenskwaliteit in swart Suid-Afrikaanse vroue met hardlywigheid-predominante prikkelderm-sindroom.

### **Metodes**

Proses-evaluasiedataversameling en prosedures is ingesluit in die huidige voorstudie om die uitvoerbaarheid van die hoof-kliniese proef te toets. Twintig swart vroulike deelnemers tussen 18 en 60 jaar oud wat geneig is tot hardlywigheid-predominante prikkelderm-sindroom is gewerf by die praktyke van gastro-enteroloë, spesialis-interniste en mediese dokters. Deelnemers wat aan die Rome III-kriteria voldoen, is willekeurig geplaas in 'n vierweke-, parallelegroep-, dubbelblind, placebo-gekontroleerde studie. Die placebo-groep het ongegeurde versoete wit basisjogurt ontvang en die intervensiegroep soortgelyke jogurt met probiotika *Bifidobacterium animalis* DN-173010 [ $>3,4 \times 10^7$  CFU/g]. Daar is van deelnemers verwag om hulle stoelgang daagliks en hulle prikkelderm-sindroomsimptome weekliks aan te teken in 'n vraelysboekie gedurende die vier weke van die studie. Hulle lewenskwaliteit is by die aanvang van die studie bepaal en weer aan die einde van die intervensieperiode. Dwarsdeur die studietydperk het deelnemers die studie-eenheid weekliks besoek om die produk af te haal en die voltooide vraelys in te handig.

### **Resultate**

Sewentien deelnemers het die studie voltooi (agt intervensie en nege placebo). Op grond van 'n omvattende evaluasie van die implementeringsproses is geen probleme ondervind wat die voorstudie gekompliseer het nie, maar dit kan steeds

verbeter word voor die hoof- kliniese proef. Daar was geen beduidende verskille in die algemene simptome van prikkelbarederm-sindroom tussen die twee groepe nie, maar verskille is mettertyd binne die groepe opgemerk. Die telling vir die hewigheid van abdominale pyn binne 'n groep was statisties beduidend ( $p=0.004$ ) en die aantal dae dat pyn ondervind is, was ook statisties beduidend ( $p=0.00001$ ). Die gereeldheid van normale stoelgang wat gerapporteer is, was statisties beduidend verskillend vergeleke met al die ander tipes stoelgange (hardlywigheid en los stoelgang) dwarsdeur die studietydperk van vier weke in sowel die intervensie- en placebo-groep. Daar was geen statisties beduidende verskil in die lewenskwaliteit van die intervensie-groep in vergelyking met die placebo-groep nie.

### **Gevolgtrekking**

Proses-evaluasie maak voorsiening vir die monitering van die program en die regstelling van probleme soos hulle voorkom. Die intervensie is lewensvatbaar om te implementeer, aanvaarbaar en veilig vir deelnemers. Die studie toon aan dat die inname van probiotiese *Bifidobacterium animalis* DN-173010 vir vier weke nie beter is as die placebo vir die verligting van die simptome van prikkelbarederm-sindroom nie.

### **Sleutelwoorde**

Prikkelbarederm-sindroom (spastiese dikderm), hardlywigheid, probiotika, *Bifidobacterium animalis*.

# TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS</b> .....	<b>ii</b>
<b>ABSTRACT</b> .....	<b>iv</b>
<b>OPSOMMING</b> .....	<b>vi</b>
<b>TABLE OF CONTENTS</b> .....	<b>viii</b>
<b>LIST OF TABLES</b> .....	Error! Bookmark not defined.
<b>LIST OF FIGURES</b> .....	Error! Bookmark not defined.
<b>CHAPTER 1: INTRODUCTION</b> .....	<b>0</b>
1.1 BACKGROUND AND MOTIVATION.....	0
1.2 RATIONALE FOR CONDUCTING A PILOT STUDY .....	1
1.3 IMPLEMENTATION OF PROCESS EVALUATION IN INTERVENTION TRIALS.....	2
1.4 AIMS AND OBJECTIVES .....	2
1.5 STRUCTURE OF THE MINI-DISSERTATION .....	3
<b>CHAPTER 2: REVIEW OF THE LITERATURE</b> .....	<b>4</b>
<b>PART 1: PROCESS EVALUATION</b> .....	<b>4</b>
<b>PART 2: IRRITABLE BOWEL SYNDROME AND PROBIOTICS</b> .....	<b>6</b>
2.1 INTRODUCTION .....	6
2.2 IRRITABLE BOWEL SYNDROME DIAGNOSIS AND CLINICAL SYMPTOMS.....	7
2.3 EPIDEMIOLOGY OF IBS.....	8
2.4 IBS PATHOPHYSIOLOGY .....	9
2.6 PROBIOTICS .....	13
2.7 MECHANISMS OF ACTION OF PROBIOTICS AND THERAPEUTIC BENEFITS OF PROBIOTIC FOR SOME OF THE IBS MECHANISMS.....	15
2.8 GENUS BIFIDOBACTERIUM AND GENUS LACTOBACILLI .....	17
2.9 VIABILITY OF BIFIDOBACTERIA IN FERMENTED PRODUCTS.....	19
2.10 EVIDENCE OF PROBIOTIC EFFICACY IN IBS .....	20
2.11 META-ANALYSES AND REVIEWS OF PROBIOTIC EFFECTS IN IBS.....	23
2.12 SAFETY OF PROBIOTICS.....	24
2.13 INTEGRATED APPROACH TO TREATMENT OF IBS .....	24
2.14 CONCLUSION .....	30
<b>CHAPTER 3: METHODOLOGY</b> .....	<b>31</b>
3.1 INTRODUCTION .....	31

3.2	STUDY DESIGN.....	32
3.4	RECRUITMENT PROCEDURE .....	34
3.5	RANDOMISATION.....	34
3.6	STUDY PRODUCT .....	35
3.7	ETHICAL CONSIDERATIONS.....	35
3.8	PROCESS EVALUATION AND ASSESSMENT .....	35
3.9	MEASURING INSTRUMENTS AND MODE OF ADMINISTRATION .....	37
3.10	STATISTICAL ANALYSIS.....	40
3.11	CONCLUDING REMARKS.....	40
	<b>CHAPTER 4: RESULTS .....</b>	<b>41</b>
4.1	OUTCOME OF THE PILOT STUDY .....	41
4.2	PROCESS EVALUATION.....	41
4.3	RESULTS INDICATING EFFECTIVENESS OF THE INTERVENTION.....	46
	<b>CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATION.....</b>	<b>58</b>
5.1	DISCUSSION.....	58
5.2	LESSONS LEARNED.....	61
5.3	CONCLUSION .....	61
5.4	LIMITATIONS.....	62
5.5	RECOMMENDATIONS .....	62
	<b>REFERENCES.....</b>	<b>63</b>
	<b>Appendix 1 Letter of ethical approval.....</b>	<b>86</b>
	<b>Appendix 2 Informed written consent form.....</b>	<b>87</b>
	<b>Appendix 3 Study process.....</b>	<b>88</b>
	<b>Appendix 4 Procedure Flow Chart .....</b>	<b>89</b>
	<b>Appendix 5 Demographic questionnaire.....</b>	<b>90</b>
	<b>Appendix 6 IBS-C screening.....</b>	<b>91</b>

## LIST OF TABLES

## Pages

<b>Table 2.1</b>	Different criteria for IBS	<b>11</b>
<b>Table 2.2</b>	Clinical trial with probiotic use	<b>25</b>
<b>Table 2.3</b>	Meta-analyses and systematic reviews of probiotic in IBS	<b>26</b>
<b>Table 2.4</b>	Summary of treatment strategies for IBS-C	<b>29</b>
<b>Table 3.1</b>	Process evaluation components	<b>40</b>
<b>Table 3.2</b>	Measuring instruments and mode of administration	<b>41</b>
<b>Table 4.1</b>	Process evaluation components results	<b>50</b>
<b>Table 4.2</b>	Baseline characteristics of the intervention and control group	<b>52</b>

## LIST OF FIGURES

## Pages

<b>Figure 2.1</b>	Criteria for classifying bacteria strain as probiotic	<b>18</b>
<b>Figure 2.2</b>	<i>Bifidobacterium</i> benefits in human health	<b>21</b>
<b>Figure 3.1</b>	Study flow design	<b>36</b>
<b>Figure 3.2</b>	Bristol stool form scale	<b>43</b>
<b>Figure 4.1</b>	Pilot study participants flow chart	<b>47</b>
<b>Figure 4.2</b>	Study logistics	<b>49</b>
<b>Figure 4.3</b>	Average number of yoghurt consumption over four weeks	<b>52</b>
<b>Figure 4.4</b>	Average number of bowel emptying over four weeks	<b>53</b>
<b>Figure 4.5</b>	Frequency of constipation stool type reporting per week over four weeks	<b>53</b>
<b>Figure 4.6</b>	Frequency of normal stool type reporting per week over four weeks	<b>54</b>
<b>Figure 4.7</b>	Frequency of loose stool type reporting per week over four weeks	<b>54</b>
<b>Figure 4.8</b>	Frequency of straining to open bowels per week over four weeks	<b>55</b>
<b>Figure 4.9</b>	Frequency of feeling an incomplete bowel emptying per week over four weeks	<b>55</b>
<b>Figure 4.10</b>	Participants experiencing abdominal pain over the four-week study period	<b>56</b>
<b>Figure 4.11</b>	Average number of days participants experienced pain over four weeks	<b>56</b>
<b>Figure 4.12</b>	Average number of days participants experienced pain over four weeks	<b>56</b>
<b>Figure 4.13</b>	Participants experiencing abdominal distention over four weeks	<b>58</b>
<b>Figure 4.14</b>	Percentage of severe abdominal distention over four weeks	<b>58</b>
<b>Figure 4.15</b>	Participants experiencing satisfactory relief of symptoms over four weeks	<b>59</b>
<b>Figure 4.16</b>	First week stool comparison	<b>60</b>
<b>Figure 4.17</b>	Second week stool comparison	<b>60</b>
<b>Figure 4.18</b>	Third week stool comparison	<b>60</b>
<b>Figure 4.19</b>	Fourth week stool comparison	<b>61</b>

## LIST OF ABBREVIATIONS

ACG	American College of Gastroenterology
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMI	Body mass index
BSS	Bristol stool scale
CBT	Cognitive behavioural therapy
CNS	Central nervous system
ENS	Enteric nervous system
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
FODMAPs	Fermentable oligosaccharides, disaccharides and polyols
GIT	Gastrointestinal tract
GRAS	Generally regarded as safe
GSS	General symptoms score
HRQOL	Health-related quality of life
IBS	Irritable bowel syndrome
IBS-A	Irritable bowel syndrome with alternating bowel movement
IBS-C	Constipation-predominant irritable bowel syndrome
IBS-D	Diarrhoea-predominant irritable bowel syndrome
IBS-M	Irritable bowel syndrome with mixed types
IBS QOL	Irritable bowel syndrome quality of life
IBS SSS	Irritable bowel syndrome severity scoring system
NICE	National Institute for Health and Clinical Excellence
PI-IBS	Post-infectious irritable bowel syndrome
RCT	Randomised controlled trial
SERT	Serotonin re-uptake inhibitor
SIBO	Small intestinal bacterial overgrowth
SSRIs	Selective serotonin re-uptake inhibitors
TCAs	Tricyclic antidepressants
PEG	Polyethylene glycol
UFE	Utilisation focused evaluation
WHO	World Health Organization

# CHAPTER 1: INTRODUCTION

## 1.1 BACKGROUND AND MOTIVATION

The incidence of gastrointestinal (GI) disturbances such as inflammatory bowel disease and irritable bowel syndrome (IBS) is on the rise worldwide, particularly in industrialised countries (Armitage *et al.*, 2001; Loftus *et al.*, 2002). IBS is a functional GI disorder in which abdominal pain or discomfort is associated with altered bowel habits (McFarland & Dublin, 2008). Other IBS symptoms typical include bloating, flatulence, stool urgency or straining and a feeling of incomplete evacuation (Drossman *et al.*, 2002). The clinical features of IBS are formalised according to the Rome II and III criteria, which are widely accepted and by definition, the diagnosis excludes structural or biochemical abnormalities of the gut (Longstreth *et al.*, 1999). IBS itself is a heterogeneous condition which, according to the modality of bowel alterations, presents as three main subtypes: constipation-predominant IBS (IBS-C), diarrhoea-predominant IBS (IBS-D) and IBS with alternating bowel movement (IBS-A) (Tillisch *et al.*, 2005). The recent Rome III group has also established diagnostic criteria for IBS-C and IBS-D. IBS-C is described as the passage of fewer than three stools per week, hard or lumpy stools, frequent straining and incomplete evacuation (Irvine *et al.*, 2006). IBS-D is described as more than three bowel movements a day, urgency and loose or watery stools. IBS-A is described as alternating constipation and diarrhoea (Longstreth *et al.*, 2006).

IBS is the most common chronic GI disorder and may affect as many as 25% of the adolescent and adult population in the Western countries (Cremonini & Talley, 2005). The incidence of IBS is higher in females than in males and it is ranked as their most bothersome condition (Sondergaard *et al.*, 2011). The pathophysiology of IBS is still not well understood (Andresen & Baumgart, 2006) and may include motor and sensory dysfunction, immune responses, food sensitivities and genetic predisposition (Cremonini & Talley, 2005). IBS can have a significant impact on an individual's quality of life (QOL), high rates of absenteeism with economic consequences and increased health care costs (Talley, 2008).

The prevalence of IBS in South Africa is unknown: however, the progressive transition to Westernisation of diets, increased sedentary lifestyles, increased use of drugs and higher stress levels among the black South African population are likely to be associated with an increased incidence of IBS (Stevenson & Blaauw, 2011). The African diet, in which traditionally a variety of wild and cultivated vegetables and fruits made a substantial contribution to the diet, is now low in fruit and vegetable intake owing to the migration of Africans to urban areas (Van Eeden & Gericke, 1996), where plant cultivation is difficult. Urban, westernised diets, high in energy and animal products, which are high in salt, fat and cholesterol, yet low in fibre, could impair QOL and are also associated with cardiovascular and GI disease (Gralnek *et al.*, 2000; Inadomi *et al.*, 2003; Amouretti *et al.*, 2006; Berg *et al.*, 2006).

The efficacy of most drug therapies in the treatment of IBS is weak (Quartero *et al.*, 2005) and not optimal (Vahedi *et al.*, 2005). Drug therapies are mainly symptom-orientated and do not address the underlying causes. However, the use of functional foods has been a matter of intense and growing scientific interest since the first scientist (Metchnikoff, 1907) reported the potential beneficial effects of lactic acid bacteria, such as those found in yoghurt. Evidence has shown that balanced intestinal microflora is necessary for maintaining health and preventing disease (Kassinen *et al.*, 2007). Research suggests that overgrowth of pathogenic gut bacteria, among other factors, plays a role in IBS and that probiotics may be effective in alleviating the symptoms of this condition (Moayyedi *et al.*, 2010).

A recent systematic review has shown that division of the IBS population into subcategories, as IBS-C or IBS-D, is recommended to identify IBS patients who are likely to benefit most from probiotics treatment (Hoveyda *et al.*, 2009). Studies where constipation-predominant IBS was treated with *Bifidobacterium lactis* DN-173 010, showed that the health-related quality of life (HRQOL) score improved within three to four weeks following intervention, but was not different from the placebo at the end of the trial (Guyonnet *et al.*, 2007; Roberts *et al.*, 2013). *Bifidobacteria* strains are widely used in food items and have been added to various products including fermented milks, cottage cheese and pharmaceutical preparations (Bouvier *et al.*, 2001; Tamine *et al.*, 1995) to speed up intestinal transit or shorten the colonic transit time to manage constipation.

To date, there has been no study that investigated the effects of *Bifidobacterium animalis* DN-173 010 on South African subjects with IBS-C. The aim of the main clinical trial is to investigate the effect of *Bifidobacterium animalis* DN-173 010 on bowel movements and IBS symptoms, such as abdominal pain and bloating and as well as the effect on the QOL within four weeks, in black South African women with IBS-C, aged 18 to 60.

The research team planned to conduct a pilot study to establish the parameters and operational standards for the main clinical trial, which will continue with the original protocol and evaluate the effectiveness of the intervention. The purpose of the pilot study is to increase the likelihood of success of interventions in subsequent larger studies by ensuring they are appropriate and effective in practice (Leon *et al.*, 2007; Rounsaville & Carol, 2001).

## **1.2 RATIONALE FOR CONDUCTING A PILOT STUDY**

Pilot studies are a crucial step in the research process (Lancaster *et al.*, 2004). They fulfil a range of important functions, such as developing and testing the adequacy of research instruments and assessing the feasibility of a full-scale study and people's willingness to participate, and provide valuable insights for other researchers (Gardner *et al.*, 2003). Evidence suggests that testing the adequacy of measuring

instruments (questionnaires) is probably the most valuable function of the pilot study (VanTeijlingen & Hundley, 2001).

A pilot study can be either internal or external. An internal pilot study is a study that is incorporated into the main study design of the randomised controlled trial (RCT). An external pilot study is defined as a stand-alone piece of work planned and carried out independently from the main study (Lancaster *et al.*, 2004). In the present study we used an external pilot study.

### **1.3 IMPLEMENTATION OF PROCESS EVALUATION IN INTERVENTION TRIALS**

Process evaluation is the systematic documentation of key aspects of programme performance that indicates whether and how well the designed programme is operating and whether it is delivered as intended to the recipients (Rossi *et al.*, 2004; Saunders *et al.*, 2005). It is defined as a form of programme monitoring (Rossi *et al.*, 2004). The use of process evaluation in intervention studies can help identify breaks in the chain of the implementation pathway, which can provide useful information in future studies (Habicht *et al.*, 2008).

A process evaluation approach was employed during the pilot study to examine and understand the feasibility of implementing the study and to explore the factors facilitating implementation.

### **1.4 AIMS AND OBJECTIVES**

The aim of the main clinical trial is to determine whether ingestion of fermented milk containing *Bifidobacterium animalis* DN-173 010 is associated with improved defecation frequency, stool consistency and QOL in IBS-C black South African females. The aim of this study also includes process evaluation and testing the methodology and measuring instruments for the main clinical trial.

#### **1.4.1 Objectives of process evaluation**

The objectives of the process evaluation are to:

- Validate the recruitment procedure;
- Monitor the operational process;
- Test the eligibility criteria;
- Test the feasibility of product delivery;
- Estimate the consent rate for the main study;
- Test the randomisation procedure;
- Test investigator and assistant skills in the procedure;
- Determine whether measuring instruments are completed correctly;

- Test the acceptability of the intervention; and
- Test the processes of data collection, data quality and data analysis.

#### **1.4.2 Objectives of intervention effectiveness**

The objectives in determining the effectiveness of the treatment are to:

- Investigate the effect of the probiotic, *Bifidobacterium animalis* DN-173 010 on defecation frequency within four weeks;
- Investigate the effect of consuming fermented milk containing the probiotic, *Bifidobacterium animalis* DN-173 010 on IBS symptoms such as abdominal pain and bloating within four weeks;
- Determine the effect of probiotic *Bifidobacterium lactis* DN-173 010 on stool consistency and stool type within a period of four week; and
- Investigate the effect of the probiotic *Bifidobacterium animalis* DN-173 010 on QOL within four weeks.

### **1.5 STRUCTURE OF THE MINI-DISSERTATION**

This mini dissertation consists of five chapters. Chapter 1 explains background information on the study, aims and objectives of the study. In Chapter 2 a literature review is explored, to provide an overview of the topic. Chapter 3 describes the design, methodology, study participants and questionnaires used and the possible evaluation process. Chapter 4 summarizes the results and the main findings. Chapter 5 comprises of the discussion and recommendations followed by list of references.

# CHAPTER 2: REVIEW OF THE LITERATURE

## PART 1: PROCESS EVALUATION

Process evaluation is defined by Rossi *et al.*, (2004) as a research procedure to systematically investigate the effectiveness of social intervention programmes that are adapted to their political and organisational environments and designed to inform social action in ways that improve social conditions. Process evaluation within a trial explores the implementation, setting of an intervention, monitoring and documenting programme implementation and can aid in understanding the relationship between specific program outcomes (Saunders *et al.*, 2005). It may aim to examine the views of participants in the intervention, study how the intervention is implemented, distinguish between components of the intervention, investigate contextual factors that affect an intervention and study the way effects vary in subgroups (Wight & Obasi, 2002).

Process evaluation helps to avoid the error of drawing conclusions about the effectiveness of a study without knowing whether or not the programme has been adequately implemented (Bartholomew *et al.*, 2001). Evidence has also shown that process evaluation provides a clear, descriptive picture of the quality of programme elements and what is happening as the programme proceeds (Two Feathers *et al.*, 2007). Process evaluation may also contribute to an understanding of why participants may have benefited from a programme. Process evaluations have been conducted in several studies for a variety of practical reasons: To aid in making decisions on whether programmes should be continued, improved, expanded or curtailed; to assess the utility of new programmes or initiatives and to increase the effectiveness of programme management and administration (Lovestam *et al.*, 2013; Wandersman, 2009). To measure the outcomes of interventions accurately, it is important to ensure that the implementation is completed as originally designed (Lee *et al.*, 2013).

Stectler & Linnan (2002) established that process evaluation is an important part of any public health intervention research and listed the components that a process evaluation should examine. The key components are summarised by Saunders *et al.*, (2005) as follows: Context, extent of reach of the programme, dose of intervention delivered, dose received and fidelity and the nature of the recruitment process. Process evaluation for reach, recruitment and context has been shown to involve documentation and record-keeping, with part of the planning involving specifying the specific elements to document or monitor (Saunders *et al.*, 2005). The authors further reported that specifying the dose delivered is simple and straight forward after the components of the intervention have been defined, whereas the dose received, which is the expected participant reaction or involvement is reported to be more challenging in that it requires thoughtful consideration of the expected

reactions in participants. Fidelity of intervention delivery includes the extent to which the intervention is delivered as intended.

Process evaluation has been performed in different settings, using both quantitative and qualitative methods and has increasingly been incorporated into health intervention studies (Martens *et al.*, 2006; Matthews *et al.*, 2012; Schneider *et al.*, 2009; Story *et al.*, 2000). Quantitative methods have the advantage of being amenable to quick analyses, brief reports and relatively straightforward interpretation, but are often not capable of answering questions such as why and how a particular intervention component was not being received as intended. Qualitative methods have the advantage of being able to elicit unanticipated information, suggested solutions or innovations that address these kinds of questions, as well as the diverse perspectives of different groups participating in the study (that is principal investigator, research assistants, medical practitioners and participants). In all studies, process evaluation offered valuable insights into reasons why the programmes may have been more or less effective in achieving their goals.

The conceptual model for the process evaluation of the study was modified from the model proposed by Saunders *et al.*, (2005) and is described in Chapter 3. To ensure comprehensive coverage of study procedures, we compiled a list of components derived from the CONSORT 2010 statement (Moher *et al.*, 2010). For each component potential methods of monitoring procedures and potential outcomes from that monitoring process were considered, covering the following:

- Feasibility and appropriateness of the study design.
- Feasibility and appropriateness of the intervention, management and safety of intervention.
- Acceptability and efficiency of implementing the research procedures.

## PART 2: IRRITABLE BOWEL SYNDROME AND PROBIOTICS

### 2.1 INTRODUCTION

The World Health Organization (WHO) and the Food and Agricultural Organization (FAO) have defined probiotics as “live microorganisms, that when administered in adequate amounts, have beneficial effects for the host” (WHO/FAO, 2001). The use of probiotics in the treatment IBS was reviewed in a recent meta-analysis, which suggested favourable therapeutic effects of probiotics and recommended further studies on the use of probiotics for IBS, particularly given the chronic nature of the condition, weak evidence of the efficacy of drug therapies and the impact of the condition on patients’ QOL (McFarland & Dublin, 2008; Moayyedi *et al.*, 2010). Recent systematic reviews have also demonstrated a positive role in alleviating symptoms of IBS and recommend further studies that focus on the type, optimal dose of probiotics and the subgroups of patients who are likely to benefit the most (Hoveyda, *et al.*, 2009; McFarland & Dublin, 2008).

IBS, a functional bowel disorder, is associated with significantly reduced QOL (Farndale & Roberts, 2011) and many patients seek alternative strategies for relief of symptoms (Harris & Roberts, 2008). One of the simplest and attractive options for patients is the inclusion of functional foods in their diet (Salonen *et al.*, 2010), and among the functional foods, probiotics have gained interest since gut microbiota may be involved in gastrointestinal functions and alterations in gut microbiota have been shown in IBS (Hoveyda *et al.*, 2009; Moayyedi *et al.*, 2010).

*Lactobacilli* and *Bifidobacteria* are the most common microbes used as probiotics and are widely added to yoghurts and other dairy products. Within the *Lactobacillus* and *Bifidobacterium* genus there are different species, for example *Lactobacillus plantarum* and *Bifidobacterium animalis* and within each species there are different strains, for example, genus (*Bifidobacterium*); species (*animalis*) and strain (DN-173 010) and genus (*Lactobacillus*); species (*plantarum*) and strain (299v). Some probiotic strains, either single or in combination, have been associated with significant alleviation in IBS symptoms (Kajander *et al.*, 2008; Kim *et al.*, 2005; Nobaek *et al.*, 2000; O’Mahony *et al.*, 2005; Whorewell *et al.*, 2006), while others proved ineffective (Camilleri, 2006; Floch, 2005; Niv *et al.*, 2005). Probiotics from the *Lactobacillus* and *Bifidobacterium* genus offered promise for the treatment of IBS in clinical trials (Hoveyda *et al.*, 2009). Reviews uniformly conclude that despite a number of confounding variables, probiotics promise benefits to IBS patients but there are many variables affecting the results such as the type, dose and formulation of bacteria comprising the probiotic preparation, the outcome measured, size and IBS subtype or characteristics of the IBS population studied (McFarland & Dublin, 2008; Moayyedi *et al.*, 2010).

A well investigated fermented milk probiotic product, containing the probiotic *Bifidobacterium* strain, *Bifidobacterium animalis* DN-173010 now also known as *Bifidobacterium lactis* CNCM 1-2494, has shown beneficial effects on gut functions in

several small randomised controlled studies (Agrawal *et al.*, 2009; Guyonnet *et al.*, 2007; Marteau *et al.*, 2002; Roberts *et al.*, 2013). In a meta-analysis of 74 studies and 84 clinical trials (10 351 patients) in which the effect of one or more *Bifidobacteria* strains (alone or in combination with other lactic acid bacteria) was studied, positive effects on IBS, pouchitis, infectious diarrhoea, helicobacter pylori infection, Clostridium difficile diarrhoea, and antibiotic-associated diarrhoea were significantly reduced (Ritchie & Romanuk, 2012). *Bifidobacterium* is one of the dominant species in intestinal microflora (Marteau *et al.*, 2001) and its supplementation improves microflora while the harmful bacteria decrease. It furthermore facilitates bowel movement, and the increased defecation frequency relieves constipation (Katsuno *et al.*, 2003). It has also been well documented that *Bifidobacterium animalis* DN-173 010 has a high resistance to gastric and bile acids (Berrada *et al.*, 1991; Pochart *et al.*, 1992) and high resistance to aggression of digestive enzymes, as well as the ability to travel through the human digestive tract and arrive at the large intestines alive (Pochart *et al.*, 1992). Beneficial effects of *bifidobacteria* or *bifidobacteria*-containing products on human health have been observed, namely improving discomfort in patients suffering from IBS, including a reduction in bloating, abdominal pain, abdominal discomfort and transit time (Hoveyda *et al.*, 2009). *Bifidobacteria* has been one of the targets of the functional food industry, generally being administered as adjunct cultures in functional dairy products (Masco *et al.*, 2005).

## **2.2 IRRITABLE BOWEL SYNDROME DIAGNOSIS AND CLINICAL SYMPTOMS**

The symptoms of IBS comprise chronic or recurrent abdominal discomfort or pain, abnormal bowel habit and abdominal bloating and flatulence or straining and a feeling of incomplete evacuation (Drossman *et al.*, 2002). Diagnosis of IBS is based on symptom representation and a thorough initial evaluation of any organic abnormalities (Grundmann & Yoon, 2009). Hahn *et al.*, (2008) showed that IBS symptoms typically fluctuate. They reported that over 12 weeks, symptoms arose a mean of 12 times with a maximum duration of five days, affecting patients on about 50% of days; 75% of patients remained symptomatic five years later. IBS has a good prognosis and at least in a few individuals symptoms can be resolved (Janssen *et al.*, 1998; Owens *et al.*, 1998).

The following symptoms cumulatively support the diagnosis of IBS, according to the Rome II and Rome III criteria (Longstreth *et al.*, 2006):

- Abnormal stool frequency (abnormal may be defined as >3 bowel movements per day and <3 bowel movements per week);
- Abnormal stool form (lumpy/hard or loose/watery stool);
- Abnormal stool passage (straining, urgency or feeling of incomplete evacuation);
- Passage of mucus; and

- Bloating or a feeling of abdominal distention.

Diagnosis of IBS in the current study is based on the Rome III diagnostic criteria (Table 2.1), which measure the IBS symptoms on frequency scales rather than presence/absence scales, as was done for Rome I and Rome II (Drossman & Whitehead, 2010). The Rome foundation continues its mission to improve knowledge of the science and practice relating to functional GI disorders and has received support from academic organisations, investigators and clinicians, pharmaceutical regulatory agencies, pharmaceutical companies and federal research agencies.

The Rome III revised IBS subtyping and recommended that patients be grouped into different subtypes based on stool consistency. Different subtypes are thought to reflect different pathophysiological mechanisms (Engsbro *et al.*, 2011). Three subtypes of IBS have been defined: IBS-C, IBS-D and IBS mixed types (IBS-M). IBS-C and IBS-D are the two dominant subtypes of IBS; a mixed subtype (IBS-M) occurs less frequently (Grundmann & Yoon, 2010).

**Table 2.1** Different diagnostic criteria for irritable bowel syndrome

<b>Manning criteria (1978)</b>	<b>Rome II criteria (1999)</b>	<b>Rome III criteria (2006)</b>
	Pain or discomfort for 12 weeks of the previous 12 months associated with:	Fewer than three bowel movements a week
Pain relieved by defecation	<ul style="list-style-type: none"> <li>• Relief with defecation</li> </ul>	More than three bowel movements a day
Looser stools at onset of pain	<ul style="list-style-type: none"> <li>• Looser or more frequent stools</li> </ul>	Loose (mushy) or watery stools
More frequent painful stools	<ul style="list-style-type: none"> <li>• Harder or less frequent stools</li> </ul>	Hard or lumpy stools
Visible abdominal distention		Straining during a bowel movement
Passage of mucus	Symptoms that cumulatively lend support to the diagnosis	Urgency (having to rush to a bowel movement)
Feeling of incomplete rectal emptying	Abnormal stool frequency > 3 bowel movements per day and <3 bowel movements per week	Feeling of incomplete bowel movement
	Abnormal stool form (hard or loose/watery)	Passing mucus (white matter)
	Abnormal stool passage (straining, urgency, or feeling of incomplete rectal emptying)	Abdominal fullness, bloating or swelling
	Passage of mucus	
	Bloating or feeling of abdominal distention	

## 2.3 EPIDEMIOLOGY OF IBS

Gastrointestinal symptoms are frequent in the general population with 60-70% of people reporting one or more symptoms (Drossman *et al.*, 2006). IBS is one of the

most frequent explanations for chronic GIT symptoms, although its prevalence varies, depending on the criteria used, from 3% to 22% (Drossman *et al.*, 2002; Kay *et al.*, 1996; Saito *et al.*, 2000) and it is increasing in countries with developing economies (Lannitti & Palmieri, 2010). The incidence of IBS peaks in the third and fourth decade of life and reduces slightly in elderly people (Brandt *et al.*, 2009). Irrespective of the IBS definition given, IBS has a substantial effect on QOL and health care costs (Talley & Spiller, 2002).

Results of population-based studies (Kay *et al.*, 1996; Talley *et al.*, 1998) applying factor analysis and cluster analysis have shown that a frequent entity that accords with IBS arises similarly in different countries. Female predominance in the Western world has been noted, where women are three to four times more likely than men to be diagnosed with IBS. Race does not appear to be a factor in the prevalence - the prevalence is similar in whites and blacks (Podovei & Kuo, 2006).

## **2.4 IBS PATHOPHYSIOLOGY**

The pathogenesis of IBS is not completely understood, but is evolving (Crowell *et al.*, 2005), hence drug development has been difficult (Whorwell *et al.*, 2006). The pathophysiology of IBS is multifactorial and most hypotheses centre on one or more of the following: Alteration in intraluminal milieu, immune activation, brain-gut axis dysregulation and enteric neuromuscular dysfunction (Cremonini & Talley, 2005).

Mechanisms such as altering of intestinal luminal environment, maintenance of the mucosal barrier function and modulation of the immune system may explain the reduction in IBS symptoms after treatment with probiotics (Lee & Bak, 2011). Findings pertaining to the pathogenesis and treatment of IBS are the following:

### **2.5.1 Alteration in the intestinal milieu**

Various studies have suggested qualitative changes in the colonic flora in IBS patients, a relative decrease in the population of *Bifidobacteria* being the most consistent finding (Madden *et al.*, 2005; Malinen *et al.*, 2005). The indigenous colonic microbial bacterial flora plays an important physiological role in the gut. Firstly, the resident flora contributes to enhancing the intestinal barrier function, thus preventing the adhesion of pathologic bacteria and inhibiting the invasion of pathogenic agents into the body (Baumgart & Dignass, 2002; Van der Waaij *et al.*, 1991). There is evidence that post-infectious IBS patients may experience increased gut permeability (Spiller, 2004).

More recently, the role of the gut flora in IBS has been taken a stage further with the suggestion that some IBS patients may harbour quantitative changes in the indigenous flora, in the small intestine, developing small intestinal bacterial overgrowth (SIBO) (Lin, 2004). The occurrence of SIBO has been associated with abnormalities in small intestinal motor function (Pimentel *et al.*, 2002) and its

eradication with symptomatic relief (Pimentel *et al.*, 2003). Further evidence of the role of altered indigenous flora in IBS has been shown by a study that described improvements in IBS symptomatology, in the short term, following administration of the non-absorbable antibiotic Rifaximin to a group of patients who did not have SIBO at baseline (Sharara *et al.*, 2006).

### **2.5.2 Immune system activation**

There is accumulating clinical evidence that suggests an association between IBS in general and immune activation. Slow onset of IBS can follow a GI infection (Mayer *et al.*, 2001), in which case it is classified as post-infectious IBS (PI-IBS). Mucosal biopsies in patients with post-infectious (PI)-IBS indicate a persistent, mild inflammatory state, defined by increased numbers of inflammatory cells and serotonin-releasing entero-endocrine cells in the mucosa (Jenkins *et al.*, 2000; Spiller, 2004; Spiller *et al.*, 2000). It has also been noted that constipation is associated with inflammatory activation of the colonic mucosa (Khalif *et al.*, 2005). Another mucosal immune system alteration found in IBS patients is an increased number of activated mast-cells in the proximity of colonic nerves in the lamina propria, where mast cells secreted mediators such as tryptase and histamine may contribute to the development of abdominal pain (Stangellini *et al.*, 2004). Since serotonin is one of the most important neurotransmitters of the enteric nervous system (ENS), mucosal changes in serotonin levels would affect both sensory motor functions possibly contributing to IBS symptoms (Borman, 2001).

### **2.5.3 Altered colonic motility**

Altered gut motility is considered to be an important underlying factor of IBS. More than 50% of patients with IBS report exacerbation of symptoms after eating (Kellow *et al.*, 2003). Eating is of course a major stimulus to colonic motility; the outcome of this stimulation depends on the balance between the mixing of motor patterns, which predominates in healthy people (Tache *et al.*, 2001) and propulsive contractions, which seem to be exaggerated in IBS-D (Whitehead *et al.*, 1992). Researchers have reported that patients with IBS have increased sensitivity to cholecystokinin and an exaggerated response to eating (Mayer *et al.*, 2001). A study by Levy *et al.*, (2000) has shown that cholecystokinin-1 antagonist, loxiglumide, selectively slowed proximal colonic transit in patients with IBS. By contrast, patients with IBS-C have been shown to have fewer propulsive contractions after eating (Levy *et al.*, 2000) and patient with IBS-D experience shorter small bowel and colonic transit than those with constipation (Hahn *et al.*, 2008).

### **2.5.4 Gender differences and IBS symptoms**

Gender-related differences in the pathophysiology of IBS have increasingly been identified with the development of new pharmacologic agents (Crowel *et al.*, 2005). In both population-based studies and clinic-based surveys, the prevalence of IBS have been shown to be higher among women. Several studies have demonstrated

that women are more sensitive to experimental pain than men, but most of these have been limited to somatic thresholds. Observation by Lee *et al.*, (2001) has suggested that non-painful gastrointestinal symptoms, such as constipation and somatic discomfort, are more commonly reported by female IBS patients. Relatively few data are available that demonstrate the consistent physiologic differences in visceral thresholds or motor patterns in women compared with men (Crowel *et al.*, 2005). Gender-related differences in sympathetic nervous system response to rectosigmoid stimulation have been reported more consistently (Mayer *et al.*, 2001; Waring *et al.*, 2004). There is also evidence that men and women with IBS may have fundamental differences in the brain response to aversive pelvic visceral stimuli and men with IBS showed greater activation of brain regions that may be involved in endogenous pain inhibition, whereas women with IBS showed greater activation of limbic and paralimbic regions, which are thought to be part of the pain facilitation circuit (Naliboff *et al.*, 2003).

Studies have begun to suggest that women exhibit greater temporal summation of heat and mechanically evoked somatic pain. It is further proposed that increased temporal pain summation in women compared with men might suggest a central nociceptive hyperexcitability in women, at least for somatic pain thresholds (Sarhani *et al.*, 2004).

### **2.5.5 Hormonal hypothesis**

The gender differences observed might in part be explained by the role of hormones. The hypothesis of oestrogen involvement in the aetiology of IBS is raised by clinical observations that IBS is more likely to be found in women and reports of symptom exacerbation during the menstrual cycle by some patients (Podovei & Kuo, 2006). Significant improvement in abdominal pain in functional bowel disorders by inhibition of the hypothalamopituitary-ovarian axis with leuprolide acetate was observed (Mathias *et al.*, 1998a; Mathias *et al.*, 1998b). Hormone replacement therapy is also associated with an increased risk of IBS (Ruigomez *et al.*, 2003). Heitkemper *et al.*, (2003) followed the symptom profile of IBS and controls during the menstrual cycle and found the IBS group to have not only more GI symptoms, but also more somatic and menstrual symptoms.

### **2.5.6 Role of stress in IBS**

Stress is widely believed to play a major role in the pathophysiology, clinical presentation and therapeutic outcome in IBS. Stress has been associated with symptom onset, exacerbation and severity (Crowell *et al.*, 2005). The slow onset of IBS over weeks and months shows strong correlation with stress disorders such as depression and anxiety (Mayer *et al.*, 2001). Even though the effects of stress on gut function appear universal, patients with IBS may have greater reactivity to stress compared with healthy individuals (Dickhaus *et al.*, 2003). Although most patients with IBS agree that stress aggravates the disorder, the variance in bowel symptoms attributable to acute stress is only 11% (Whitehead *et al.*, 2000).

The relationship between IBS symptoms and stress reactivity may be modulated by differential neuroendocrine responsiveness (Crowell *et al.*, 2005). Corticotropin-releasing hormone is a major mediator of the stress response in the brain-gut axis (Sagami *et al.*, 2003). One study compared the responses of IBS patients to rectal balloon distensions before, during and after mental stress with responses in healthy control subjects (Posserud *et al.*, 2004). The investigators concluded that both neuroendocrine responses and visceral perception are altered in IBS patients during and after stress, and that the observations may explain some of the stress-related GI symptoms in IBS.

Diagnosis with consideration of stress disorders and explanation to the patient about the relationship between altered central nervous system (CNS) signalling and IBS development may aid in establishing positive health-care provider-patient rapport, with consistently better clinical outcomes (Dhaliwal *et al.*, 2004; Lee *et al.*, 2001).

### **2.5.7 Genetic factors in IBS and the serotonin re-uptake transporter**

Following the Rome III criteria, a patient can be diagnosed with IBS by considering the family and clinical history (colon cancer, onset of symptoms later than age 50 years), representation of gradual onset and consistency of symptoms, with no specific warning signs indicative of a specific pathophysiology (including rectal bleeding, anaemia, weight loss, fever) and normal laboratory results (Grundmann & Yoon, 2010).

The regulation of serotonin and the role of the serotonin re-uptake inhibitor (SERT) have stimulated most genetic research (Harris & Roberts, 2008). SERT is one of the primary mechanisms that the body has for regulating the availability of serotonin in the extracellular space (Crowell *et al.*, 2005). SERT is present in the brain and gut. It has been shown that a decrease in SERT consistently leads to dysfunction of GI motility in animals and in humans through increased serotonin concentrations (Coates *et al.*, 2004). Elevated serotonin concentrations stimulate 5-HT<sub>3</sub> and 5-HT<sub>2</sub> receptors, leading to dysregulated contraction and dilation of the intestinal tract. Attenuation of this signalling cascade is employed for treatment of IBS and various other GI disorders (Grundmann & Yoon, 2010). A few studies have explored the association of IBS and SERT polymorphisms and reported several observations (Kim, *et al.*, 2005; Pata *et al.*, 2002; Yeo *et al.*, 2004; Kim). Firstly, the distribution of the 1/s genotype seems heterogeneous in different populations. This observation has important implications when comparing studies from different ethnic groups. Secondly, associations of SERT polymorphisms with either IBS in general or a subtype of IBS in particular have not been shown consistently. Lastly, certain SERT polymorphisms have been associated with high somatic symptom scores. The findings are consistent with studies linking SERT polymorphisms with certain behavioural disorders, such as anxiety and depression (two conditions commonly seen in association with IBS). Although SERT may not equal IBS, SERT dysfunction may have gastrointestinal and behavioural phenotypes (Crowell *et al.*, 2005).

### **2.5.8 Brain-gut interaction and visceral hypersensitivity**

Visceral hypersensitivity and increased viscerosomatic referral are frequently present in patients with IBS and may provide an important biomarker for the assessment of pharmacologic interventions aimed at improving pain and discomfort in IBS patients (Camilleri, 2006). Bidirectional communication from the brain to the ENS occurs through sympathetic and parasympathetic pathways (Coulie *et al.*, 2002).

Experimental evidence suggests that a variety of perceptual alterations exist in patients with IBS that involve visceral hypersensitivity of the upper and lower gastrointestinal tract (GIT) (Crowell *et al.*, 2005). Boulin *et al.*, (2004) reported that IBS patients with concomitant functional dyspepsia had decreased gastric and rectal sensory thresholds, whereas IBS patients without dyspeptic symptoms displayed hypersensitivity to rectal distention only. They then concluded that visceral hypersensitivity to distention can be pan-intestinal in patients with multiple upper and lower gastrointestinal symptoms, but patients with more specific complaints tend to have organ-specific hypersensitivity (Crowell *et al.*, 2005).

### **2.5.9 Intestinal gas**

Bloating is one of the most common and bothersome abdominal complaints in subjects with IBS and is reported by up to 96% of IBS sufferers (Lembo *et al.*, 1999). Some cases of bloating have been reported to stem from dysfunctional somatic muscular activity in the abdominal wall (Azpiroz & Malagelada, 2005). Perez *et al.*, (2005) postulated roles for weak abdominal muscles or exaggerated diaphragmatic descent. Bloating is a notoriously difficult to treat IBS symptom (Quigley, 2003); it is also one of the most important supportive signs of IBS diagnosis (Thompson *et al.*, 1999).

The volume of intestinal gas and its composition are amongst others determined by the colonic microflora and food residue in the colon. Bacterial populations appear to vary substantially among individuals, although modifying this population with probiotics may be efficacious in treating the intestinal gas in the short term (Di Stephano *et al.*, 2000; Di Stephano *et al.*, 2004). A more prudent approach would be clinical scrutiny to identify aerophagia, malabsorption syndromes and ingestion of gas-producing foods, including starches, poorly absorbed sugars, such as sorbitol and fructose, oligosaccharides, such as raffinose and stachyose and fermentable fibres such as pectin (Crowel *et al.*, 2005).

## **2.6 PROBIOTICS**

Probiotics, are defined as “live microorganisms, that when administered in adequate amounts, have beneficial effects for the host” (WHO/FAO, 2001). The probiotic concept suggests that supplementation of the intestinal microbiota with the right

types and numbers of live microorganisms can improve the microbiota characteristics and promote health (Reid *et al.*, 2011).

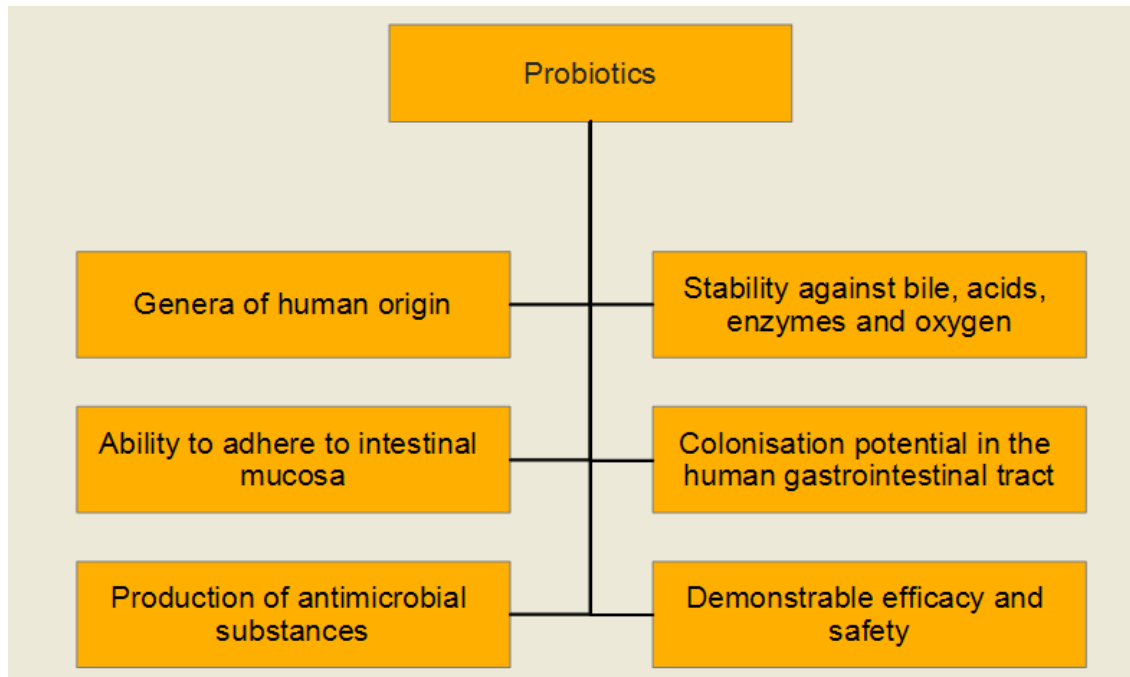
To understand the role of probiotics, it is important to be aware that many types of bacteria inhabit the human large intestine and play a role in mediating the digestive process (Neish, 2009). These bacteria have traditionally been classified into groups such as *Eubacteria*, *Clostridia*, *Bifidobacteria* and *Lactobacilli*. The two key groups of probiotic bacteria found in the large intestines are *Bifidobacteria* which make up approximately 5% of the large intestinal bacteria and *Lactobacilli* which although present as less than 1% of the bacteria, are very important in terms of their probiotic effect (Lannitti & Palmieri, 2010). Each group involves different species (*Lactobacillus acidophilus*, *Bifidobacterium bifidus* and many more), which include different strains.

According to Kaur *et al.* (2002) a good probiotic must satisfy the following requirements:

- Being able to adhere to cells.
- Excluding or reducing pathogenic adherence.
- Being able to persist, multiply and produce acids and hydrogen peroxide.
- Being safe, noninvasive, noncarcinogenic and non-pathogenic.
- Being able to coaggregate so as to form a normal balanced flora.

It has also been suggested that these microorganisms survive in hibernation during storage and must survive gastric and bile acids in order to reach the intestinal tract, colonise the host epithelium, and exhibit a beneficial effect (Mcfarlane *et al.*, 1999).

A product containing probiotic organisms should contain a number of viable cells that have been proven to be efficacious, generally  $>10^6$  to  $10^8$  CFU/g or  $>10^8$  to  $10^9$  CFU/day (Champagne *et al.*, 2011). Although no cell count level has been recognised to guarantee a health effect (Reid, 2008), the Canadian Food Inspection Agency (2009) recommends a level of  $10^9$  CFU per serving to be able to present generic health claims. Figure 2.1 indicates criteria for classifying a bacterial strain as probiotic.



**Figure 2.1** Criteria for classifying a bacteria strain as probiotic (Lannitti & Palmieri, 2010)

Research has been done on several preparations of these bacterial species, but findings from one strain should not be extrapolated to other strains (Camilleri, 2006). There are great differences in the content and medium in which a probiotic is administered (e.g. tablets or capsules, yoghurt or yoghurt drinks), and the bowel capability of the preparations (Camilleri, 2006). Probiotics selected for commercial use must survive industrial manufacturing and storage to ensure long-term viability and activity. Commercially available probiotic preparations contain different bacteria and varying bacterial colony counts and are found as a single microbial strain or as a mixture of multiple strains. To some extent these vary with clinical indications (Camilleri, 2006). For example, *Clostridium butyricum*, and selected *E. coli* strains (e.g. Nissle 1917) are used in inflammatory bowel disease research studies (Kruis *et al.*, 2004), in addition to the *Lactobacilli* and *Bifidobacteria* species used in IBS and multi-strain probiotics such as *Bifidobacterium* and *Lactobacillus acidophilus* used in treating the critically ill (Deshpande *et al.*, 2007). Probiotics benefits have not been shown if they are clearly strain-specific and disease-specific and the success of one probiotic in one clinical situation does not presuppose success in another.

## **2.7 MECHANISMS OF ACTION OF PROBIOTICS AND THERAPEUTIC BENEFITS OF PROBIOTIC FOR SOME OF THE IBS MECHANISMS**

The mechanisms by which probiotics exert biological effects are still poorly understood, but nonspecific terms such as colonisation resistance or competitive exclusion are often used to explain their mode of action (Elo *et al.*, 1991).

Colonisation resistance or competitive exclusion describes a phenomenon whereby the indigenous anaerobic flora limits the concentration of potentially pathogenic flora in the digestive tract (Volaard *et al.*, 1994). Beneficial effects of probiotics on IBS symptoms are likely to include modulation of the immune system, maintenance of mucosal barrier function, alteration of the intestinal luminal environment and sensory functions (Lee & Bak, 2011).

### **2.7.1 Modulation of immune system**

Probiotics might be able to modulate the host's defences, including the innate as well as the acquired immune system. This mode of action is most likely important for the prevention and treatment of infectious diseases, but also for the treatment of chronic inflammation of the digestive tract (Oelschlaeger *et al.*, 2010). In addition, this probiotic action could be important for the eradication of neoplastic host cells. O'Mahony *et al.*, (2005) reported that patients with IBS have an abnormal ratio of IL-10/IL-12, which is an indicator of a pro-inflammatory state. The ratio was normalised after eight weeks treatment with *Bifidobacterium infantis* 35624, which suggested that probiotics may have an immune-modulating role in the treatment of IBS.

### **2.7.2 Maintenance of mucosal barrier function**

Probiotics could influence IBS symptoms directly through balancing the microbiota and thus normalising aberrant gas production (Korpela & Niittynen, 2012). Probiotic administration has the potential to shift the microbiota composition from a pathogenic predominance towards a more beneficial microbiota ecosystem (Zhu *et al.*, 2012). Kajander *et al.*, (2008) and Nobaek *et al.*, (2000) have shown that probiotics can alter the gut microbiota, resulting in improvement in the symptoms of IBS. Studies have also confirmed that probiotics are an effective treatment to re-establish a balanced commensal flora after an intestinal infection or antibiotic treatment (Cremonini *et al.*, 2005; Sartor, 2004). It has been suggested that alteration of the colonic flora with administration of probiotics may modify fermentation processes (Andrea & Baumgart, 2006) and, abdominal bloating, distention and flatulence have been shown to improve significantly after probiotic treatment in placebo-controlled trials (Bausserman & Michail, 2005; O'Mahony, 2005).

### **2.7.3 Altering intestinal luminal environment**

There is growing evidence that disturbance of barrier function may play a role in the development of IBS and probiotics have proven to improve barrier function (Lee & Bak, 2011). *Lactobacilli* and *Bifidobacteria* subspecies are able to deconjugate and absorb bile acids (Camilleri, 2005), which may result in a reduced bile salt load in the colon. Another role of probiotics reported is that they may serve to inactivate the bile salts delivered to the colon and thus avoid the potential colonic secretions and mucosal permeability changes induced by the bile salts (Saggiaro, 2004).

#### 2.7.4 Sensory functions

Visceral hypersensitivity has been associated with IBS and is suggested to play a pathogenic role in the symptom of abdominal pain (Camilleri, 2005). Although the evidence suggesting potential effects of probiotics on sensory neurotransmission is limited, it has been suggested that probiotics may modulate disturbed visceral perception (Andrea & Baumgart, 2006). These beneficial effects of probiotics on sensory mechanisms are suggested by clinical trials that demonstrate an improvement in abdominal pain in IBS patients after treatment with different *Lactobacilli* or *Bifidobacteria* (Niedzielin *et al.*, 2001; Saggiaro, 2004; O'Mahony, 2005).

### 2.8 GENUS BIFIDOBACTERIUM AND GENUS LACTOBACILLI

*Bifidobacteria* are Gram-positive, non-spore-forming, non-motile and catalyase-negative anaerobes, which naturally inhabit the GIT of humans and other warm-blooded animals (Sgorbati *et al.*, 1995). They are sensitive microorganisms with low survival upon exposure to acid and temperature stress or exposure to oxygen encountered during production, storage and consumption (Dave, 1998). *Bifidobacterium* was recognised as a genus in its own right consisting of 11 species (Buchnan & Gibbons, 1974). At present 37 species are included in the genus *Bifidobacterium*, 13 of which are from human origin (GIT and genito-urinary tracts), the exact ratio of which is determined by age and diet (Soccol *et al.*, 2010), 18 from animal intestinal tracts, two from waste water, three from the bumblebee digestive tract and one from fermented milk (Turroni *et al.*, 2011).

Figure 2.2 shows more benefits of *Bifidobacteria* for human health (Lannitti & Palmieri, 2010). The number of *Bifidobacteria* decreases with increasing age.



**Figure 2.2** *Bifidobacteria* benefits for human health

All members of the genus *Bifidobacterium* show a bacillar form (Dave, 1998). Some strains have been shown to develop ramifications giving short, curved rods, club-shaped rods and bifurcated Y-shaped ones (Ishibashi *et al.*, 1997). Generally, *Bifidobacterium* are considered to be strict anaerobes. However, their ability to tolerate and survive the presence of oxygen depends on the species or strain and the composition of the culture medium (Gome & Malcata, 1999). Upon exposure to aerobic conditions from an anaerobic environment, various species of *Bifidobacteria* can produce different types of response. The optimum growth temperature of the species of human origin is  $37 \pm 1^\circ\text{C}$  and that of those of animal origin is  $42 \pm 1^\circ\text{C}$  (Dave, 2008). Most *Bifidobacteria* have been reported to die at  $60^\circ\text{C}$  (Rasi & Kurman, 1983). The optimum growth pH is 6.5 to 7 and no growth occurs below 5 or above 8 (Lankaputhra *et al.*, 1996b). Below pH 4.1, most species die within a week and at a pH below 2.5 most species die within three hours (Lankaputhra & Shah, 1995). Most randomised, placebo-controlled studies have suggested that *Bifidobacterium* have beneficial effects on IBS symptoms (Guglielmetti *et al.*, 2011; Guyonnet *et al.*, 2007; O'Mahony *et al.*, 2005 & 2006).

*Lactobacilli* are in general characterised as Gram-positive, non-spore-forming and non-flagellated rods or coccobacilli (Hammes & Vogel, 1995). *Lactobacilli* strains vary in their fermentation process, hydrogen peroxide and bacteriocin production (Soccol *et al.*, 2010). These different features make them a versatile group suitable

for different conditions. Gomes and Malcata (1999) reported that 56 species of the genus *Lactobacillus* have been recognised.

*Lactobacilli* are also distributed in various ecological niches throughout the gastrointestinal and genital tracts and constitute an important part of the indigenous microflora of man and animals. Their distribution is affected by several environmental factors, which include pH, availability of oxygen, level of specific substrates, presence of secretions and bacterial interactions. *Lactobacilli* have the reputation of health promoters, especially in the human gastrointestinal and genitor-urinary tracts (Salminen *et al.*, 1996). Ingestion of *Lactobacilli* has been suggested to confer a range of health benefits including immune system modulation (Isolauri *et al.*, 2001), increased resistance to malignancy (Roller *et al.*, 2004) and infectious illness (Nomoto, 2005).

In this study we focused on the genus *Bifidobacterium* and strain DN-173 010.

## **2.9 VIABILITY OF BIFIDOBACTERIA IN FERMENTED PRODUCTS**

The need to monitor survival of yoghurt bacteria and probiotic bacteria has often been neglected (Dave, 1998) and as a result, a number of products reach the market with few viable bacteria, ranging from several hundreds to a few per gram of product (Shah *et al.*, 1995). Several factors have been claimed to affect the viability of yoghurt and probiotic cultures in fermented milk products (Bertoni *et al.*, 1994; Costello, 1993). The viability of probiotic bacteria in yoghurt depends on:

- The strains used;
- Interaction between species present;
- Culture conditions;
- Chemical composition of the fermentation medium;
- Hydrogen peroxide content due to bacterial metabolism;
- Final titratable acidity;
- Concentrations of lactic and acetic acids;
- pH;
- Milk solids content;
- Availability of nutrients;
- Growth promoters and inhibitors;
- Concentration of sugars (osmotic pressure);
- Dissolved oxygen, especially for *Bifidobacterium* spp.;
- Level of inoculation;
- Incubation temperature;
- Fermentation time; and
- Storage temperature.

Several researchers have reported the effects of some of these factors (Klaver *et al.*, 1993; Martin & Chou, 1992; Patidar *et al.*, 1994; Samona & Robinson, 1994). However, it seems that the effects of all the parameters on the viability of probiotic bacteria have not been studied simultaneously.

## 2.10 EVIDENCE OF PROBIOTIC EFFICACY IN IBS

Numerous reports and uncontrolled trials suggest beneficial effects of probiotics on IBS symptoms. Table 2.2 summarises randomised placebo-controlled clinical trials, which evaluated the effects of various probiotics on IBS in general or the relief of specific IBS symptoms. Several studies tested *Lactobacillus plantarum* 299v, with conflicting results. In a study conducted by Niedzielin *et al.* (2001), *Lactobacillus plantarum* significantly improved pain and had a responder rate for overall IBS symptom improvement of 95% compared with only 15% in the placebo group. Nobaek *et al.* (2000) observed a significant improvement of flatulence in IBS patients treated with *Lactobacillus plantarum* 299v, while pain was improved in both the probiotic and the placebo-treated group. This study incorporated a 12-month follow-up questionnaire according to which the probiotic-treated patients maintained better overall GI functions than the control patients. This might indicate a possible long-term beneficial alteration in colonic flora.

Saggiaro (2004) administered a combination of *Lactobacillus plantarum* with either *Lactobacillus acidophilus* or *Bifidobacterium breve*, also observed a significant improvement in pain and overall IBS symptom score by both probiotic combinations compared to a placebo. Two studies using *Lactobacillus casei* failed to provide convincing evidence of the efficacy of this probiotic in IBS (O'Sullivan & O'Morain, 2000). However, the researchers reported a trend towards improving stool frequency and consistency in a small subgroup of IBS patients with diarrhoea.

In children with IBS, *Lactobacillus casei* was not superior to a placebo in the treatment of pain and bowel symptoms. A study by Bausserman and Michail (2005) reported a beneficial effect on perceived abdominal distention, but the observed difference in the treatment group was solely due to worsening of this symptom in the placebo group. Whorwell *et al.*, (2006) investigated *Bifidobacterium infantis* 35624 in three different doses and showed beneficial effects on IBS symptoms. In addition, *Bifidobacterium bifidum* MIMBb75 was investigated by Guglielmetti *et al.* (2011) on the severity of IBS symptoms and effected an improvement in IBS symptoms and a significant improvement in QOL.

The Camilleri group conducted two trials with VSL#3, a probiotic cocktail containing eight strains of different *Lactobacilli* (*L.acidophilus*, *L.casei*, *L.bulgaricus*, *L.plantarum*), *Bifidobacteria* (*B.longum*, *B. infantis*, *B. breve*) and *Streptococcus salivarius* subspecies *termophilus*. In addition to effects on IBS symptoms, these studies also evaluated the influence of VSL#3 on gastrointestinal transit time (Camilleri *et al.*, 2006a; 2006b). A study by Kim *et al.* (2003) focused on diarrhoea-

predominant IBS patients and observed a borderline significant improvement of abdominal bloating, but no effects on other IBS symptoms or gastrointestinal transit time. Based on this study, a second study focused on IBS patients with bloating (Kim *et al.*, 2005). This study detected a significant improvement in flatulence in the VSL#3 group, but differences tending towards greater improvement in bloating, pain and urgency score in the VSL#3 group did not reach statistical significance.

O'Mahony *et al.* (2005) compared the effects of two different probiotics, *Lactobacillus salivarius* and *Bifidobacterium infantis*, on IBS symptoms with weekly assessments over eight weeks of treatment and four weeks of washout. This study included an assessment of QOL and of cytokine profiles. *Bifidobacterium infantis* was superior to a placebo in relieving all the individual and composite symptom scores, except for stool frequency and stool consistency. Beneficial effects on the weekly symptom scores were significant in all eight treatment weeks and in two out of two washout weeks. In contrast, *Lactobacillus salivarius* showed significant symptom improvement over placebo only in the second treatment week, and was significantly less effective than *Bifidobacterium infantis* in four out of eight treatment weeks. Despite the significant symptom improvement brought about by *Bifidobacterium infantis*, the overall QOL scores were not significantly different between the three treatment groups (O'Mahony *et al.* 2005).

### **2.10.1 Supportive evidence for *bifidobacterium animalis* DN 173-010 efficacy in patients with IBS-C**

Three studies in Table 2.2 investigated the probiotic *Bifidobacterium animalis* DN 173-010, evaluating overall IBS symptoms as secondary end point in patients with IBS-C (Agrawal *et al.*, 2008; Guyonnet *et al.*, 2007; Roberts *et al.*, 2013). Guyonnet *et al.* (2007) demonstrated significant beneficial effects in stool frequency over six weeks in the intervention versus placebo group and improvements in bloating, but not in abdominal pain. This six-week study did not detect any effects on the feeling of incomplete evacuation; however, the probiotic group had a significantly greater proportion of responders in the discomfort dimension score than the placebo group at week 3. Roberts *et al.* (2013) demonstrated significant improvement across a range of symptoms and QOL outcomes, but improvement did not differ between the intervention and placebo group. Agrawal *et al.* (2008) reported significant improvements in oro-caecal transit time, colonic transit time and urgency, bloating and abdominal pain, but no significant effects on stool frequency and consistency, straining during evacuation and feelings of incomplete evacuation.

**Table 2.2** Clinical trials with probiotic use

Author	Probiotic	Subjects	Duration	Results
Niedzielin <i>et al.</i> , 2001	<i>L. plantarum</i> 299v	40 Author-defined IBS	4 weeks	Pain resolution
Nobaek <i>et al.</i> , 2000	<i>L. plantarum</i> 299v	60 Rome I IBS	4 weeks	Flatulence and pain reduction
Saggioro, 2004	<i>L. plantarum</i> LP01 & <i>B. breve</i> or <i>L. Plantarum</i> LP01 and <i>L. acidophilus</i> LA02 or placebo	70 Rome II IBS	4 weeks	Pain and overall symptom score
O'Sullivan & O'Morain, 2000	<i>Lactobacillus casei</i> GG	24 Rome I IBS	20 weeks	No significance difference between the two groups
Kim <i>et al.</i> , 2003	VSL#3	25 Rome II IBS-D	8 weeks	Abdominal bloating improvement, but no significant difference between the two groups
Kim <i>et al.</i> , 2005	VSL#3	48 Rome II IBS	4-8 weeks	Flatulence reduction
O'Mahony <i>et al.</i> , 2005	<i>L. salivarius</i> UCC4331 or <i>B. Infantis</i> 35624	80 Rome II IBS	8 weeks	<i>B. infantis</i> reduced GSS, while <i>L. Salivarius</i> reduced abdominal pain and discomfort, bloating and straining
Kajander, 2005	<i>L. rhamnosus</i> GG, <i>L. rhamnosus</i> LC705, <i>B. Breve</i> Bb99, <i>P. freudenreichii</i> spp. <i>Shermanii</i> JS	103 Rome I & II IBS	6 months	Significant reduction in global IBS symptoms
Kajander, 2008	<i>L. rhamnosus</i> GG, <i>L. rhamnosus</i> LC705, <i>B. Breve</i> Bb99, <i>P. freudenreichii</i> spp. <i>Shermanii</i> JS	86 Rome II IBS	5 months	Significant reduction in global IBS symptoms
Whorwell <i>et al.</i> , 2006	<i>B. infantis</i> 35624 in 3 different doses	362 Rome II IBS	4 weeks	With 10 CFU, significant improvement in global IBS symptom
Sinn <i>et al.</i> , 2008	<i>L. acidophilus</i> -SDC 2012,2013	40 Rome III IBS	4 weeks	Improvement in abdominal pain
Guyonnet <i>et al.</i> , 2007	<i>B. animalis</i> DN-173 010, <i>S. Thermophilus</i> & <i>L. Bulgaricus</i>	274 Rome II IBS	6 weeks	Significant improvement in QOL, bloating and stool frequency
Roberts <i>et al.</i> , 2013	<i>B. lactis</i> DN 173 010 (CNCM 1-2494)	184 Rome III IBS	12 weeks	Improvement in bloating, flatulence, ease of bowel movement QOL were experienced in both placebo and intervention groups
Bausserman & Michal, 2005	<i>Lactobacillus</i> GG	50 Rome II	6 weeks	Perceived abdominal distention
Agrawal <i>et al.</i> , 2008	<i>B. lactis</i> DN- 173 010	Rome III	4 weeks	Significant improvements in abdominal girth, GI transit time and reduced IBS symptoms
Gugliemetti <i>et al.</i> , 2011	<i>B. bifidum</i> MIMBb75	122 Rome III	4 weeks	Significant improvement in GSS and QOL in probiotic group
Marteau <i>et al.</i> , 2007	<i>Bifido longum</i> <i>L. acidophilus</i> <i>Lactobacillus lactis</i>	52 Rome II	6 weeks	Relief of discomfort

(GI) Gastrointestinal, (CFU) Colony forming unit, (GSS) General symptom score, (QOL) Quality of life

## 2.11 META-ANALYSES AND REVIEWS OF PROBIOTIC EFFECTS IN IBS

Several randomised control trials comparing the effects of probiotics versus placebo in IBS have been published. Comparing and summarising these studies is difficult because of considerable differences in study design, dosing regimens, probiotic species used and reported clinical end points (Ringel & Ringel-kulka, 2011). Despite these limitations, all meta-analyses conducted indicated that probiotics were beneficial to varying degrees. Table 2.3 summarises the meta-analyses and systematic reviews of probiotics in IBS. Moayyedi *et al.* (2010) demonstrated a trend in symptom improvement following treatment with *Bifidobacteria*. The beneficial impact of probiotics on global symptoms was also reported in these meta-analyses. Hoveyda *et al.* (2009) included 14 randomised placebo-controlled trials in their meta-analyses and reported a beneficial impact on both abdominal pain and flatulence. The meta-analyses concluded that probiotics may play a role in alleviating some IBS symptoms, but the optimal type and dose of probiotics and especially the subgroups of patients who are likely to benefit most, still have to be clarified.

McFarland and Dublin (2008) reviewed 20 randomised placebo-controlled trials that included a total of 1 404 subjects and suggested that probiotic use was associated with improvements in global IBS symptoms and less abdominal pain compared with placebo. The study suggested that longer studies were needed to confirm this beneficial effect. Nikfar *et al.*, (2008) reported improvement of IBS symptoms compared to placebo in eight randomised trials with a total of 1 011 subjects. The National Institute for Health and Clinical Excellence in the UK conducted a meta-analysis in 2008 and observed improvement in general IBS symptoms and abdominal pain (NICE, 2008).

**Table 2.3** Meta-analyses and systematic reviews of probiotics in IBS

Author	Trials included	Analysis	General symptoms	Abdominal pain	Bloating	Flatulence
McFarland & Dublin, 2008	20 RCTs Adults and children	Dichotomous	Reduced risk of symptoms P< 0.001	Reduced risk of symptoms	Not analysed	Not analysed
NICE, 2008	13 RCTs Adults only	Dichotomous Continuous	Increased risk of improvements P< 0.001 No effect	Increased risk of improvement	No effect	Not analysed
Hoveyda <i>et al.</i> , 2009	14 RCTs Adults and children	Dichotomous Continuous	Increased odds of improvement P<0.001 Improvement in symptom score P=0.004	Increased odds of improvement	Increased odds of improvement	Increased odds of improvement
Nikfar <i>et al.</i> , 2008	8 RCTs Adults only	Dichotomous	Increased odds of improvement P<0.0042	Reduced risk of Symptoms	Not analysed	Not analysed
Moayyedi <i>et al.</i> , 2010	19 RCTs Adults only	Dichotomous Continuous	Reduced risk of symptoms P=0.002 Reduction in symptom score P=0.01	Reduction in symptom score	Not analysed	Not analysed
Hungin <i>et al.</i> , 2013	37 RCTs	Dichotomous	Reduced risk of symptoms P < 0.05	Reduced risk of symptoms	Reduced risk of symptom	Not analysed

(NICE) National Institute for Health and Clinical Excellence (RCTs) Randomised controlled trials

## 2.12 SAFETY OF PROBIOTICS

Probiotics have generally regarded as safe (GRAS) status (Gupta & Garg, 2009) and are marketed as dietary supplements; as such they do not need to fulfil safety and efficacy norms set by the Food and Drug Administration (FDA) (Theodorakopoulou *et al.*, 2013). Snyderman (2008) reported three theoretical concerns regarding the safety of probiotics: firstly the occurrence of disease due to bacteraemia, sepsis or endocarditis; secondly the toxic or metabolic effects on the GI tract and thirdly the transfer of antibiotic resistance in the GI flora. The study further underlined that although rare cases of bacteraemia or fungemia related to the use of saprophytic probiotics occur, epidemiologic evidence through clinical trials and epidemiologic survey suggests that they can be used safely. Wassenaar and Klein (2008) highlighted that bacteria used for probiotic formulation are safe, although there could be some flaws: sometimes virulence factors have been detected in probiotic bacterial strains; horizontal gene transfer could result in the acquisition of virulen genes or antimicrobial resistance in probiotic bacteria and antimicrobial resistance in these bacteria can possibly aid the spread of unwanted resistance in endogenous bacterial populations.

A study by Liong (2008) observed that probiotics, such as *Lactobacillus*, *Bifidobacterium* and *Enterococcus*, could translocate during an infection. The study underlined that probiotic translocation hardly occurs in healthy humans and if it happens, detrimental effects are rare. But health-damaging effects of probiotic translocation can occur in immunocompromised patients, the critically ill, premature neonates and transplant recipients (Lolis *et al.*, 2008). Although further investigations are still required to understand the mechanism of probiotic translocation and infection, in immunocompromised patients probiotics have been reported to theoretically cause infections that need to be treated with antibiotics but unfortunately the antibiotic resistance of some strains has increased the complexity of their eradication (Lannitti & Palmieri, 2010). Adverse effects of dyspepsia, headache and nausea have infrequently been reported (Hoveyda *et al.*, 2009). Most studies have shown that probiotics are well tolerated and quite safe to use, but routine use in the critically ill and in severe pancreatitis is not recommended (Besselink *et al.*, 2009; Theodorakopoulou *et al.*, 2013).

## 2.13 INTEGRATED APPROACH TO TREATMENT OF IBS

Since physiological and psychosocial factors interact to influence the severity of symptoms, illness behaviour and outcome, it is rational to consider both these factors in planning treatment. Current therapy for IBS-C comprises non-drug interventions and drug interventions. Table 2.4 summarizes the treatment strategies of IBS.

### **2.13.1 The physician-patient therapeutic relationship**

The value of an effective physician-patient relationship is supported by the fact that some studies involving IBS patients have a placebo effect rate of up to 42.6% (Dorn *et al.*, 2007). It may not be what is done, but how it is done that makes a difference. To establish a therapeutic relationship, the physician has to apply the following guidelines given by Drossman *et al.* (2002):

- Obtain the history through a non-judgemental, patient-centred interview.
- Conduct a careful examination and cost-efficient investigation.
- Determine the patients' understanding of the illness and his or her concerns. ('What do you think is causing your symptoms?')
- Provide a thorough explanation of the disorder.
- Identify and respond realistically to the patient's expectations of improvement. (How do you feel I can be helpful to you?)
- Set consistent limits. ('I appreciate how bad the pain is, but narcotic medication is not indicated.')
- Involve the patient in the treatment. ('Let me suggest some treatment for you to consider.')
- Establish a long-term relationship with a primary care provider.

### **2.13.2 Non-drug interventions**

#### **2.13.2.1 Psychological and behavioural therapies**

It is not known what events predispose patients to developing IBS. Adverse environmental factors include family influence, trauma, abuse or exposure to infections and may set the stage for the development of IBS (Ford *et al.*, 2009). Early developmental influences, including paediatric conditions such as recurrent abdominal pain, may portend experiencing IBS in adulthood (Jarret *et al.*, 2003). Higher levels of psychological stress in patients, particularly women, have been linked to gastrointestinal symptoms (Hertig *et al.*, 2007). For example, the severity of bloating in IBS patients has been associated with distress (anxiety and depression) (Park *et al.*, 2008).

Evidence supports the use of psychological and behavioural therapies. Two meta-analyses each concluded that psychological interventions may be slightly superior to usual care for IBS-C (Ford *et al.*, 2009; Zijdenbos *et al.*, 2009). No adverse effects have been reported with psychological and behavioural therapies in the treatment of IBS-C (ACGTF, 2009).

#### **2.13.2.2 Hypnotherapy**

Hypnotherapy may be beneficial for some patients with IBS, possibly because of its effects on the CNS pain-processing regions (Rainville *et al.*, 2007) and influence on colorectal sensitivity. Hypnotherapy has been reported to improve psychological

factors associated with IBS (Simren, 2006). The American College of Gastroenterology Task Force (ACGTF) however does not support hypnotherapy as a recommended treatment for IBS-C (ACGTF, 2009).

### 2.13.2.3 Psychotherapy, cognitive behavioural therapy and relaxation therapy

A single study of IBS-C patients evaluated self-administered cognitive behavioural therapy (CBT) in combination with stress management and found that it had a small effect on IBS symptoms; however, stress management was observed to reduce the risk of continuing IBS symptoms over time (Shaw *et al.*, 1991; Sander *et al.*, 2007). Another self-management programme with integrated elements of CBT, reassurance and education, and dietary counselling in women with IBS-C, demonstrated improved HRQOL, reduced gastrointestinal symptoms and reduced psychological distress after 12 months (Heitkemper *et al.*, 2004).

**Table 2.4** Summary of treatment strategies for IBS-C

Treatment type	Evidence	Adverse effects
<b>PROBIOTICS</b> <i>Lactobacillus</i> and <i>Bifidobacteria</i>	Some IBS symptom improvement with <i>bifidobacteria</i> alone or in combination with <i>lactobacillus</i> bacteria, which alone are not efficacious	Dyspepsia headache Nausea
<b>BULKING AGENTS/ FIBRE</b> (Psyllium Husk)	Some evidence to suggest that psyllium fibre is effective for improving constipation	Flatulence or bloating if initiated rapidly
<b>OSMOTIC LAXATIVES</b> (Lactulose)	Mostly studied in patients with chronic constipation; insufficient evidence to support benefit for IBS-C patients	Could worsen bloating and abdominal distention
<b>HYPNOTHERAPY</b>	Meta-analysis of four studies supports hypnotherapy as beneficial short-term therapy with global symptom relief	None
<b>PSYCHOTHERAPY</b>	Expert opinion supports both multicomponent and dynamic psychotherapy as superior to usual care for global IBS symptom improvement	None
<b>COGNITIVE BEHAVIORAL THERAPY</b>	Expert opinion supports CBT as superior to usual care for global IBS symptom improvement	None
<b>EXCLUSION DIETS</b>	Responses to exclusion diets vary widely; little evidence to support benefit for IBS-C	None
<b>PROSTONE</b>	Global symptom relief, improvements in individual abdominal pain measures, and HRQOL.	Nausea Abdominal pain Distention & diarrhea

Treatment type	Evidence	Adverse effects
<b>SELECTIVE SEROTONIN RE- UPTAKE INHIBITORS</b> (SSRIs)	Global symptom relief, improvements in individual abdominal pain measures and HRQOL may have a prokinetic effect	Dizziness Drowsiness
<b>TRICYCLIC ANTIDEPRESSANTS</b> (TCAs)	Improves abdominal pain and HRQOL	Dizziness Drowsiness

(IBS-C) Constipation -predominant irritable bowel syndrome, (CBT) Cognitive behavioural therapy, (HRQOL) Health-related quality of life, (SSRIs) Selective serotonin re-uptake inhibitors, (TCAs) Tricyclic antidepressants.

### 2.13.2.5 Dietary management

Fibre supplementation is the most extensively and carefully studied dietary treatment for IBS (Bijkert *et al.*, 2003) and its deficiency was widely believed to be the cause of IBS despite numerous studies that reported that cereal fibre worsened symptoms in 55% of secondary care and 22% of primary care IBS patients (Francis *et al.*, 1997; Lea & Whorwell, 2005; Miller *et al.*, 1994). Soluble fibres studied are psyllium, partially hydrolysed guar gum, fructo-oligosaccharide, oligosaccharide and calcium polycarbophil. Products rich in insoluble fibre that have been studied are wheat bran, corn bran and ground flaxseeds (Hasler, 2007). The use of fibre has been reviewed in meta-analyses by Ford *et al.* (2009), a systematic review by Quatero *et al.* (2005) and narrative review by Zuckerman (2006). The reviews uniformly concluded that fibre has either no efficacy in the treatment of IBS or possibly limited benefits for patients with IBS-C. Meta-analyses by Ford *et al.*, (2009) concluded that wheat bran was not effective for treating IBS but the soluble fibre, psyllium husk, was probably effective. Lastly the results of meta-analysis by Talley *et al.* (1992) concluded that fibre supplements have not been proven to be superior to placebo, but could improve constipation.

It has been reported that patients with IBS-C may improve their symptoms by increasing the intake of soluble fibre by 10 to 20 g/day in the form of supplements such as psyllium and probably other supplements and foods high in soluble fibre. If fibre supplements are prescribed, they should be introduced gradually, accompanied by probiotics in the form of foods such as live-culture yoghurt (Heizer *et al.*, 2009). It should also be noted that a diet that relieves IBS symptoms will not be the same for all IBS sufferers, and since there are no reliable laboratory tests or other simple, clinically applicable methods to determine what dietary restrictions will be helpful for a particular patient, a careful dietary history is essential and a diary of foods eaten and symptoms experienced will be helpful. Most IBS patients believe that their symptoms are triggered by diet and that avoiding specific foods reduces symptoms (Halpert *et al.*, 2007). One study found that 57% of patients with IBS-C reported differences in stool form after consuming certain foods (Muller *et al.*, 2005). Foods that patients identified included chocolate, bananas, bread and coffee.

While general restriction diets are not advisable, specific avoidance of unabsorbed, highly fermentable, short-chain carbohydrates collectively termed FODMAPs

(fermentable oligosaccharides, disaccharides, monosaccharides and polyols) may be useful in IBS patients with severe bloating and diarrhoea (Eduard, 2011). A diet low in FODMAPs was recently shown to change microbiota composition in parallel with improvement in GI symptoms in a group of IBS patients (Staudacher *et al.*, 2012). FODMAPs can include fructose and lactose in patients in whom these are malabsorbed, polyols (such as sorbitol and xylitol) because they are generally poorly absorbed by humans, fructo-oligosaccharides (fructans) and galacto-oligosaccharides (such as raffinose), for which humans do not express suitable hydrolases and which are always poorly absorbed. Lactose is mainly found in dairy products. Common dietary sources of fructose are fruit, honey and high-fructose corn syrup and sources of fructans are wheat and onions (Fernandez *et al.*, 2006; Lomer *et al.*, 2008).

Dietary changes that have shown to relieve IBS symptoms are the following (Heizer *et al.*, 2009):

- Avoid large meal;
- Reduce fat to no more than 40 to 50 g/day;
- Reduce fructose in all forms, including high-fructose corn syrup, honey, high-fructose fruits;
- Reduce gas producing-foods such as beans, broccoli, cabbage and bran;
- Eliminate all wheat and wheat-containing products; and
- Eliminate banana, corn, potato, eggs, milk and coffee.

Recommendations regarding diet changes for IBS symptoms (Parker *et al.*, 1995):

- If a patient believes foods triggers symptoms but cannot identify one or more specific foods, recommend a food and symptom diary for two to four weeks.
- Do not conclude that a food causes symptoms unless the symptoms occur within three days of eating the food and occur consistently on at least three separate occasions after eating it.
- Encourage specificity. If pizza causes symptoms, each component should be tested separately (e.g. cheese, tomato, pastry, spices).
- To test a diet change, patients should follow it carefully for two weeks then return to the previous diet. If it is unclear whether the diet change helped, either conclude that the change is unhelpful or recommend that the patient repeat the process.
- If it is concluded that elimination of a food or following of a particular diet helps reduce symptoms, help the patient realise that the need for the change may not be permanent. The change should be continued for three to six months, and then the patient should return to a normal diet to determine if symptoms recur or worsen.

## **2.13.3 Drug therapies**

### **2.13.3.1 5-HT<sub>4</sub> agonist**

Newer drug therapies for IBS-C have focused on the neurotransmitter serotonin. Tegaserod, 5-HT<sub>4</sub> agonist, was withdrawn in 2007 because of safety concerns (Heitkemper, 2009). Currently, it is available through the FDA under an emergency investigational drug protocol. Future 5-HT<sub>4</sub> receptor agonists may provide greater selectivity and may be safer (Heitkemper, 2009).

### **2.13.3.2 Lubiprostone**

Lubiprostone is a selective chloride channel-2 activator that appears to act topically in the small intestine and may stimulate colonic motility by promoting a higher intraluminal volume (Rivkin & Chagn, 2006). It is believed to work by opening the chloride channels in the small and large intestines, as well as by opening bicarbonate secretory pathways in the duodenum (Camilleri *et al.*, 2006). It is the only FDA-approved treatment for adult women aged 18 and older with IBS-C (ACGTF, 2009). It is also approved for adult men and women with chronic idiopathic constipation, at a higher dose, which is accompanied by more frequent adverse effects (Johanson *et al.*, 2008).

### **2.13.3.3 Antidepressants**

Antidepressants have been postulated to improve IBS symptoms by both central and peripheral mechanisms (Clouse, 2003a; 2003b; Talley & Spiller, 2002). Serotonin and its receptors may influence a broad range of gastrointestinal functions, including colonic motility. In IBS patients, selective serotonin re-uptake inhibitors (SSRIs) have been observed to have a moderate effect on reducing abdominal pain and bloating, increasing patient satisfaction and increasing stool frequency (Ford *et al.*, 2009; Masand *et al.*, 2005; Vahedi *et al.*, 2005). Tricyclic antidepressants (TCAs) have also been shown to relieve a number of IBS symptoms. However, SSRIs differ in their prokinetic effect, compared to the anticholinergic properties of TCAs that may worsen constipation (Gorard *et al.*, 1994). The ACGTF suggests that SSRIs may therefore work better in patients with IBS-C than in those with IBS-D (ACGTF, 2009). Drowsiness and dizziness were the most common adverse events reported in a 2009 meta-analysis of antidepressant therapies for IBS symptoms (Ford *et al.*, 2009).

### **2.13.3.4 Antispasmodics**

Abdominal pain is a major symptom of IBS. A meta-analysis that included 23 randomised controlled trials concluded that anticholinergics and antispasmodics were superior to the placebo in treatment of IBS (Tache *et al.*, 2001). However the quality of most of the trial included in the meta-analysis was very poor; results were mixed and publication bias was not excluded.

## 2.14 CONCLUSION

IBS is the most common gastrointestinal disorder seen in primary care and gastroenterology practice (Drossman *et al.*, 2006). The diagnostic approach and treatment methods have changed in response to new and growing knowledge of its pathophysiology (Quigley & Flourie, 2006).

Literature shows that the therapeutic potential of probiotic bacteria, especially *Lactobacilli* and *Bifidobacteria*, is the centre of considerable interest in a number of fields, including gastroenterology. Some of these organisms appear to have properties that might be advantageous, especially in conditions in which there may be an infectious trigger or an inflammatory component, such as IBS. Factors determining the response to treatment include probiotic strains, dose and mode of administration, health status of the patient and medication such as antibiotics and antacids.

# CHAPTER 3: METHODOLOGY

## 3.1 INTRODUCTION

A pilot study is conducted to collect initial data for the primary outcome measure, in order to perform a sample size calculation for a larger trial (Ross-McGill *et al.*, 2000; Stevinson & Ernest, 2000). The number of participants to be included in the pilot study depends on the parameter(s) to be estimated (Lancaster, 2002). A general rule of thumb is to include 30 patients or more to estimate a parameter (Browne, 1995). A conservative approach has been suggested when estimating a standard deviation, using at least an 80% upper one-sided confidence limit rather than the estimate itself (Browne, 1995).

In preparation for a possible large, multicentred trial, a randomised pilot study can be treated as a dummy run (Burrows *et al.*, 2001; Ross-McGill *et al.*, 2000). This approach enables all procedures to be put in place, including inclusion/exclusion criteria, study product delivery, storage, testing of measuring instruments, demographics questionnaire, the consent form, training of an assistant and assessment of intervention. Lastly it enables the research team to confirm the enrolment procedure and pilot data collection and determine the number of assistants necessary for a large trial.

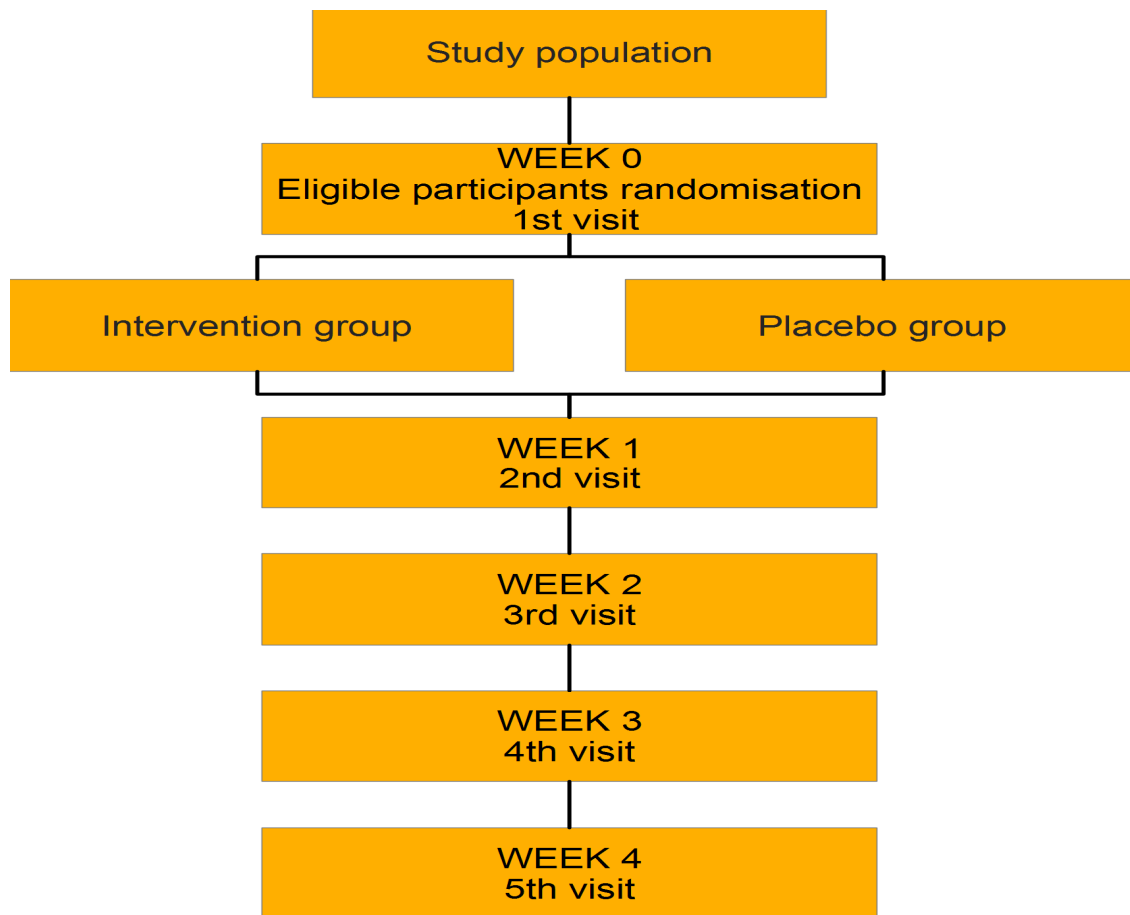
Piloting of data collection questionnaires is particularly important, especially when the participants have to self-administer the questionnaires (Carfoot *et al.*, 2002). It ensures that the questionnaire is comprehensible and appropriate and that questions are well defined and clearly understood by the subject and presented in a consistent manner. In the present study the following questionnaires will be tested: IBS Severity Scoring System (IBS SSS) by Francis *et al.* (1997); IBSQOL by Patrick *et al.* (1998); weekly question by Kellow *et al.* (2003) and daily record form, derived from the IBS SSS.

The measuring instruments to be used in this pilot project were validated in Western countries but not in a South African population. Providing a validated disease questionnaire to participants belonging to another population group requires adaptation, modification and establishment of its validity in a different cultural context (Streiner & Norman, 1995). According to Burrows *et al.* (2001) and Ross-McGill *et al.* (2000), the way in which a randomisation procedure is to be implemented has to be tested. Determining what the consent rate will be for patients entering the trial is important, as it will have a direct impact on planning how long it will take to recruit enough patients for the trial (Ross-McGill *et al.*, 2003; Burrows *et al.*, 2001; Carfoot *et al.*, 2002). Failure to recruit sufficient numbers in a trial will reduce statistical power and it is one of the main reasons for abandoning trials early (Ross *et al.*, 1999). It is wise to determine the acceptability of an intervention in a pilot sample to see whether the intervention is difficult to administer (Ross-McGill *et al.*, 2000).

The rest of this chapter describes the methodology used for the pilot study.

### 3.2 STUDY DESIGN

The study was a four-week, parallel-group, randomised, double blind, placebo-controlled study. Figure 3.1 indicates the flow of the study from screening and randomisation to the four-week parallel group treatment period. The pilot study was undertaken over a seven-week period using the methodology of the main study. The seven-week time period was used for data collection and excluded the initial preparation of the study material, printing, formulation of questionnaires and data interpretation.



**Figure 3.1** Study flow design.

### **3.3 STUDY POPULATION**

Black females aged between 18 and 60 years with a diagnosis of IBS-C according to the Rome III criteria were recruited to participate in this study and were randomly allocated to an intervention group or placebo group. The sample size was 20 participants, 10 in each group. Collaboration with general practitioners from health care centres, specialist physicians and a gastroenterologist from a private clinic in Soweto who were the main referrals of the patients to be studied took place. Participants were recruited from these healthcare providers and a confirmatory screening questionnaire based on Rome III criteria was sent to all health providers. Eligibility for the study was assessed for the second time at the study unit based on the information provided in the screening questionnaire. All suitable consenting participants were entered into a four-week parallel group treatment period, after they had been verbally briefed on the double-blind nature of the study, the treatment conditions, evaluation methods and the study procedures.

During the four-week parallel group treatment period, participants were required to visit the study unit weekly to hand in completed evaluation forms for the previous week and receive another week's supply of the study products together with different products for the rest of the family members to prevent them from consuming the participants' study product.

Throughout the entire four-week study period, participants were not allowed to consume any other probiotic or any fermented dairy products (amasi) or fermented pap (ting) other than the fermented study product. They were encouraged to continue their other dietary habits and physical activity to prevent results from being affected by any change in diets. Dietary counselling and a nutrition assessment were given to each participant only during the last visit of the study period.

#### **3.3.1 Inclusion criteria**

The inclusion criteria determined that participants had to:

- Be black females aged between 18-60 years;
- Be able to read and write;
- Present with symptoms of digestive disturbances with irregular bowel movements of fewer than three times per week or hard stools or straining two to three times per week and incomplete evacuation, lower abdominal pain and bloating at least twice per week for the past three months, according to the Rome III criteria.
- Be prepared to consume two 100g tubs of yoghurt daily; provided to them;
- Be willing to come for the required number of follow-up visits;
- Be willing to complete daily records;
- Have refrigerators for storing the study product supplied to them; and
- Agree to sign the informed consent form.

### 3.3.2 Exclusion criteria

Subjects were excluded from the study if they:

- Had a diarrhoea-predominant IBS type and irritable bowel syndrome alternating type, according to the medical history in the demographic profile;
- Had a total score lower than 75 in the IBS SSS.
- Had dietary habits that could interfere with the study products, e.g. a slimming or vegetarian diet;
- Used any probiotic product, including probiotic-containing yoghurt, in the four-week study period;
- Were using drugs that could cause constipation, antidepressants or psychotropic drugs (drugs listed in the literature review);
- Had received IBS treatment in the month prior to the commencement of the study;
- Displayed GI symptoms requiring treatment or had a history of an abdominal or GI surgical procedure;
- Had a known intolerance to the study product, such as lactose intolerance or allergy or hypersensitivity to milk proteins; or
- Routinely used oral cleansing regimens such as herbs, dietary supplements or laxatives and performed bowel enemas for colon cleansing.

### 3.4 RECRUITMENT PROCEDURE

Participants were recruited through gastroenterologists, specialist physicians and medical doctors after they had been briefed thoroughly about the study and its procedures. Medical practitioners were requested not to prescribe any drug therapy aimed at reducing the IBS symptoms or bulking agents that affect bowel movements to any of the patients they referred for dietary management. They were to follow the inclusion and exclusion criteria list sent to them for all patients they referred and diagnose participants as IBS-C according to the Rome III criteria. Medical practitioners were to refer IBS-C patients for inclusion in the study within the period stipulated in the study manual.

### 3.5 RANDOMISATION

Participants fulfilling the eligibility criteria were randomised and entered the four-week parallel group treatment period. Participants received either fermented *Bifidobacterium animalis* DN-173 010 twice a day (1x100g in the morning + 1x100g in the evening = 200g/day) or placebo. Participants were assigned by using two randomisation numbers (416 and 258) in an alternative order. Randomisation numbers were produced by the product provider. Only the product provider knew which code was the intervention product or placebo product and this information was

withheld from the research team, referring doctors and participants throughout the study period.

### **3.6 STUDY PRODUCT**

The intervention product was fermented milk unflavoured white base of DANONE Activia. Activia contains Actiregularis™ *Bifidobacterium animalis* DN-173 010 [ $>3,4 \times 10^7$  CFU/g], full cream milk, sugar, skim milk powder, thickener (maize starch), yoghurt cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and preservative, potassium sorbate. The placebo product was Nutriday sweetened white base, which contains low fat milk, sugar, reconstituted whey powder, modified maize starch, gelatin, potassium sorbate and yoghurt cultures with no probiotic. Both the intervention and placebo products were supplied in the same colour 2 kg containers, which were coded by the product provider with the two randomisation numbers. Both products were filled into 100 g tubs in the study unit by the principal investigator and an assistant. The intervention and placebo products looked the same and were identical in weight, colour, smell, taste and package. For quality control measures and to ensure equal stability of the *Bifidobacterium lactis* and its CFU count in all study products, products were manufactured weekly, collected from the product provider every Tuesday in cooler boxes and taken to the study unit, where they were stored in refrigerators to ensure that the characteristics that gave rise to their health benefits were retained throughout the study period. The portion size of the yoghurt was 100 g and the nutritional composition per 100 g was as follows: Energy 483 KJ; carbohydrates 17.7 g, of which 14.2 g was sugar; total fat 3.3 g, saturated fat 2.1 g; protein 3.5 g and fibre 0 g.

### **3.7 ETHICAL CONSIDERATIONS**

The ethics committee of the North-West University (Potchefstroom campus) approved both the pilot and main study (NWU-00069-12-S1). All participants were fully informed about the objectives and procedures of the study and provided an informed consent for inclusion in the study.

### **3.8 PROCESS EVALUATION AND ASSESSMENT**

Process evaluation was used to monitor and document programme implementation. Including a process evaluation during a clinical trial allows for silent indicators to be examined on the pathway through which an intervention is expected to work and brings about understanding of outcome results (Baranowski *et al.*, 2000; Steckler *et al.*, 2002). Table 3.1 provides a summary of the process components evaluated during the trial period.

**Table 3.1** Process evaluation components

<b>Components</b>	<b>Monitoring methods</b>
Recruitment procedure	Visits to medical practitioners informing them of the study and the importance of their involvement Assessment of participants flow
Consent procedures	Consulting with eligible participants after being thoroughly briefed about the study
Reach	The number of target group referred to participate in the intervention
Blinding	Testing whether research team can be kept blinded throughout the study
Randomisation	Testing the randomization procedure provided by the product provider
Dose delivered	Recording the number of study products provided by the product provider
Dose received	Recording the number of study products delivered to the participants and extent to which they actively engage with study protocol
Cold chain (from provider in the study unit and from the study to the participants homes)	Checking refrigeration system and temperature and availability of cooler boxes
Intervention fidelity	Measuring and assessing compliance with intervention
Research protocol adherence	Research assistant and principal investigator to test adherence as widely as possible
Tolerability of the product	Acceptability record form
Compliance with scheduled appointment	Attendance record form
Data analysis	Testing analysis plan on pilot data

### 3.8.1 Compliance

Participants were requested to record the number of 100 g tubs of yoghurt they consumed daily and also to keep a count of the remaining containers of yoghurt in their daily record form between visits. Participants were sent text messages every third day to remind and encourage them to continue taking their two 100 g tubs of yoghurt. A reminder about their weekly visits was sent through text messaging a day before the visit and telephonic follow-up was used to verify responses or re-schedule appointments. Compliance with treatment was monitored at every visit by checking the recorded diaries. Fermented foods, fermented dairy products and any other product known to have a laxative effect were to be avoided during the study period and a dietary list with such items was provided at the beginning of the trial and monitored at every visit to ensure dietary compliance. Participants were encouraged to continue consuming the study product twice daily. A compliance rate above 80% was set as minimum. The family members of the participants were provided with enough of the 75 g fruit-flavoured Nutriday snack packs of yoghurt to prevent them from consuming the participants' study products. The Nutriday snack packs of yoghurt were provided to the participants during each weekly visit.

### 3.8.2 Tolerability, safety and adverse events

Participants were asked to report all unusual symptoms and any adverse events during the course of the study. They were also asked if they were tolerating the intervention product at each weekly visit during the study period.

## 3.9 MEASURING INSTRUMENTS AND MODE OF ADMINISTRATION

In a four-week parallel group treatment period, participants were requested to complete the questionnaires listed below and keep a daily diary of study product consumption and adverse effects. Table 3.1 indicates what was measured, measuring instruments and measurement frequency.

### 3.9.1 Demographic questionnaire

At the screening visit, participants completed the demographic questionnaire, including the following particulars: age, height, weight, family size, medical history, source of recruitment, time since IBS-C diagnosis, healthcare provider seen, bowel-cleansing habit and employment status.

**Table 3.2** Measuring instruments and mode of administration

What to measure	Measuring instruments	Reference	Frequency
Presence and severity of abdominal pain	IBS SSS	Francis <i>et al.</i> , 1997	Weekly
Presence and severity of abdominal distension or tightness	IBS SSS	Francis <i>et al.</i> , 1997	Weekly
Bowel habit satisfaction	IBS SSS	Francis <i>et al.</i> , 1997	Weekly
Bowel habit interference with life	IBS SSS	Francis <i>et al.</i> , 1997	Weekly
34 items of quality of life affected by IBS	IBS-34 QOL questionnaire	Patrick <i>et al.</i> , 1998	Baseline and end
Satisfactory relief of IBS symptoms	Question on relief of IBS symptoms	Kellow <i>et al.</i> , 2003	Weekly
Stool pattern	Bristol Stool Scale	Lewis S.J; 1997	Daily
IBS-C screening	Screening questionnaire	Rome III (2006)	Screening day
Demographic profile	Demographic questionnaire	Y. Rammwa	Screening day

### 3.9.2 IBS-C screening questionnaire

The Rome III diagnostic criteria for IBS-C were followed during the screening period at the referring doctors' rooms and confirmed by the researcher at the study unit. Recurrent abdominal pain or discomfort of at least three days per month in the last three months, with onset of symptoms at least six months previously was essential

for suspecting IBS. In addition to diagnosing a person as having IBS, the pain needed to be associated with at least two out of three features, which included improvement of pain or discomfort associated with a change in frequency or form of stool.

Patients were further sub-classified into IBS-C if they had hard or lumpy stools with no loose, watery, mushy or water stools in the past three months (indicated in Appendix 6).

### **3.9.3 Irritable Bowel Syndrome Severity Scoring System (Francis *et al.*, 1997)**

The IBS SSS is a validated tool for evaluating IBS symptoms (Francis *et al.*, 1997). The questionnaire comprises of four visual analogue scales examining the intensity of abdominal pain, severity of abdominal distention, satisfaction with bowel habits and finally the influence of IBS on the patient's life in general. The scale ranges from 0-100, with a minimum score of 0 indicating the absence of symptoms and a maximum score of 100 indicating severe symptoms. A total IBS SSS score is calculated by multiplying the number of days when pain is experienced by 10 and adding the score of each of the four visual analogue scales, producing a score ranging from 0 to 500. Scores <75, 75-175, 175-300 and >300 indicate no IBS symptoms, mild, moderate and severe symptoms of IBS, respectively. The scoring system is specifically designed to assess the severity of IBS symptom at a particular point in time. The questionnaire contains demographic information, instructions for the patient on how to use the questionnaire, actual severity scoring questions, which include ones on abdominal pain, bloating, defecation frequency, dissatisfaction with bowel habits, stool consistency and shape.

All participants received the IBS SSS questionnaire validated by Francis *et al.* (1997), to be self-administered at baseline and weekly during the entire four-week period. Questionnaires completed during the week were handed in during the weekly visits. Some IBS SSS questions were included in the daily record form, including questions on improvement in bloating, abdominal discomfort, abdominal pain and defecation frequency.

### **3.9.4 IBS QOL questionnaire (Patrick *et al.*, 1998)**

The IBS-QOL questionnaire was developed and well validated by Patrick *et al.* (1998) and consist of 34 items in a self-administered, condition-specific questionnaire that uses the following response scale: 1= not at all; 2= slightly; 3= moderately; 4= quite a bit; 5=extremely. The items contribute to the following subscale structures: Dysphoria, interference with activity, body image, health concerns, food avoidance, social reaction, sex and relationships. A request for approval to use the IBS-QOL questionnaire was sent to the authors and granted. The IBS-34 QOL questionnaire was administered at baseline and at the end of the four-week parallel group treatment period.








### 3.9.5 Daily diary/record form

The daily diary developed for this study included a record form for recording consumption of the study product, adverse effects, any medication started during the study period and consumption of any other fermented products. The daily diary is a form with short questions about IBS symptoms, which has been extracted from the IBS SSS. To my knowledge the daily diary for measuring IBS symptom has not been used before in the South African population.

### 3.9.6 Bristol Stool Scale (Heaton *et al.*, 1991)

Participants completed the Bristol Stool Scale (BSS) chart daily in their daily record form. The different stool forms were described in words and also illustrated by pictures in Figure 3.2. Stool forms 1 and 2 were classified as constipation, 3-5 as normal/easy to pass stools and 6 and 7 as diarrhoea. The Rome III criteria committee recommended the use of the BSS for subtyping the IBS by self-report of stool pattern (Heaton *et al.*, 1991).

## Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. <b>Entirely Liquid</b>

**Figure 3.2** The Bristol Stool Scale chart

### **3.9.7 Satisfactory relief of IBS score (Kellow *et al.*, 2003)**

Participants were required to record a weekly response to a question on the satisfactory relief of IBS during the four-week treatment period. Satisfactory relief measures satisfactory relief for the past seven days only. 'Have you experienced satisfactory relief of your IBS symptoms over the past seven days?'

The satisfactory relief question has been used before in a randomised control trial of a probiotic, VSL#3 on gut transit symptoms in IBS-D by Kim *et al.* (2003).

### **3.10 STATISTICAL ANALYSIS**

The statistical analysis was done in consultation with the statistical consultation service of NWU. The analyses were conducted only on the 17 participants who completed the study as per protocol. Statistical significance was set at  $p < 0.05$ . Descriptive data were presented as mean and standard deviation or median and range. Analysis was done using the Wilcoxon signed rank test or Fishers exact test, as appropriate. Cramer's V measured the difference in proportions of participants in the intervention group compared to those in the control group.

Repeated measures of analysis of variance (ANOVA) assessed the difference in the average values on the scores of severity of pain and number of days with pain. The ANOVA method was also applied to follow two groups over time, looking within and between the intervention and control groups. Post hoc analysis for the repeated measure ANOVA was adjusted using the Bonferroni method.

To see if the two groups were comparable before intervention, both an independent T-test and a dependent T-test were applied. Comparison of QOL within groups was assessed by the paired T-test. Differences between groups were analysed using a non-parametric test, the Wilcoxon signed rank test. As this was a pilot study, a sample size calculation was not performed.

### **3.11 CONCLUDING REMARKS**

In conducting a pilot study, a research team must focus on process outcomes to ensure successful operational conditions of the main study. A review of the literature reveals that many reports on pilot studies are related to research, rather than the process and outcomes. This presents an unrealistic expectation for researchers that pilot studies are a small-scale mimic of the research projects and will in themselves produce meaningful statistical results.

# CHAPTER 4: RESULTS

## 4.1 OUTCOME OF THE PILOT STUDY

This chapter presents the results of the process evaluation and the effectiveness of the intervention of the pilot study. The results are therefore presented in two parts, namely the process evaluation and the effectiveness of the intervention.

## 4.2 PROCESS EVALUATION

### 4.2.1 Recruitment to the study

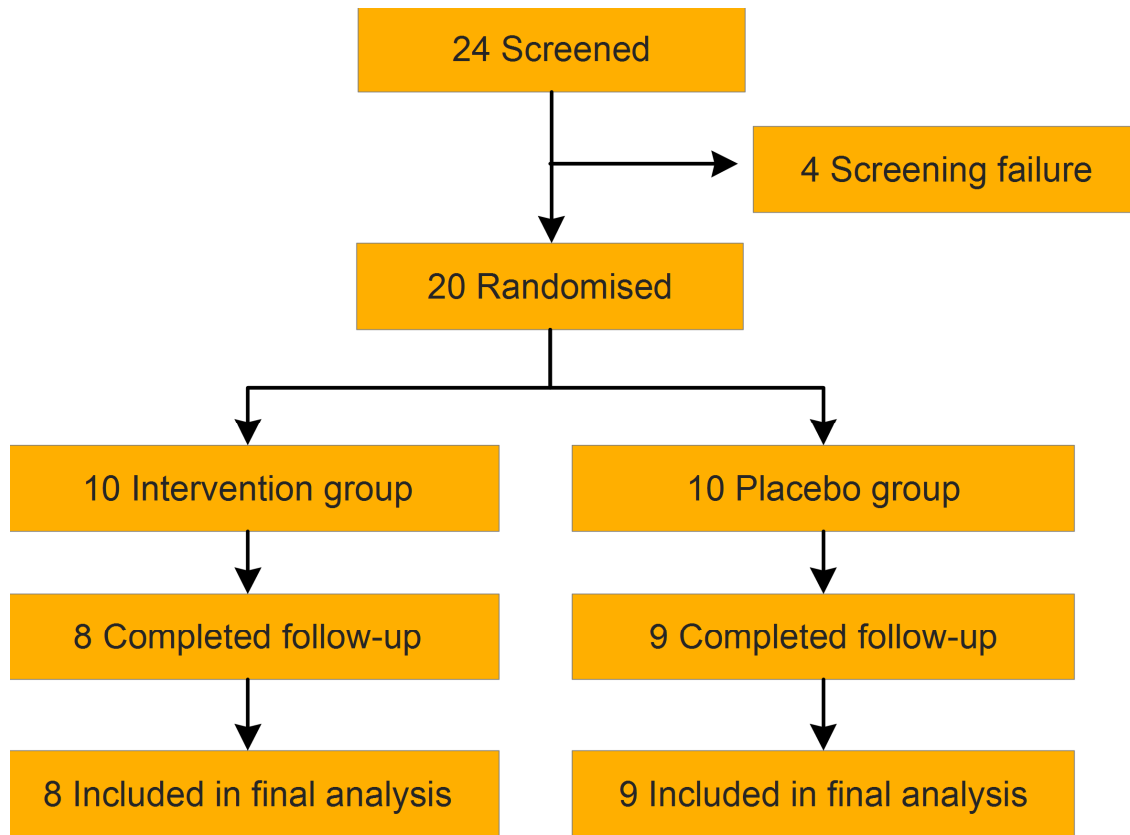
A total of 24 patients were referred by the doctors who were visited and informed of the study by the principal investigator. Patients were screened based on the Rome III criteria using the IBS module and 20 participants were randomised, 10 participants into the intervention group and 10 in the placebo group. The main reason for screening failure of four patients was reporting of alternating bowel movements. A total of 17 participants completed the study as per protocol. A study flow diagram is shown in Figure 4.1. During the enrolment process, participants were given the option to make their weekly visit during the week or the weekend and a few chose weekend days. No alterations were made to the study protocol during the trial.

### 4.2.2 Operational process

The study unit had all the equipment needed for the study, namely two refrigerators and a food scale, tubs for filling with yoghurt, a scale and booklets containing all the questionnaires. Cooler boxes were made available to the principal investigator for transporting study products from the product provider to the study unit and to participants for transporting the study products from the study unit to their homes.

### 4.2.3 Feasibility of product delivery

Enough study products were manufactured weekly, collected by the principal investigator and taken to the study unit, where they were distributed to the participants. Figure 4.2 shows the product workflow. Transportation of products from provider was implemented as planned with minimal adjustment at the beginning of the study. Enough cooler boxes were available for the participants to transport products to their homes.



**Figure 4.1** Pilot study participants flow chart

#### 4.2.4 Eligibility criteria confirmation

Referred patients came to the study unit with a referral letter from their referring medical practitioners. The principal investigator screened the patients according to the study entry criteria. Some medical practitioners showed unanticipated reluctance to complete the IBS module form confirming IBS-C according to the Rome III criteria for the patients they referred; they would send a patient with a referral letter confirming the IBS-C diagnosis and the principal investigator confirmed the IBS-C with an IBS module form. The demographic questionnaire was completed by the research assistant. Eligible participants were thoroughly briefed about the study and provided with an informed consent form to sign, if they agreed to participate in the study. While the principal investigator was completing the baseline QOL questionnaire with the participant, the research assistant weighed the products for the participants to save time.

#### 4.2.5 Consent rate confirmation

The consent rate was an average of five participants per week with a total of 20 participants over a four-week study period and complete data collection was performed over a seven-week period, which was the anticipated period.

#### **4.2.6 Randomisation**

The randomisation procedure was dealt with by the product provider and withheld from the study members throughout the study period. Since 2kg products from provider were clearly marked as 416 and 258, the filling into the 100g tub was done in an alternating the 2marked products in the study unit. Randomisation was effective and not difficult to administer.

#### **4.2.7 Role of the research assistant**

The principal investigator and the research assistant were competent in performing their tasks and were able to administer the protocol successfully as designed. The assistant did very well throughout the study. Training and educating her about the purpose of the study and participants' recruitment was critical, as she was involved in the operational processes of the pilot study, such as administration, telephoning all participants to remind them of their appointments and encouraging them to continue consuming their study products through text messaging, weighing the study products and checking that all questionnaires had been completed correctly and in full.



**Figure 4.2** Study logistics

**Table 4.1** Process evaluation components results

<b>Components</b>	<b>Outcomes</b>
Recruitment procedure	Collaboration with medical practitioners to refer eligible participants took place. Participants were recruited through the practitioners.
Consent procedures	Participants were not hesitant to sign the consent form.
Reach	A total of 24 IBS patients were screened, but only 20 were eligible.
Blinding	Research team was kept blinded throughout the study, the randomization numbers were kept by the product provider until the end of the study.
Randomisation	Randomisation was done by the product provider and the research team had no access to the randomisation procedure
Dose delivered	Study products were enough for the duration of the study which was completed after a total of seven weeks.
Dose received	Intervention was well received by the participants and the study protocol was implemented as planned
Cold chain (From provider, in the study unit and from the study to the participants homes)	Refrigeration system at the unit was adequate. Seventeen-litre cooler boxes were big enough for carrying study products to the unit. Participants' cooler boxes were big enough to carry both their products and family members' products to their homes.
Intervention fidelity	Intervention was successfully implemented.
Research protocol adherence	Research protocol was executed as planned.
Tolerability of the product	No adverse event reported throughout the study.
Compliance with scheduled appointment	Appointments were well kept by all the participants who completed the study.
Data analysis	Data analysed with the statistical consultation service of NWU .

#### 4.2.8 Questionnaire completion

All participants understood and answered the questions correctly. All questionnaires were first tried on a pilot group of 10 psychiatric ward patients using both the self-administration and the interviewer-administration techniques before they were used in the current pilot study to test if questionnaires were easy to understand and not difficult to administer.

The daily questionnaire needed to be adjusted and some questions on the IBS SSS needed to be rephrased for use in the self-administration technique. The adaptation of all three questionnaires was established in the current study's cultural context and both a self-administration and an interviewer technique were shown to be feasible in the context of the study.

#### **4.2.9 Acceptability of the intervention**

Intervention was not difficult to administer and the consumption of the study product was well accepted for the four weeks of the study period. Participants liked the taste of the yoghurt. No serious adverse events were reported in either group. Two adverse events were reported during the study, but no participants withdrew from the study because of the adverse events. One participant from the intervention group and one from the placebo group reported diarrhoea immediately after consumption of the study products in the third week of the study period.

Overall compliance in both the intervention and placebo group was 96.4%.

#### **4.2.10 Dropouts**

Three participants could not complete the study. One participant from the intervention group withdrew from the study within the first week because she was tired of consuming the study product. The second participant from the intervention group was involved in a car accident in the third week of the study period and was excluded from the study because of her low compliance. The third participant from the placebo group withdrew from the study because her baby was admitted to a hospital far from her home and she had to move to her parent's house next to the hospital.

#### **4.2.11 Data capturing and analysis**

It was important to conduct a pilot trial of the methods of data collection and analysis to ensure the necessary data would be available to answer the research question. Extraneous variables, which were not considered in the design, became apparent during the data analysis, such as grouping the stool forms in the daily questionnaires into normal, constipation or diarrhoea. Grouping of the stool form will improve the accuracy of the data set for the main study.

### **4.3 RESULTS INDICATING EFFECTIVENESS OF THE INTERVENTION**

The baseline characteristics of the participants in the intervention and placebo group are shown in Table 4.2. There was no statistically significant difference between the groups concerning any of the baseline characteristics; the two groups were similar in terms of gender, age and body mass index (BMI).

**Table 4.2** Baseline characteristics of the intervention and control group

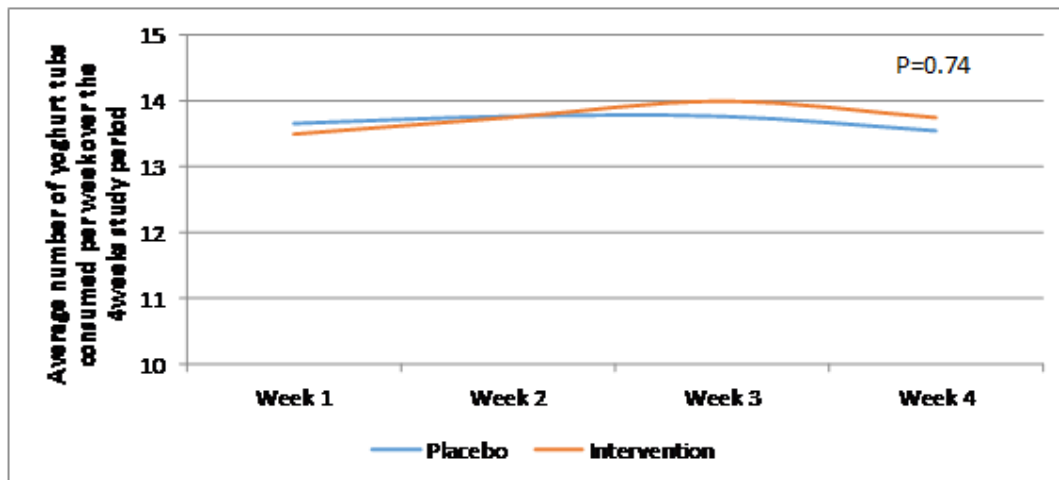
	<b>Intervention group n = 8</b>	<b>Placebo group n = 9</b>
Characteristics	Mean-(SD)	Mean-(SD)
Age	38.9 (10.5)	40.5 (11.2)
BMI	31.7 (6.63)	29.1 (3.8)

### 4.3.1 Daily record form

The results of the ANOVA test for the difference in the average values on the scores of yoghurt consumption, fermented products consumption, bowel emptying, degree of straining and feeling of incomplete emptying are shown in the graphs below.

#### 4.3.1.1 Yoghurt consumption

Figure 4.3 shows the average yoghurt consumption over the four-week study period.



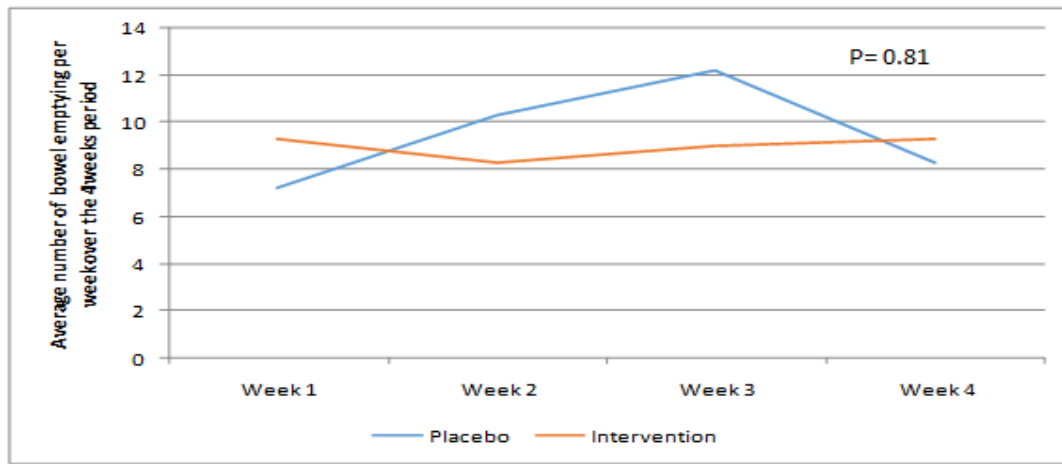
**Figure 4.3** Tubs of yoghurt consumed over the four-week study

Average yoghurt consumption was more than 13.5 tubs (96.4%) per week in both the placebo and intervention group. There was no difference in yoghurt consumption between the groups ( $p=0.74$ ), nor was there any change over time ( $p=0.16$ ).

A very small quantity of other fermented products was consumed by the participants, with no differences between the groups ( $p=0.72$ ) and over time ( $p=0.39$ ).

### 4.3.1.2 Daily bowel emptying

Figure 4.4 shows the average number of instances of bowel emptying over the four-week study.

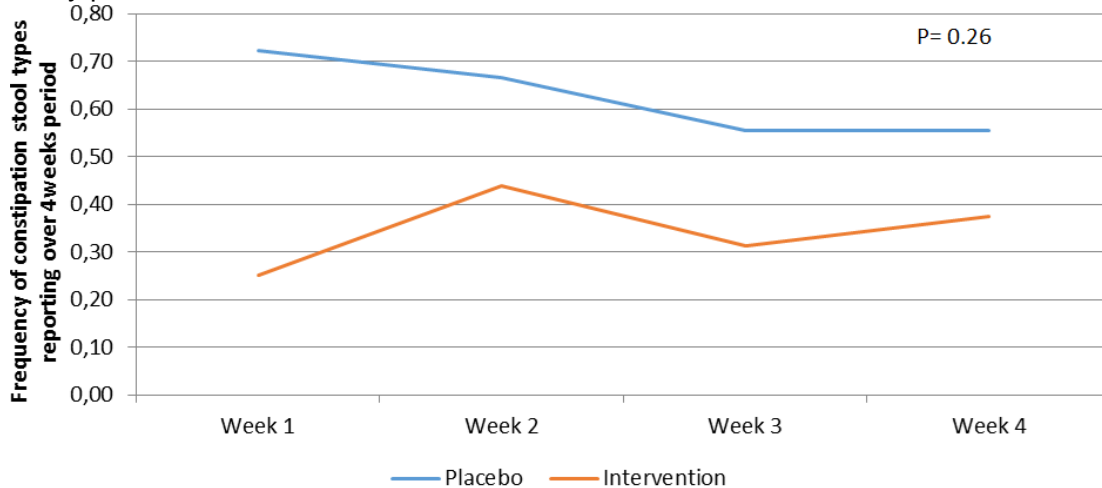


**Figure 4.4** Average number of bowel emptying events over the four-week study period

The difference in the average values on the scores of bowel emptying was analysed by repeated measures ANOVA. Repeated ANOVA showed no statistically significant difference between the two groups ( $p=0.81$ ) and no change was observed over time ( $p=0.6$ ).

### 4.3.2 Stool types frequency

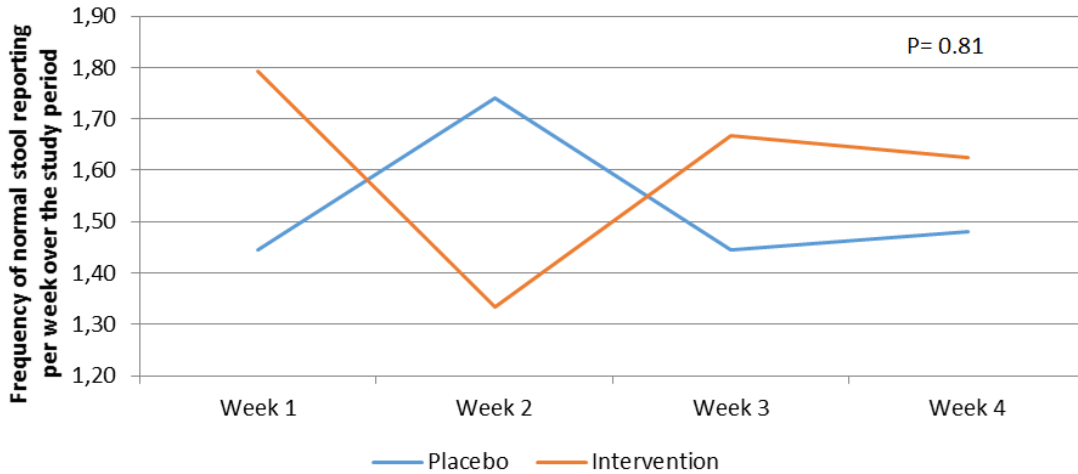
Figure 4.5 shows the frequency of stool types 1-2 (constipation) in the four-week study period



**Figure 4.5** Frequency of constipation stool type reporting per week over four-week study period

Stool types associated with constipation were more frequently reported throughout the four-week study period in the placebo group than in the intervention group. The difference between the groups was not statistically significant ( $p=0.26$ ), nor was it statistically significant over time ( $p=0.94$ ).

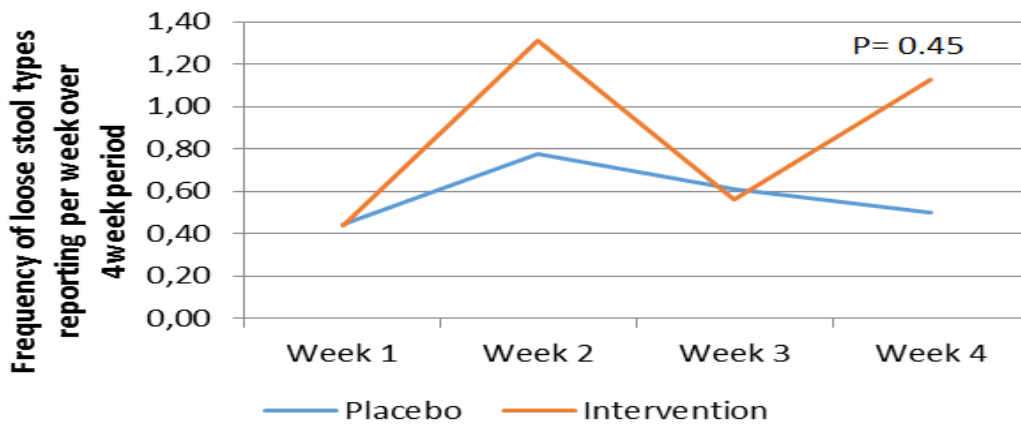
Figure 4.6 shows the frequency of stool type 3-5 (normal stools) in the four-week study period .



**Figure 4.6** Frequency of normal stool type reporting per week over four-week period

Normal stool type reporting remained relatively variable throughout the study period in both the intervention and placebo group, with no statistically significant difference between groups ( $p=0.81$ ) and over time ( $p=0.98$ ).

Figure 4.7 shows the frequency of stool types 6-7 (loose stool type) in the four-week study period.

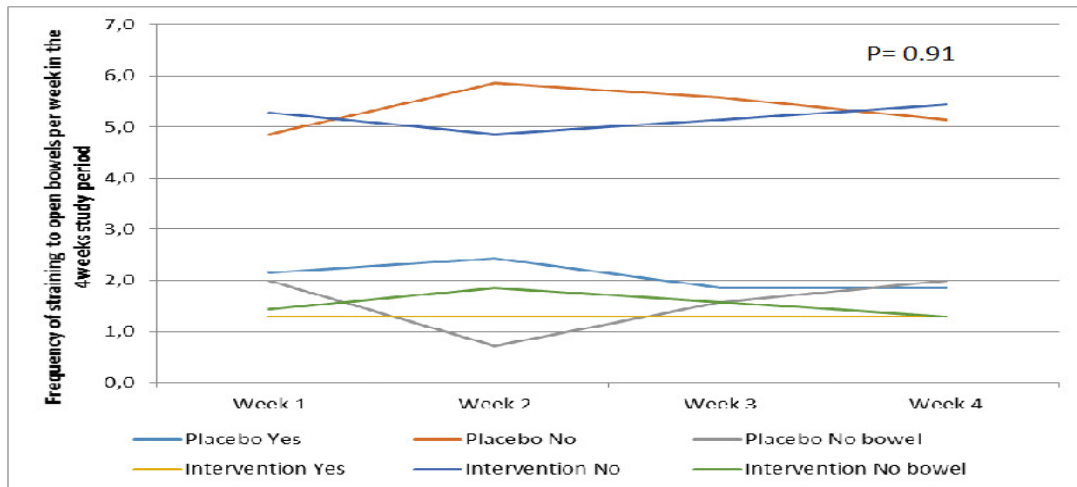


**Figure 4.7** Frequency of loose stool type reporting per week over four-week period

A loose stool type was frequently reported during the second and fourth week in the intervention group, but was not statistically significant between groups ( $p=0.45$ ).

### 4.3.3 Straining effects

Figure 4.8 shows the frequency of straining to open bowels per week over four-week period.

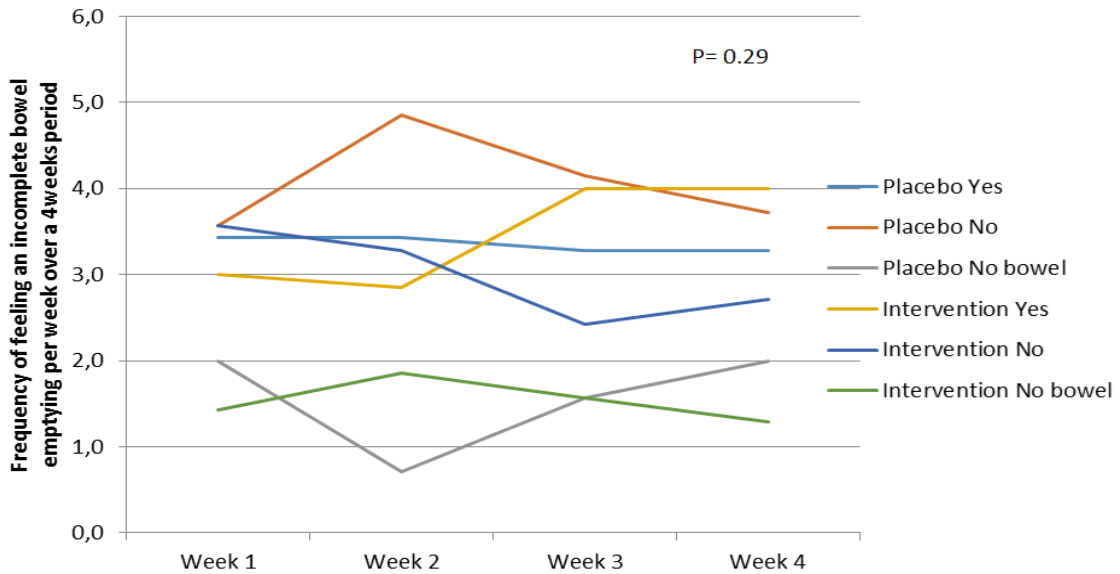


**Figure 4.8** Frequency of straining to open bowels per week over four-week period

Using repeated ANOVA, we did not find a statistically significant difference in the frequency of straining to open bowels between groups ( $p=0.91$ ), nor was there any change over time ( $p=0.67$ ).

### 4.3.4 Feeling of incomplete bowel emptying

Figure 4.9 shows the frequency of a feeling of incomplete bowel emptying after visiting the toilet per week over the four-week period.



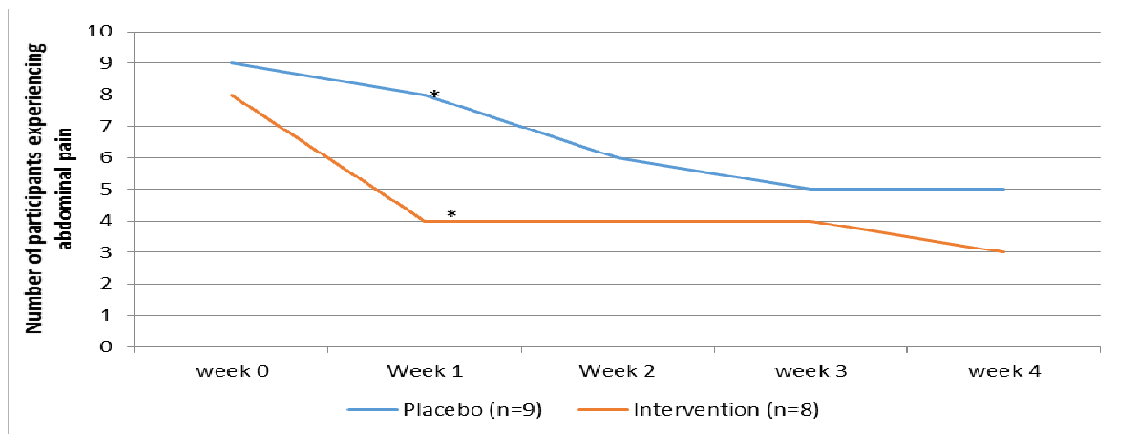
**Figure 4.9** Frequency of feeling of incomplete bowel emptying per week over the four-week period

Using repeated ANOVA, we did not find a statistically significant difference in the frequency of a feeling of incomplete bowel emptying between groups ( $p=0.29$ ), nor was there any change over time ( $p=0.57$ ).

### 4.3.5 IBS symptoms and severity of symptoms

#### 4.3.5.1 Abdominal pain

Figure 4.10 shows the number of participants experiencing abdominal pain throughout the study.



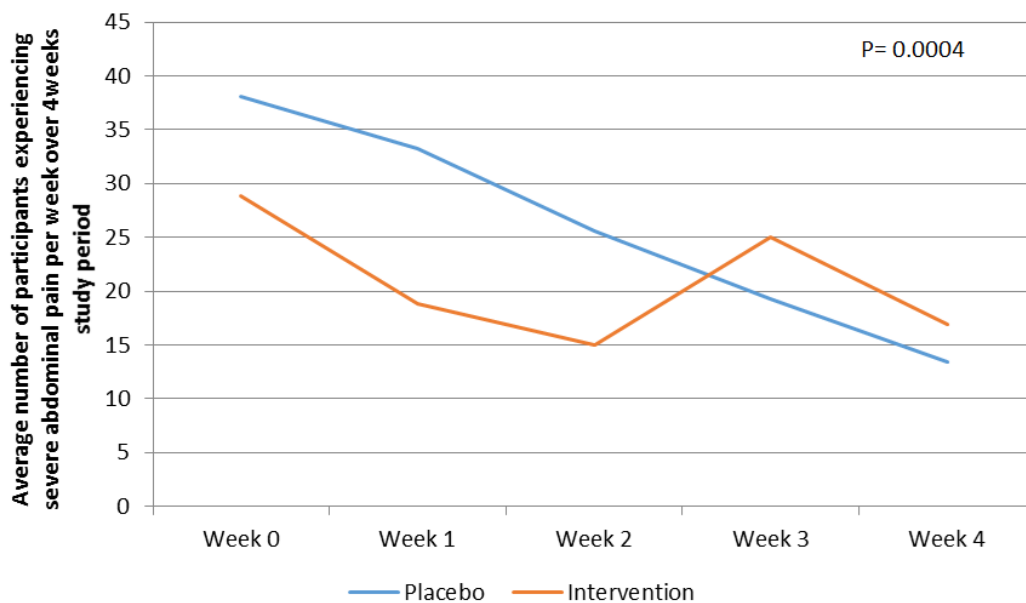
**Figure 4.10** Participants experiencing abdominal pain over the four week study period

As indicated by the asterisk (\*) in the figure, a significantly larger proportion of participants in the placebo group still suffered from abdominal pain after one week. A decrease in the proportion of participants with abdominal pain was observed at the

end of the study in both the intervention and the placebo group. The proportion of participants in the intervention group experiencing pain remained relatively stable during the study and a trend of participants reporting adequate relief in the placebo group in the first three weeks was observed but at no time was this statistically significant (five versus three for placebo and intervention respectively). The difference in the number of participants experiencing abdominal pain between groups after one week is of practical importance ( $d=0.6$ ).

#### 4.3.5.2 Severity of abdominal pain

Figure 4.11 shows the number of participants experiencing severe abdominal pain throughout the four-week study.

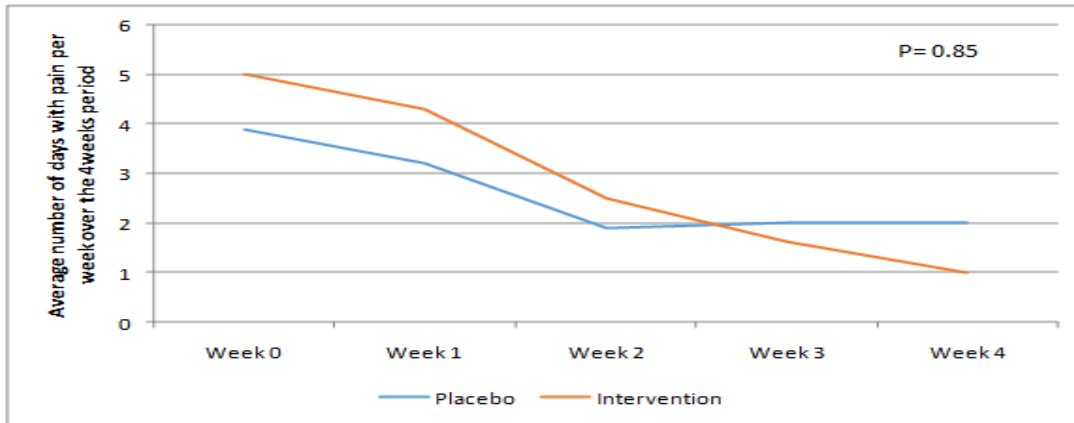


**Figure 4.11** Average number of participants experiencing severe abdominal pain over the four-week study period

Using repeated ANOVA we did not find a statistically significant difference between the groups in terms of the severity of pain experienced by participants ( $p=0.74$ ), but the improvement over time in both groups was statistically significant ( $p=0.004$ ).

#### 4.3.5.4 Number of days with pain

Figure 4.12 shows the number of days participants experienced abdominal pain throughout the study.

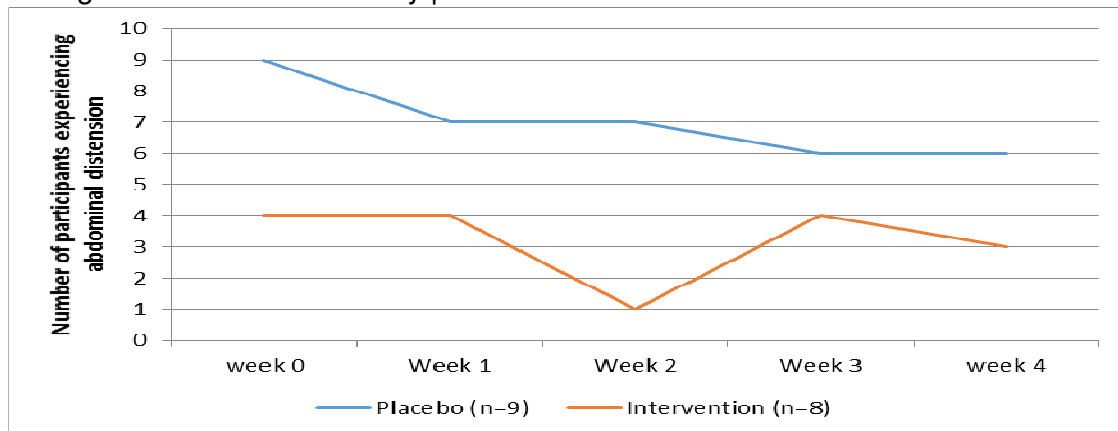


**Figure 4.12** Average number of days participants experienced pain over four-week study period

The repeated ANOVA did not show a statistically significant difference between the groups in terms of the number of days on which participants experienced pain ( $p=0.85$ ), but significant improvement was reported in both groups over time ( $p=0.00001$ ).

#### 4.3.5.5 Abdominal distention

Figure 4.13 shows the number of participants experiencing abdominal distention throughout the four-week study period.



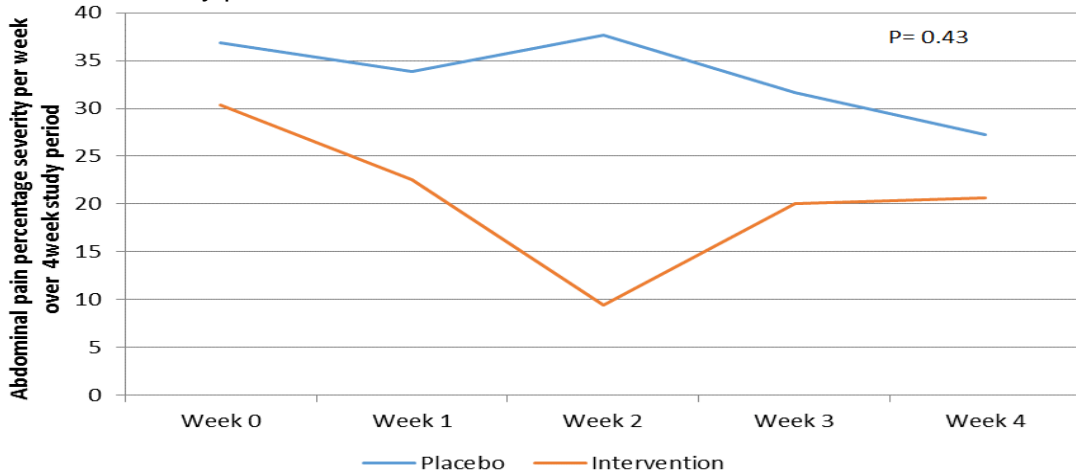
**Figure 4.13** Participants experiencing abdominal distention over four-week study period

A larger proportion of participants in the placebo group suffered from abdominal distention at baseline and a minor decrease in the proportion of participants with abdominal distention was observed at the end of the study in both the intervention and the placebo group. By week 4, the number of participants with abdominal distention was not significantly different between the two groups (six versus three for placebo and intervention respectively). At the end of week 2 the differences in the

number of participants experiencing abdominal distention between groups was of large practical importance ( $d= 0.7$ ).

#### 4.3.5.6 Severity of abdominal distention

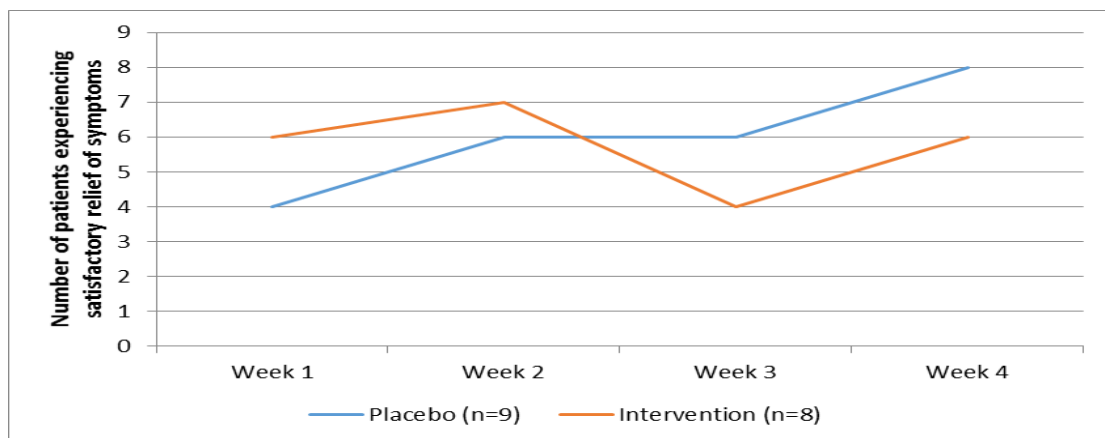
Figure 4.14 shows the percentage of severity of abdominal distention throughout the four-week study period.



**Figure 4.14** Percentage of severity of abdominal distention over four-week period. Repeated ANOVA did not show a statistically significant difference between the groups in terms of bloating severity ( $p=0.43$ ), nor was there any difference over time in both groups.

#### 4.3.6 Satisfactory relief of symptoms

Figure 4.15 shows the satisfactory relief of symptoms participants experienced throughout the study.



**Figure 4.15** Participants experiencing satisfactory relief of symptoms over four-week period

There was no difference in the proportion of participants experiencing satisfactory relief of overall IBS symptoms between groups ( $d<0.5$ ).

### 4.3.7 Weekly comparison of the stool types from Bristol stool form scale

The proportion of bowel movements was evaluated using the stool evaluation by Heaton *et al.*, (1991), which classified stool types 1-2 as constipation, stool type 3-5 as normal and stool type 6-7 as diarrhoea. The p-value for comparisons between groups and stool types was analysed by using the Bonferroni test.

Figure 4.16 shows stool type comparison in the first week of the four-week study period

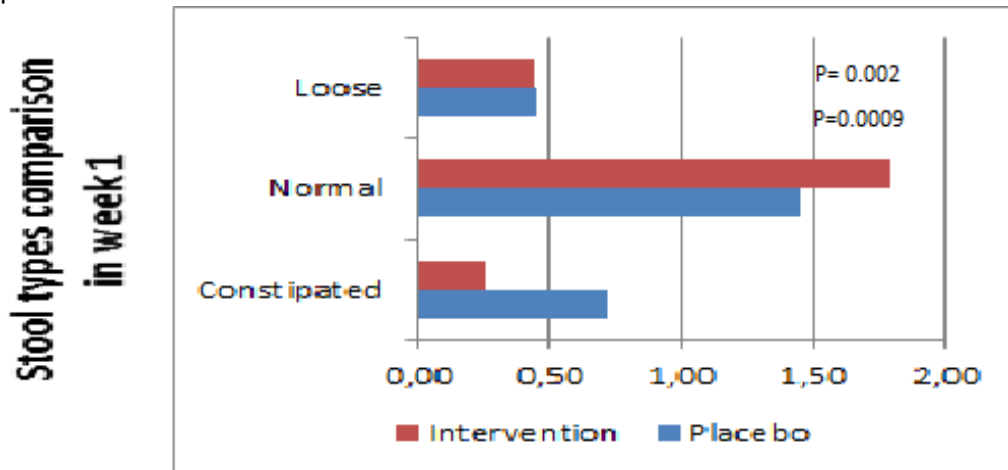


Figure 4.16 First week stool comparison

Figure 4.17 shows the stool type comparison in the second week of the four-week study period.

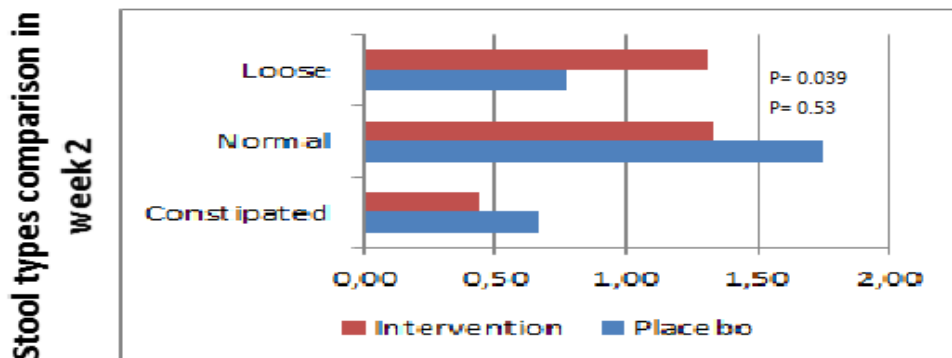
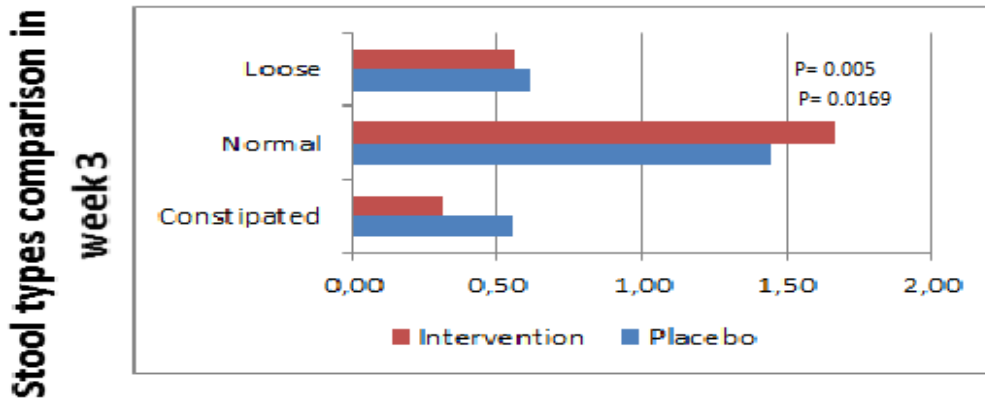
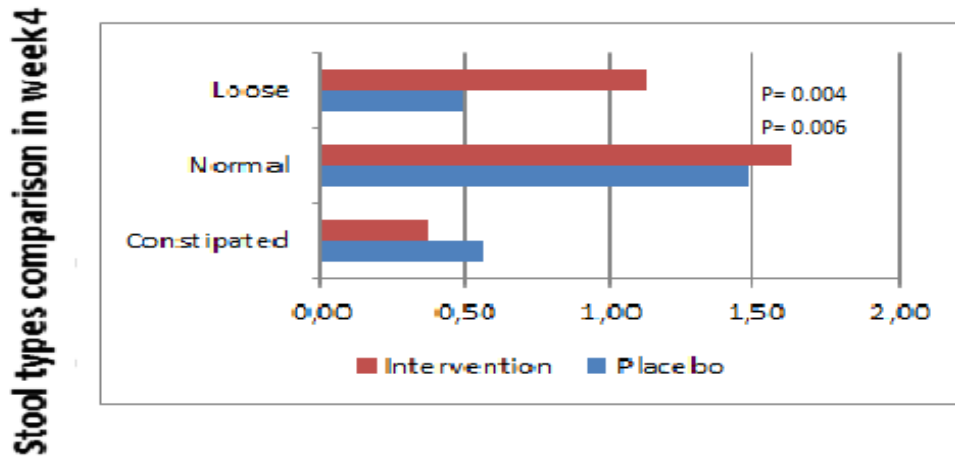


Figure 4.17 Second week stool comparison



**Figure 4.18** Third week stool comparison

Figure 4.19 shows the stool type comparison in the fourth week of the four-week study period.



**Figure 4.19** Fourth week stool comparison

The frequency of the normal stool type was significantly higher between the loose stool type and constipation stool type in both the intervention and placebo group throughout the four week study period.

#### 4.3.8 Quality of Life (QOL)

The changes in IBS QOL scores before and after treatments were compared between the two groups. The Wilcoxon signed rank test (non-parametric) analysed the mean QOL score. In the placebo group the mean QOL score improved significantly by -1.37 points ( $p=0.017$ ). The mean improvement in the QOL in the intervention group of -1.22 points was not statistically significant ( $p=0.093$ ). A large effect size ( $d= 0.87$ ) in the placebo group and ( $d= 0.92$ ) in the intervention group indicates that the improvement in QOL in each group is likely to be of practical

importance. There was no statistically significant difference in the intervention group compared to the improvement in the placebo group ( $p=0.744$ ).

# CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATION

## 5.1 DISCUSSION

The aim of this pilot study was to conduct a process evaluation and investigate the effectiveness of probiotic *Bifidobacterium animalis* DN-173 010 intervention in black females with IBS-C. The findings of this study reflect the results of both the process evaluation and the effectiveness of the intervention.

### Process evaluation

Process evaluation enabled us to assess the results that can be produced when a programme is delivered under optimal conditions and the effect that can be expected. Process evaluation assisted in keeping the programme on track and in monitoring the extent to which the intervention was implemented and reached the intended participants. The implementation of the pilot study was considered successful, with no barriers and major difficulties encountered. The number of participants to be recruited was reached in more or less the expected time after confirmation of the eligibility criteria. The close working relationship between the principal investigator and medical practitioners was key to smooth running of the study.

For the sake of consistency and applicability of the results of this study, the Rome III criteria for IBS were used. Participants willingly signed the consent form after being thoroughly briefed about the study. The randomisation that was done by the product provider was accurate and effective, with the research team being kept blinded throughout the study period. The principal investigator and research assistant were able to administer the protocol as designed throughout the study period. The process evaluation methods and procedures used to deliver the key components combined both qualitative and quantitative methods to document the intervention as it was delivered, identify areas where delivery was a problem and contribute to development of strategies to address problems with delivery of the intervention. Process evaluation in pilot studies allows the growth and improvement of data collection and computer databases (Musil, 2006). Piloting the methods of data collection and analysis enabled us to ensure the necessary data were collected to answer the research question.

With respect to the question of the feasibility of intervention implementation, it was possible to administer the intervention effectively throughout the study period. The overall product consumption rate was very good (96.4%) throughout the four-week study period; the minimum compliance rate was set at 80%. From the results it is clear that the intervention product was highly acceptable. Participants frequently mentioned their appreciation for the supply of yoghurt to their family members.

Process evaluation questionnaires were also used to identify possible adverse events specific to the intervention. None of the participants recorded any side-effects or adverse events.

In this study we evaluated all the questionnaires that were used to see if participants understood them and had completed them correctly. The interview technique applied in the QOL questionnaire proved to give a better yield of answers and a minimal rate of unanswered questions, which was noted in the self-administered IBS SSS questionnaire. The higher yield of correctly completed daily record forms than in the IBS SSS questionnaire when the self-administered technique was applied could be because of the Bristol stool picture chart. Participants seemed to understand the Bristol stool chart better; they also mentioned that it had taught them always to check their stools' shape and form after bowel movement. Participants completed the questionnaires correctly.

The process evaluation components assessed in this study included:

- Dose delivered pertaining to the intervention delivered to the participants;
- Dose received pertaining to participants' receptiveness, compliance with the study procedures and consumption of study products;
- Reach;
- Recruitment; and
- Context.

The above components have been reported as key elements in process evaluation (Linnan *et al.*, 2008). Using this clearly articulated model for the process evaluation was one of the strengths of this study that enabled us to execute the operational process as planned. The process evaluation of the current study had the function not only of providing contextual answers to questions about why interventions work, but more critically, examining how the intervention was received and whether it could be repeated (Parry-Langdon *et al.*, 2003).

### **Effectiveness of the intervention findings**

The use of probiotic *Bifidobacterium animalis* DN-173 010 in managing IBS-C appeared both feasible and acceptable in black South African women. In addition to the high acceptability and feasibility, a placebo effect was observed in the placebo group, which confirms well-known data reported in IBS placebo-controlled trials showing an average placebo effect rate of 40-45% (Enck & Klosterhalfen, 2005; Pitz *et al.*, 2005). The results in both the intervention and the placebo groups showed improvement across a range of IBS symptoms, but the improvements were not statistically significant. Reductions in symptoms were reported at similar levels in both groups. A recent study by Roberts *et al.* (2013) reported similar results with the same probiotic; IBS symptoms improved in both the intervention and the placebo group, but it was not significant between the groups.

The fact that similar improvements were observed in ease of stool passage suggests that the participants may benefit from regular consumption of yoghurt with or without probiotics. The frequency of loose stools in the intervention group during the second and fourth week could also be because of the prevalence of lactose intolerance among people of African descent, which could have been increased by regular consumption of 200 g of yoghurt per day. The most common carbohydrate maldigestion syndrome, lactose intolerance, results from insufficient enterocyte lactase, which hydrolyses lactose into glucose and galactose. Lactase deficiency occurs in 21% of Caucasians but is more prevalent in those of African (75%) and Native American (79%) descent (Schrimshaw & Murray, 2001). Most lactase-deficient people have residual enzymes and tolerate small amounts of lactose. It is also a known fact that such people may tolerate yoghurt with active cultures because of the bacterial  $\beta$ -galactosidase (Kolars *et al.*, 1984), but the frequency of consumption of such products has not yet been established. The difference between the normal and constipation stool type reporting was not statistically significant in both groups.

Other studies suggested that IBS-C sufferers might benefit from consumption of fermented milk with added *Bifidobacterium animalis* DN-173010, as it alleviates bloating and improve transit and QOL (Bouvier *et al.*, 2001; Guyonnet *et al.*, 2009; Meance *et al.*, 2001; Meance *et al.*, 2003). In our study there was no improvement in abdominal distention from the first week to the third week, but a minor improvement was observed at the end of the fourth week in both groups, although not significant. This is different from what has been reported by Marteau *et al.* (1996), that dairy products containing lactase-producing *Lactobacillus acidophilus*, *Bifidobacterium* species, and *Lactobacillus* reduce bloating in some with a risk of lactose intolerance. Severity of pain and number of days with pain improved significantly over time of yoghurt consumption in both groups but not between groups. A large effect size in the placebo group and in the intervention group indicates that the improvement in QOL in each group is likely to be of significant practical importance but the improvement was not statistically significant in the intervention group compared to the improvement in the placebo group, which is a different finding from what was reported by Guyonnet *et al.* (2007).

Although participants were reporting a feeling of satisfactory relief of symptoms, the difference between the groups was not statistically significant. The frequency of straining and a feeling of incomplete bowel emptying remained unchanged in both groups.

## 5.2 LESSONS LEARNED

The current study was planned and conducted in a way that would make it possible to systematically measure effects and capture the experience and lessons learned during the pilot study.

- It is not advisable to include the Easter month in the data-collection period. The Easter holiday falls in one of the main holiday months on the South African calendar; people travel from their work places (cities) to their hometowns/villages and also to their yearly church conferences, which could disturb the daily consumption of yoghurt.
- It has to be made clear to all the eligible participants that they are participating voluntarily and will not be charged or paid for their participation.
- Transportation of the study products and boxes of yoghurt tubs needs to be taken into consideration before the implementation of the study.
- Referring doctors are reluctant to complete the IBS screening questionnaire provided to them; confirming the diagnosis by using the IBS module questionnaire in the study unit was critical.
- Participants do not like long waiting periods during their weekly visits, which were usually caused by going through their questionnaires to check if these had been fully completed and weighing their next intervention supply.
- Participants found the project very appealing because of the provision of extra yoghurt to their family members.
- Unflavoured yoghurt was highly acceptable to both the intervention and placebo group. Participants compared the taste with sour milk (amasi/inkomazi), to which sugar has been added and they did not get bored with consuming it daily.

## 5.3 CONCLUSION

- Conducting a pilot study was an extremely useful strategy for testing our recruitment, research process, implementation, measuring instruments and data collection methods. Pilot study results inform feasibility, which in turn points to modifications needed in the planning and design of the main clinical trial. IBS symptom improvements reported were not different between the groups, which does not support evidence reported by other researchers, but our study was small and short which may have limited the ability to detect the effect of the intervention on individual symptoms. The intervention has shown to be safe, acceptable and feasible to administer, which could support the implementation of a larger randomised controlled trial.

## 5.4 LIMITATIONS

- Our short pilot study may have limited the ability to detect the effect of the intervention on individual symptoms.
- The sample size was small; as a result, our study was not adequately powered on clinical outcomes. While we acknowledge this as a limitation, we also believe it is always necessary first to demonstrate the feasibility of an approach.
- Another limitation is that the entire population was female, so the effects of the probiotic *Bifidobacterium animalis* DN-173 010 in IBS-C may not be generalised to male IBS-C patients.
- The study mostly relied on self-administration of questionnaires, which is considered unbiased for the patient and time-saving for the investigators, but vulnerable to social desirability bias and measurement error.

## 5.5 RECOMMENDATIONS

The current pilot study highlights important considerations for the design of future studies on probiotic *Bifidobacterium animalis* DN-173010 in treatment for IBS-C among the black South African population.

- Results from our hypothesis testing should be treated as preliminary and interpreted with caution, as no formal power calculation was carried out.
- The temptation not to proceed with the main clinical trial when no significant differences are found should be avoided, given the sample size of our pilot study.
- Studies that include both males and females are needed.
- Larger studies with longer duration of treatment and follow-up are necessary.
- More well-designed trials testing the same probiotic strain should be undertaken.
- Researchers should adhere to intention to treat principles, analysing all subjects within the group to which they were originally assigned, regardless of compliance with treatment and response to treatment.
- Given that IBS is not a single entity, it is recommended that therapies be customised to specific IBS subtypes.
- Rewarding the research assistant is vital to successful implementation.

## REFERENCES

Abrahamsson, H. 1987. Gastrointestinal motility in patients with the irritable bowel syndrome. *Scandinavian journal of gastroenterology*, 130(1):21-26.

Agrawal, A., Houghton, L.A, Morris, J., Reilly, B., Guyonnets, D., Goupil Feuillerats, N., Schlumbergers, A., Jakobs, S. & Whorwell, P.J. 2008. Clinical trial: The effects of a fermented milk product containing *Bifidobacterium lactis* DN-173 010 on abdominal distention and gastrointestinal transit in irritable bowel syndrome with constipation. *Gastroenterology*, 29:104-114.

American College of Gastroenterology Task Force on IBS. 2009. An evidence-based systematic review on the management of irritable bowel syndrome. *American journal of gastroenterology*, 104:S1-S35.

Amouretti, M., Le Pen, C. & Gaudin, A.F. 2006. Impact of irritable bowel syndrome on health-related quality of life (HRQOL). *Gastroenterology and clinical biology*, 30:241-246.

Armitage, E., Drummond, H.E., Wilson, D.C. & Ghosh, S. 2001. Increasing incidence of both juvenile-onset Crohn's disease and ulcerative colitis in Scotland. *European journal of gastroenterology and hepatology*, 13:1439-1447.

Andresen, V. & Baumgart, D.C. 2006. Role of probiotics in the treatment of irritable bowel syndrome: Potential mechanisms and current clinical evidence. *International journal of probiotics and prebiotics*, 1:11-18.

Anon. 1992. Yoghurt and probiotics. *Choice*, 11:32.

Azpiroz, F. & Malagelada, J.R. 2005. Abdominal bloating. *Gastroenterology*, 129:1060-1078.

Bartholomew, L.K., Parcel, G.S., Kok, G. & Gottlieb, N.H. 2001. Intervention mapping: Designing theory and evidence-based health promotion programs. *New York: McGraw-Hill*.

Baumgart, D.C. & Dignass, A.U. 2004. Current biological therapies for inflammatory bowel disease. *Current pharmaceutical design*, 10:4127-4147.

Bausserman, M. & Michail, S. 2005. The use of *Lactobacillus GG* in irritable bowel syndrome in children: A double blind randomized controlled trial. *Journal of paediatrics*. 147:197-201.

Berg, M.J., Goettsch, W.G., Van Den, B.G., Smout, A.J. & Herings, R.M. 2006. Quality of life of patients with irritable bowel syndrome is low compared to others with

chronic diseases. *European journal of gastroenterology and hepatology*, 18:475-481.

Berrada, N., Lemeland, J.F., Laroche, G., Thouvenot, P. & Piaia, M. 1991. *Bifidobacterium* from fermented milks: Survival during gastric transit time. *Journal of dairy & science*, 74:409-413.

Bertoni, J., Calamary, L., Malamti, M.G. & Azzoni, A. 1994. Factor modifying the acidification rate of milk. *Lait*, 17:941.

Besselink, M.G., Van Santvoort, H.C., Renooij, W., De Smet, M.B., Boermeester, M.A. & Fischer, K. 2009. Intestinal barrier dysfunction in a randomized trial of a specific probiotic composition in acute pancreatitis. *Ann Surgery*, 250: 712-719.

Biavati, B. & Mattarelli, P. 2001. The family *bifidobacteriaceae*. *The prokaryotes*, 1-70.

Bijkerk, C.J., De Wit, N.J., Stalman, W.A., Knottnerus, J.A., Hoes, A.W. & Muris, J.W. 2003. Irritable bowel syndrome in primary care: The patients' and doctors' views on symptoms, etiology and management. *Canadian journal of gastroenterology*, 17: 363-368.

Borman, R. 2001. Serotogenic modulation and irritable bowel syndrome. *Expert opinion in emerging drugs*, 6:57-68.

Bouin, M., Lupien, F. & Riberdy, M. 2004. Intolerances to visceral distention in functional dyspepsia or irritable bowel syndrome. *Pain*, 113:160-171.

Bouvier, M., Meance, S. Bouley, C., Berta, J.L. & Grimaud, J.C. 2001. Effects of consumption of a milk fermented by a probiotic *Bifidobacterium animalis* DN 173010 on colonic transit times in healthy humans. *Bioscience of microflora*, 20:43-48.

Brandt, L.J., Chey, W.D., Foxx-Orenstein, A.E., Quigley, E.M.M., Schiller, L.R. & Schoenfeld, P.S. 2009. An evidence-based systematic review on the management of irritable bowel syndrome. *American journal of gastroenterology*, 104(1):S8-S35.

Brenner, D.M, Moeller, M.J. & Chey, W.D. 2009. The utility of probiotics in the treatment of irritable bowel syndrome: A systematic review. *American journal of gastroenterology*, 104(4):1033-1049.

Browne, R.H. 1995. On the use of a pilot sample for sample size determination. *Statistics in medicine*, 14:1933-1940.

Buchnan, R.E & Gibbons, N.E. 1974. Bergy's manual of deteriorative bacteriology, 8<sup>th</sup> edition, Williams and Wilkins, Baltimore. USA.

Burrows, R.F., Gan, E.T., Gallus, A.S., Wallace, E.M. & Burrows, E.A. 2001. A randomized double-blind placebo controlled trial of low molecular weight heparin as prophylaxis in preventing venous thrombotic events after caesarean section: A pilot study. *British journal of obstetrics and gynaecology*, 108:835-839.

Cabre, E. 2011. Clinical nutrition university: Nutrition in the prevention and management of irritable bowel syndrome, constipation and diverticulosis. *European society for clinical nutrition and metabolism*, 6:85-95.

Carfoot, S., Dickson, R. & Williamson, P.R. 2002. Breastfeeding: Effects of skin to skin care trial. *Celebrating health research*, 4:234-239.

CFI (Canadian Food Inspection Agency), 2009. Probiotic claims. <http://www.inspection.gc.ca/english/fssa/labeti/guide/ch8ae.shtml> 2009 Chapter 8, section 8.7

Camilleri, M. 2006. Probiotic and irritable bowel syndrome: Rationale, putative mechanisms, and evidence of clinical efficacy. *Journal of clinical gastroenterology*, 40: 264-269.

Camilleri, M. & Talley, N.J. 2006. Pathophysiology as a basis for understanding symptom complexes and therapeutic targets. *Neuron-gastroenterology motility*, 16:135-142.

Camilleri, M., Mangel, A.L., Fehnel, S.E., Drossman, D.A, Mayer, E.A. & Nicholas, J.T. 2006(b). Primary endpoints for irritable bowel syndrome trials: A review of performance endpoints. *Clinical gastroenterology and hepatology*, 5:534-540.

Camilleri, M., Bharuda, A. & Ueno, R. 2006. Effect of a selective chloride channel activator, lubiprostone, on GI transit, gastric sensory and motor functions in healthy volunteers. *Clinical gastroenterology and hepatology*, 290(5): G941-G47.

Cash, B.D. & Chey W.D. 2005. Diagnosis of irritable bowel syndrome. *Gastroenterology clinic of North America*, 34:247-255.

Champagne, C.P., Ross, R.P., Saarela, M., Hansen, K.F. & Charalampopoulos, D. 2011. Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. *International journal of food microbiology*, 149:185-193.

Chassany, O., Marquis, P. & Scherrer, B. 1999. Validation of a specific quality of life questionnaire for functional digestive disorders. *International journal of gastroenterology & hepatology*, 44: 527-533.

Clouse, R.F. 2003(a). Managing functional bowel disorders from top to bottom: Lessons from a well-designed treatment trial. *Gastroenterology*, 125:249-253.

- Clouse, R.F. 2003(a). Antidepressants for irritable bowel syndrome. *Gastroenterology*, 52:598-599.
- Coates, M.D., Mahoney, C.R. & Linden, D.R. 2004. Molecular defects in mucosal serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology*, 126:1657-1664.
- Costello, M. 1993. Probiotic foods. In the food industry conference proceedings, Sidney convention and exhibition centre. *Publication of food and probiotics*, July 12-24.
- Coulie, B., Camilleri, M, & Gupta, V. 2002. Evidence for autonomic dysregulation in the irritable bowel syndrome. *Digestive science*, 47:1716-1722.
- Creminoni, F., Di Caro, S., Nsita, E.C., Bartolozzi, F., Capelli, G., Gasbarrini, G. & Gasbarrini, A. 2002. Meta-analyses: The effect of probiotic administration on antibiotic-associated diarrhoea. *alimentary pharmacology and therapeutics*, 16:1461-1467.
- Cremonini, F. & Talley, N.J. 2005. Irritable bowel syndrome: Epidemiology, natural history, health care seeking and emerging risk factors. *Gastroenterology*, 34: 189-204.
- Crowell, M.D., Harris, L. Jones, M.P. & Chang, L. 2005. New insights into the pathophysiology of irritable bowel syndrome: Implications for future treatments. *Current gastroenterology reports*, 7:272-279.
- Dave, R.I. 1998. Factors affecting viability of yoghurt and probiotic bacteria in commercial starter cultures. *Australian journal of dairy technology*, 4:341-363.
- Deshpande, G., Rao, S. & Patole, S. 2007. Probiotics for prevention of necrotizing enterocolitis in preterm neonates with very low birth weight: A systematic review of randomized controlled trials. *Lancet*, 369:1647-1620.
- Dhaliwal, S.K. & Hunt, R.H. 2004. Doctor-patient interaction for irritable bowel syndrome in primary care: A systematic perspective. *European journal of gastroenterology & hepatology*, 16: 1161-1166.
- Dickhaus, B., Mayer, E.A. & Firooz, N. 2003. Effect of auditory stress on perceptual and emotional ratings of visceral stimuli in IBS patients. *American journal of gastroenterology*, 98:135-143.
- Dorn, S.D., Kaptchuk, T.J., Park, J.B., Nguyen, L.T., Canenguez, K., Nam, B.H., Woods, K.B., Conboy, L.A., Stason W.B. & Lembo, A.J. 2007. A meta-analysis of the placebo response in complementary and alternative medicine trials of irritable bowel syndrome. *Neurogastroenterology and motility*, 19:630-637.

Drossman, D.A. & Whitehead, W.E. 2010. Validation of symptom-based diagnostic criteria for irritable bowel syndrome: A critical review. *American journal of gastroenterology*, 105 (4):814-820.

Drossman, D.A. 2006. The functional gastrointestinal disorders and Rome III process. *Gastroenterology*, 130: 377-1390.

Drossman, D.A., Camilleri, M., Mayer, E.A. & Whitehead, H.E. 2002. AGA technical review on irritable bowel syndrome. *Gastroenterology*, 123: 2108-2131.

Eduard, C. 2011. Clinical nutrition university: Nutrition in the prevention and management of irritable bowel syndrome, constipation and diverticulosis. *European journal of clinical nutrition and metabolism*, 6:e85-e95.

Elo, S., Saxelin, M. & Salimen, S. 1991. Attachment of Lactobacillus casei strain GG to human colon carcinoma cell line Caco-2: Comparison with other dairy strains. *Microbiology*, 13:154-156.

Enck, P. & Klosterhalfen, S. 2005. The placebo response in functional bowel disorders: Perspectives and putative mechanisms. *Neurogastroenterology and motility*, 17:325-331.

Engsbro, A.L., Simren, M. & Bytzer, P. 2011. Short-term stability of subtypes in the irritable bowel syndrome: Prospective evaluation using Rome III classification. *Alimentary pharmacology and therapeutics*, 35:350-359.

Farndale, R. & Roberts, L.M. 2011. Long-term impact of irritable bowel syndrome: A qualitative study. *Primary health care research and development*, 12:52-67.

FAO/WHO. 2001. Joint FAO/WHO expert consultation on evaluation of health and nutritional properties in food including powder milk with live lactic acid bacteria [homepage on the internet].

Fernandez, B.F., Rosinach, M., Esteve, M., Forne, M. Espinos, J.C. & Viver, J.M. 2006. Sugar malabsorption in functional abdominal bloating: A pilot study on the long term effect of dietary treatment. *Clinical nutrition*, 25: 824-831.

Floch, M.H. 2005. Use of diet and probiotic therapy in the irritable bowel syndrome: Analysis of the literature. *Journal of clinical gastroenterology*, 39: S243-S246.

Floch, M.H. 2005. Prebiotics, probiotics and dietary fibre. *Clinical nutrition: A guide to gastroenterologists*, 13:234-239.

Ford, C.A., Talley, N.S., Spiegel, B.M.R., Schiller, L., Foxx-Oreistein, A.E., Quigley, E.M.M. & Moayyedi, P. 2009. Effect of fibre, antispasmodics and peppermint oil in treatment of IBS: Systematic review and meta-analysis. *British medical journal*, 338:b1881.

Francavilla, R., Miniello, V., Magista, A.M., De Canio, A., Bucci, N. & Garliadi, F. 2010. A randomized controlled trial of *Lactobacillus* GG in children with functional abdominal pain. *Paediatrics*, 126: e1445-452.

Francis, C.Y. & Whorwell, P.J. 1994. Bran and irritable bowel syndrome: Time for reappraisal. *Lancet*, 344:39-40.

Francis, C.Y., Morris, J. & Whorwell, P.J. 1997. The irritable bowel severity scoring system: A simple method of monitoring irritable bowel syndrome and its progress. *Alimentary pharmacology & therapeutics*, 11: 395-402.

Fuller, R. 1989. Probiotics in man and animals. *Journal of application & bacteriology*, 66: 365-378.

Gardner, G., Gardner, A., Maclellan, L. & Osborne, S. 2003. Reconceptualising the objectives of a pilot study for clinical research. *International journal of nursing studies*, 40(7):719-724.

Gawronska, A., Dziechciarz, P., Horvath, A. & Szajweska, H. 2007. A randomized double-blind placebo-controlled trial of *Lactobacillus* GG for abdominal pain disorders in children. *Alimentary pharmacological therapy*, 25: 177-184.

Gome, A.M.P. & Malcata, F.X. 1999. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends of food science technology*, 10: 139-157.

Gorard, D.A., Libby, G.W. & Farthing, M.J. 1994. Influence of antidepressants on whole gut and oro-caecal transit times in health and irritable bowel syndrome. *Alimentary pharmacology therapeutics*, 8:159-166.

Gralnek, I.M., Hays, R.D., Klibourne, A., Naliboff, B. & Mayer, E.A. 2000. The impact of irritable bowel syndrome on health-related quality of life. *Gastroenterology*, 119: 654-660.

Groll, D., Vanner, S.J., Depew, W.T., Dacosta, L.R., Simon, J.B., Groll, A. 2002. The IBS-36: A new quality of life measure for irritable bowel syndrome. *The American journal of gastroenterology*, 97(4): 962-971.

Grundmann, O. & Yoon, S.L. 2009. Irritable bowel syndrome: Epidemiology, diagnosis and treatment: An update for health-care practitioners. *Journal of gastroenterology & hepatology*, 25:691-699.

Guaner, F. & Malagelada, J.R. 2003. Gut flora in health and disease. *Lancet*, 361:512-519.

Guglielmetti, S., Mora, D., Gschwender, M. & Popp, K. 2011. Randomized clinical trial: *Bifidobacterium bifidum* MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life – a double-blind placebo-controlled study. *Alimentary pharmacology therapeutics*, 33: 1123-1132.

Gupta, V. & Garg, R. 2009. Probiotics. *Indian journal of medicine and microbiology*, 27(3):202-209.

Guyonnet, D., Chassany, M., Ducrotte, P., Picard, C., Mouret, M., Mercier, C.H. & Mmatuchansky, C. 2007. Effect of fermented milk containing *Bifidobacterium animalis* DN- 173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults' primary care: a multicentre, randomized, double-blind, controlled trial. *Alimentary pharmacology and therapeutics*, 26: 475-486.

Grundmann, O. & Yoon, S.L. 2010. Irritable bowel syndrome: Epidemiology, diagnosis and treatment: An update for health-care practitioners. *Journal of gastroenterology and hepatology*, 25:691-699

Habicht, J.P., Pelto, G.H. & Lappa, J. 2008. *Methodologies to evaluate the impact of large scale nutrition projects*. Washington D.C.: World Bank.

Hammes, W.P. & Vogel. R.F. 1995. The genus *Lactobacillus*. In: *The Lactic Acid Bacteria*, Vol. 2, B.J.B. Wood, W.H. Hlzapfel (Eds.), Chapman and Hall, London, UK (1995) pp. 19-54.

Halpert, A., Dalton, C.B., Palsson, O., Morris, C., Hu, Y., Bangdiwala, S., Hankins, J., Norton, N. & Drossman D. 2007. What patients know about irritable bowel syndrome (IBS) and what they would like to know. National survey on patient educational needs questionnaire (PEQ). *American journal of gastroenterology*, 102:1972-1982.

Hahn, B., Watson, M., Yan, S., Gunput, D. & Heuwerkerjans, J. 2008. Irritable bowel syndrome symptom patterns: Frequency, duration and severity. *Digestive disease science*, 43:2715-2718.

Harris, L.R. & Roberts, L. 2008. Treatments for irritable bowel syndrome patients' attitudes and acceptability. *Complementary alternative medicine*, 8:65.

Hasler, L.W. 2007. Irritable bowel syndrome and bloating. *Best practice and research*, 21(4) 689-707.

Heaton, K.W., Ghosh, S., Braddon, F.E. 1991. How bad are the symptoms and bowel dysfunction of patients with IBS? A prospective, controlled study with

emphasis on stool form. *International journal of gastroenterology & hepatology*, 32: 73-79.

Heizer, W.D., Southern, S. & MCGovern, M.A. 2009. The role of diet in symptoms of irritable bowel syndrome in adults: A narrative review. *Journal of American dietetic association*, 109:1204-1214.

Heitkemper, M.M, Cain, K.C. & Jarret, M.E. 2003. Relationship of bloating to other GI and menstrual symptoms in women with irritable bowel syndrome. *Digestive disease science*, 49: 88-95.

Hertig, V.L., Cain, K.C., Jarret, M.E. & Burr, R.I. 2007. Daily stress and gastrointestinal symptoms in women with irritable bowel syndrome. *Nursing health*, 56:399-406.

Hoveyda, N., Heneghan, C., Mahtani, K.R., Perera, R., Roberts, N. & Glasziou, P. 2009. A systematic review and meta-analysis: Probiotic in the treatment of irritable bowel syndrome. *Gastroenterology*, 9:15.

Huang, W., Zhou, F., Bushnell, D.M., Diakite, C. & Yang., X. 2007. Cultural adaptation and application of the IBS-QOL in China: A disease-specific quality-of-life questionnaire. *Springer science & business media*, 16:1991-1996.

Hungin, A.P.S., Mulligan, C, Pot, B., Whorwell, P., Agreus, L., Fracasso, P., Lionis, C., Mendive, J., Rubin, G., Winchester, C. & De Wit, N. 2013. Systematic review: Probiotic in the management of lower gastrointestinal symptoms in clinical practice – an evidence-based international guide. *Alimentary pharmacology and therapeutics*, 38: 864-886.

Inadomi, J.M., Fennerty, M.B. & Bjorkam, D. 2003. Systematic review: The economic impact of irritable bowel syndrome. *Alimentary pharmacology and therapeutics*, 18: 671-682.

Irvine, E.J., Wittehead, W.J. & Chey, W.D. 2006. Design of treatment trials for functional gastrointestinal disorders. *Gastroenterology*, 130: 1538-1551.

Ishibashi, N. Yaeshima, T & Hayasawa, H. 1997. *Bifidobacteria*: Their significance in human intestinal health. *Malaysian journal of nutrition*, 3:149-159.

Isolauri, E., Sutus, Y., Kankaanpaa, H., Arvilommi, S. & Salminen, S. 2001. Probiotics: Effects on immunity. *American journal of clinical nutrition*, 73: 444-450.

Janssen, H.A., Muris, J.W. & Knotterus, J.A. 1998. The clinical course and prognostic determinants of the irritable bowel syndrome: A literature review. *Scandinavian journal of gastroenterology*, 33: 561-567.

Johanson, J.F., Drossman, D.A., Panas, R., Wable, A. & Ueno, R. 2008. Clinical trial: Phase 2 study for irritable bowel syndrome with constipation. *Alimentary pharmacology and therapeutics*, 27:685-696.

Jones, J., Boorman, J. & Cann, P. 2000. British Society of Gastroenterology guidelines for the management of irritable bowel syndrome. *Gastroenterology*, 47:ii1-ii19.

Kajander, K., Myllyluoma, E. Rajlic-Stojanovic, M., Kryonpalo, S.S., Rasmussen, M., Jarvenpaa, S.S., Zoetendal, E.G., Vos, W.M., De Vapaatalo, H. & Korpela, R. 2008. Clinical trial: Multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. *Alimentary pharmacology and therapeutics*. 27(1): 48-47.

Kassinen, A., Krogius-Kurikka, L. & Makivuokko, H. 2007. The faecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology*. 133: 24-33.

Katsuno, S., Okado, M., Kawai, K., Hono, H., Kogure, S., Nakazawa, Y. & Terada, A. 2003. Effects of YP97 yoghurt on the stool frequency, faecal characteristics and flora of human volunteers (II). *Chonai saikingaku zasshi*, 17: 27-34.

Kaur, I.P., Chopra, K. & Saini, A. 2002. Probiotics: Potential pharmaceutical applications. *European journal of pharmaceutical science*, 15:1-9.

Kay, L., Jorgensen, T., Schultz-Larsen, K. & Davidsen. 1996. Irritable bowel syndrome and upper dyspepsia among the elderly: A study of symptom clusters in random 70 year old population. *European journal of epidemiology*, 12: 199-204.

Kellow, J., Lee, O.Y., Chang, F.Y., Thongsawat, S., Mazlam, M.Z. & Yuen, H. 2003. An Asia Pacific, double blind, placebo controlled, randomized study to evaluate the efficacy, safety, and tolerability of tegaserod in patients with IBS. *International journal of gastroenterology & hepatology*, 52:671-676.

Khalif, I.L., Quigley, E.M., Konovitch, E.A., Maximova, I.D. 2005. Alterations in the colonic flora and intestinal permeability and evidence of immune activation in chronic constipation. *Digestive liver disease*, 37:838-849.

Khoshoo, V., Armstead, C. & Landry, L. 2006. Effect of a laxative with and without tegaserod in adolescents with constipation predominant irritable bowel syndrome. *Alimentary pharmacological therapy*, 23:191-196.

Kim, H.J., Vazquez Rooque, M.I. & Camilleri, M. 2005. A randomized controlled trial of a probiotic combination VSL# 3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterology and motility*, 17: 687-696.

- King, T.S., Elia, M. & Hunter, J.O. 1998. Abnormal colonic fermentation in irritable bowel syndrome. *Lancet*, 352:1187-1189.
- Klaver, F.A.M., Kingma, F. & Weerkamp, A.H. 1993. Growth and survival of *bifidobacteria* in milk. *Netherland milk dairy journal*, 47:157.
- Kneifel, W., Jaros, D. & Edhard, F. 1993. Microflora and acidification properties of yoghurt and yoghurt related products fermented with commercially available starter cultures. *International journal of food and microbiology*, 18:179.
- Kolars, J.C., Levitt, M.D. & Aouji, M. 1984. Yoghurt - an autodigesting source of lactose. *England journal of medicine*, 310: 1-3.
- Korpela, R. & Niittynen, L. 2012. Probiotics and irritable bowel syndrome. *Microbial ecology in health and disease*, 23: 19-25.
- Kruis, W.; Fric, P. & Pokrotnieks., J. 2004. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. *Gastroenterology*, 53:1617-1623.
- Kurdi, P., Tanaka, H. & Van Veen, H.W. 2003. Cholic acid accumulation and its diminution by short chain-chain fatty acids in *bifidobacteria*. *Microbiology*, 149:2031-2037.
- Lancaster, G.A., Dodd, S. & Williamson, P.R. 2004. Design and analysis of pilot studies: Recommendations for good practice. *Journal of evaluation in clinical practice*, 10(2):307-312.
- Lankaputhra, W.E.W. & Shah, N.P. 1994. Investigation of factors affecting viability of *Lactobacillus acidophilus* and *Bifidobacteria* in yoghurt. 24<sup>th</sup> International dairy congress, Melbourne, Australia, September. 18-22.
- Lankaputhra, W.E.W. & Shah, N.P. & Britz, M.L. 1996b. Survival of *bifidobacteria* during refrigerated storage in the presence of acid and hydrogen peroxide. *Milchwissenschaft*, 51:65.
- Lanniti, T. & Palmieri, B. 2010. Therapeutical use of probiotic formulations in clinical practice. *Clinical nutrition*, 29:701-725.
- Lea, R. & Whorwell, P.J. 2005. The role of food intolerance in irritable bowel syndrome. *Gastroenterology of North America*, 34:247-255.
- Lee, V., Guthrie, E., & Robinson, A. 2001. Functional bowel disorders in primary care: Factors associated with health-related quality of life and doctor consultation. *Journal of psychosomatic res*, 64:129-138.

- Lee, O.Y., Mayer, A.E., Schmulson, M., Chang, L. & Naliboff, B. 2001 Gender related differences in IBS symptoms. *American journal of gastroenterology*, 96:2184-2193.
- Lee, B.J. & Bak, Y.T. 2011. Irritable bowel syndrome, gut microbiota and probiotics. *Journal of neurogastroenterology & motility*, 17:252-266.
- Lembo, T., Naliboff, B. & Munakata, J. 1999. Symptom and visceral perception in patients with pain-predominant irritable bowel syndrome. *American journal of gastroenterology*, 94: 1320-1326.
- Leon, A.C, Davis, L.L & Kraemer, H.C. 2011. The role and interpretation of pilot studies in clinical research. *Journal of Psychiatry research*, 45:626-629.
- Lewis, S.J. & Heaton, K.W. 1997. Stool form scale as a useful guide to intestinal transit time. *Scandinavian journal of gastroenterology*, 32(9):920-924.
- Lin, H.C. 2004. Small intestinal bacterial overgrowth: A framework for understanding irritable bowel syndrome. *Journal of American medical association*, 292: 852-858.
- Linnan, L., Bowling, M., Childress, J., Lindsay, G., Blakey, C. & Pronk, S. 2008. Results of the 2004 National worksite health promotion survey. *American journal of public health*, 98:1503-1509.
- Liong, M.T. 2008. Safety of probiotics: Translocation and infection. *Nutrition review*, 66(4):192-202.
- Loftus, E.V. Jr. & Sandborn, W.J. 2002. Epidemiology of inflammatory bowel disease. *American journal of gastroenterology*, 31: 1-20.
- Lolis, N., Veldekis, D., Moraitou, H., Kanavaki, S., & Velegraki, A. 2008. *Saccharomyces boulardii* fungaemia in an intensive care unit patient treated with caspofungin. *Critical care*, 12: 414.
- Lomer, M.C.E, Parkes, G.C. & Sanderson, J.D. 2008. Review article: Lactose intolerance in clinical practice – myths and realities. *Alimentary pharmacology and therapeutics*, 27: 93-103.
- Longstreth, G.F., Thompson, W.C., Chey, W.D., Houghton, L.A., Mearin, F. & Spiller, R.C. 2006. Function bowel disorders. *Gastroenterology*, 130:1480-1491.
- Longstreth, G.F., Thompson, W.C., Drossman, D.A., Heaton, K.W., Irvine, E.J. & Muller-Lissner, S.A. 1999. Functional bowel disorders and functional abdominal pain. *Gastroenterology*, 45(2): 1143-1147.

- Lovestam, E., Orrevall, Y., Koochek, A., Karlstrom, B. & Andersson, A. 2013. Evaluation of a nutrition care process-based audit instrument, the Diet-NCP-Audit, for documentation of dietetic care in medical records. *Scandinavian journal of caring sciences*, 10:2-10.
- Mcfarland, L.V. & Dublin, S. 2008. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World journal of gastroenterology*. 14(17): 2650-2661.
- Mcfarlane, G.T. & Cumming, J.H. 1999. Probiotics and prebiotics: Can regulating the activities of intestinal bacteria benefit health? *British medical journal*, 318:999-1003.
- Madden, J., Plummer, S. & Tang, J. 2005. Effect of probiotics on preventing disruption of the intestinal microflora following antibiotic therapy: A double-blind, placebo-controlled pilot study. *International immunotherapy and pharmacology*, 5:1091-1097.
- Malinen, E., Rinttila, T. & Kajander, K. 2005. Analysis of the faecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *American journal of Gastroenterology*, 100:373-382.
- Manning, A.P., Wyman, J.B. & Heaton, K.W. 1976. How trustworthy are bowel histories? Comparison of recalled and recorded information. *British medical journal*, 2:213-214.
- Martens, M., Van Assema, P., Paulussen, T., Schaalma, H. & Brug, J.K. 2006. Process evaluation of a Dutch programme for lower vocational schools to promote healthful diet. *Health education review*, 21:695-704.
- Marteu, P., Vesa, T.H. & Zidi, S. 1996. Digestion and tolerance of lactose from yoghurt and different semi-solid fermented dairy products containing *Lactobacillus acidophilus* and *bifidobacteria* in lactose maldigesters-is bacterial lactase important? *European journal of clinical nutrition*, 50: 730-733.
- Marteau, P.M., Celliers, C. & Schrezenmeir, J. 2001. Protection from gastrointestinal diseases using probiotics. *American journal of clinical nutrition*, 73(2): 430S-6S.
- Marteau, P., Cuillerier, E., Meance, S., Gerhardt, M.F., Myara, A., Bouvier, M., Bouley, C., Tondu, F., Bommelaer, G. & Grimaud, J.C. 2002. *Bifidobacterium animalis* strain DN-173 010 shortens the colonic transit time in healthy women: A double-blind, randomized, controlled study. *Alimentary pharmacology & therapeutics*, 16: 587-593.

Martin, J.H. & Chou, K.M. 1992. Selection of *Bifidobacterium* spp. for use as dietary adjuncts in cultured dairy foods. I. Tolerance to PH of yoghurt. *Culture and dairy products journal*, 27(4):21.

Masand, P.S, Gupta, S. & Schwarts, T.L. 2005. Open label treatment with citalopram in patients with irritable bowel syndrome: A pilot study. *Primary care companion of journal of clinical psychiatry*, 4:12-16.

Masco, L., Huys, G., Brandt, E., Temmerman, R. & Swings, J. 2005. Culture-dependent and culture-independent qualitative analysis of probiotic products claimed to contain *bifidobacteria*. *International journal of food microbiology*, 102:221-230.

Mathews, L.B., Moodie, M.M., Simmons, A.M. & Swinburn, B.A. 2010. The process evaluation of Its your move!, an Australian adolescent community-based obesity prevention project. *British medical journal of public health*,10:448-465.

Mathias, J.R. & Clench, M.H. 1998(a). Relationship of reproductive hormones and neuromuscular disease of the gastrointestinal tract. *Digestive disease science*, 16:3-13.

Mathias, J.R., Clench, M.H. & Abell, T.L. 1998(b). Effect of leuprolide acetate in treatment of abdominal pain and nausea in premenopausal women with functional bowel disease: a double-blind, placebo-controlled, randomized study. *Digestive disease science*, 43:1347-1355.

Mayer, E.A., Craske, M. & Naliboff, B.D. 2001. Depression, anxiety, and gastrointestinal system. *Journal of clinical psychiatry*, 62:28-36.

Mcfarland, L.V. & Dublin, S. 2008. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World journal of gastroenterology*. 14(17): 2650-2661.

Mckenna, S.P. 1994. A new theoretical approach to the measurement of quality of life. *Drug information journal*, 28:13-18.

Meance, S., Cayuela, C., Turchet, P., Pietro, T., Raimondi, A. & Lucas, C. 2001. A fermented milk with *Bifidobacterium animalis* strain DN-173 010 shortened oro-faecal gut transit time in elderly. *Microbial ecology in health and disease*. 13: 217-222.

Meance, S., Cayuela, C., Raimondi, A.T. 2003. Recent advances in the use of functional food: effects of the commercial fermented milk with *Bifidobacterium animalis* strain DN-173 010 and yoghurt strains on gut transit time in the elderly. *Microbial ecology in health and disease*. 15: 15-22.

Metchkinoff, E. 1907. The prolongation of life: optimistic studies. London: Butterworth-Heinemann.

- Miller, V., Lea, R., Agrawal, A. & Whorwell, P.J. 1994. Bran and irritable bowel syndrome: The primary-care perspective. *Digestive and liver disease*, 38:737-740.
- Moayyedi, P., Ford, A.C., Talley, N.J., Cremonini, F., Foxx-Orenstein, A.E., Brandt, L.J. & Quigley, E.M.M. 2010. The efficacy of probiotic in the treatment of irritable bowel syndrome: A systematic review. *Gastroenterology*, 59: 325-332.
- Moher, D., Hopewell, S., Schulz, K., Montori, V., Devereaux, P.J., Elbourne, D., Egger, M. & Altman, D.G. 2010. Consort 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *British medical journal*, 340:869-883.
- Moser, S.A. & Savage, D.C. 2001. Bile salt hydrolase activity and resistance to toxicity of conjugated bile salts are unrelated properties in *lactobacilli*. *Applied environmental microbiology*, 67:3476-3480.
- Muller, S., Holtmann, G. & Rueegg, P. 2005. Tegaserod is effective in the initial and re-treatment of irritable bowel syndrome with constipation. *Alimentary pharmacology and therapeutics*, 21: 11-20.
- Musil, C. 2006. Pilot study. *Nursing research*, 17: 21-29.
- Naliboff, B.D., Berman, S. & Chang, L. 2003. Sex-related differences in IBS patients: Central processing of visceral stimuli. *Journal of clinical gastroenterology*, 124:1738-1747.
- National Institute for Health and Clinical Excellence (NICE). 2008. Irritable bowel syndrome in adults: Diagnosis and management of irritable bowel syndrome in primary care. National collaborating centre for nursing and supportive care.
- Neish, A.S. 2009. Microbes in gastrointestinal health and disease. *Gastroenterology*, 136:65-80.
- Nikfar, S., Rahimi, R., Rahimi, F., Derakhshani, S. & Abdollahi, M. 2008. Efficacy of probiotics in irritable bowel syndrome: A meta-analyses of randomized, controlled trials. *Disease of colon and rectum*, 51:1775-1780.
- Niedzielin, K., Kordecki, H. & Birkenfeld, B. 2001. A controlled, double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. *European journal of gastroenterology and hepatology*, 13: 1143-1147.
- Niv, E., Naftali, T., Hallak, R. & Vaisman, N. 2005. The efficacy of *lactobacillus reuteri* ATCC 55730 in the treatment of patients with irritable bowel syndrome-a double blind, placebo-controlled, randomized study. *Clinical nutrition*, 24: 925-931.

- Nobaek, S., Johansson, M.L., Molin, G., Ahrne, S. & Jeppsson, B. 2000. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *American journal of gastroenterology*, 95: 1231-1238.
- Nomoto, K. 2005. Prevention of infections by probiotics. *Journal of bioscience and bioengineering*, 100: 583-592.
- Oelschlaeger, T.A. 2010. Mechanisms of probiotic actions – A review. *International journal of medicine and microbiology*, 300:57-62.
- O'mahony, L., Mccarthy, J. & Kelly, P. 2005. *Lactobacillus and bifidobacterium* in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, 128: 541-551.
- O'sullivan, M.A. & O'morain, C.A. 2000. Bacterial supplementation in the irritable bowel syndrome. A randomized double-blind placebo-controlled crossover study. *Digestive liver disease*, 32:294-301.
- Owens, D.M., Nelson, D.K. & Talley, N.J. 2000. The irritable bowel syndrome: Long-term prognosis and the physician-patient interaction. *International medicine*, 122:107-12.
- Park, H.J., Jarret, M., Cain, K. & Heitkemper, M. 2008. Psychological distress and GI symptoms are related to severity of bloating in women with irritable bowel syndrome. *Nursing health*, 31:98-107.
- Parker, T.J., Naylor, S.J., Riordan, A.M. & Hunter, J.O. 1995. Management of patients with food intolerance in irritable bowel syndrome: The development and use of an exclusion diet. *Journal of human nutrition diet*, 8:159-166.
- Parry-Langdon, N., Bloor, M., Audrey, S. & Halliday, J. 2003. Process evaluation of health promotion interventions. *Policy and politics*, 31:207-216.
- Pata, C., Erdal, M.E., & Derci, E. 2002. Serotonin transporter gene polymorphisms in irritable bowel syndrome. *American journal of gastroenterology*, 97:1780-1784.
- Patidar, S.K., Prajapati, J.B. & Dave, R.I. 1994. Standardization and evaluation of *acidophilus lassi*. 24<sup>th</sup> *International dairy congress*, Melbourne, Australia, Sep 18-22.
- Patrick, D.L. & Erickson, P. 1993. Health status and health policy: Allocating resources to health care. New York, Oxford University press.
- Patrick, D.L., Wild, D.J., Johnson, E.S., Wagner, T.H. & Martin, M.A. 1994. Cross-cultural validation of quality of life measures. *In quality of life and pharmacoeconomics in clinical trial*, 3: 355-362.

Patrick, D.L., Drossman, D.A., Frederick, I.O. 1998. Quality of life in persons with irritable bowel syndrome: Development of a new measure. *Digestive Disease Science*, 43:400-411.

Perez, F., Accarino, A.M. & Azpiroz, F. 2005. Measurement of intestinal gas volume using a new CT-based analysis program (abstract). *Gastroenterology*, 131: 1003-1010.

Picard, C., Fioramont, J., Francois, A., Robinson, T., Neant, F. & Matuchansky, C. 2005. Review article: bifidobacteria as probiotic agents – Physiological effects and clinical benefits. *Alimentary pharmacology and therapeutics*, 22(6): 495-512.

Pimentel, M., Mayer, A.G., Park, S., Chow, E.J., Hasan, A. & Kon, Y. 2002. Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Digestive disease science*, 48: 86-92.

Pimentel, M., Chow, E.J. & Lin, H.C. 2003. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. A double blind, randomized, placebo controlled study. *American journal of gastroenterology*, 98:412-419.

Pitz, M., Cheang, M. & Bernstein, C.N. 2005. Defining the predictors of the placebo response in irritable bowel syndrome. *Clinical gastroenterology and hepatology*, 3:237-247.

Pochart, P., Marteau, P., Bouhnik, Y., Goderel, I., Bourlioux, P. & Rambaud, J.C. 1992. Survival of *Bifidobacterium* ingested via fermented milk during their passage through the human small intestine: An in vivo study using intestinal perfusion. *American journal of clinical nutrition*, 55: 78-80.

Podovei, M. & Kuo, B. 2006. Irritable bowel syndrome: A practical review. *Southern medical journal*, 99:1235-1242.

Posserud, I., Agerforz, P. & Ekman, R. 2004. Altered visceral perceptual and neuroendocrine response in patients with irritable bowel syndrome during mental stress. *Gastroenterology*, 53:1102-1108.

Quartero, A.O., Meineche-Schmidt, V., Muris, J., Rubin, G. & De Wit, N. 2005. Bulking agents, antispasmodic and antidepressant medication for the treatment of irritable bowel syndrome. *Cochrane database systematic review*, CD003460.

Rainville, P., Duncan, G.H., Price, D.D., Carrier, B. & Bushnell, M.C. 2007. Pain effect encoded in human anterior and sensory cortex. *Science*, 200:98-105.

Rasi, J.L. & Kurmen, L. 1983. The role of dairy foods containing bifido and acidophilus bacteria in nutrition and health. *Northern European dairy journal*, 4:80.

Reid, G., Younes, J.A. & Van Der Mei, H.C. 2011. Microbiota restoration: Natural and supplemented recovery of human microbial communities. *National review of microbiology*, 9: 27-38.

Reid, G. 2008. Probiotics and prebiotics – progress and challenges. *International dairy journal*, 18:969-975.

Riberdy-Poitras, M., Verrier, P. & Plourde, V. 2000. Amitriptyline for the treatments of IBS (abstract). *Gastroenterology*, 118: A617.

Richie, M.L. & Romanuk, T.N. 2012. A meta-analysis of probiotics efficacy for gastrointestinal diseases. *Plos one*, 7:e34938.

Ringel, Y. & Ringel-Kulka, T. 2011. The rationale and clinical effectiveness of probiotics in irritable bowel syndrome. *Journal of clinical gastroenterology*, 45: (3) S145-S148.

Rivkin, A. & Chagan L. 2006. Lubiprostone: Chloride channel activator for chronic constipation. *Clinical therapy*, 28: 2008-2021.

Roberts, L.M., Mccahon, D., Holder, R., Wilson, S. & Richard Hobbs, H.D. 2013. A randomized controlled trial of a probiotic 'functional food' in the management of irritable bowel syndrome. *Gastroenterology*, 13:45.

Roller, M., Femia, A.P., Caderni, G., Rechkemmer, G. & Walts, B. 2004. Intestinal immunity of rats with colon cancer is modulated by oligofructose-enriched inulin combined with *Lactobacillus rhamnosus* and *Bifidobacterium lactis*. *British journal of nutrition*, 92: 931-938.

Ross, S., Grant, A., Counsell, C., Gillespie, W., Russel, I. & Prescott, R. 1999. Barriers to participation in randomized trials: A systematic review. *Journal of clinical epidemiology*, 52:1143-1156.

Rossi, P.H., Lipsey, M.W. & Freeman, H.E. 2004. Evaluation: A Systematic Approach. 7<sup>th</sup> Ed. Thousand Oaks, CA: Sage.

Ross-McGill, H., Hewison, J., Hirst, J., Dowswell, T., Holt, A., Brunskill, P & Thornton, J.G. 2000. Antenatal home blood pressure monitoring: A pilot randomized controlled trial. *British journal of obstetrics and gynaecology*, 107:217-221.

Rounsaville, B.J. & Carroll, K.M. 2001. A stage model of behavioural therapies research: Getting started and moving on from stage I. *Clinical psychological science practice*, 8:133-142.

- Ruigomez, A., Wallander, M.A. & Johansson, S. 2008. Irritable bowel syndrome and gastroesophageal reflux disease in primary care: Is there a link? *Digestive disease science*, 11:11-20.
- Sagami, Y., Shimada, Y. & Tayama, J. 2003. Effect of a corticotrophin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. *Gastroenterology*, 53:958-964.
- Saggiro, A. 2004. Probiotics in the treatment of irritable bowel syndrome. *Journal of clinical gastroenterology* 38:S104-S106.
- Saito, Y.A., Locke, G.R., & Talley, N.J. 2000. A comparison of the Rome and Manning criteria for case identification in epidemiological investigations of irritable bowel syndrome. *American journal of gastroenterology*, 95:2816-2824.
- Salminen, S., Isolauri, E. & Salminen, E. 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. *Antonie van Leeuwenhoek*, 70:347-358.
- Salonen, A., De Vos, W.M. & Palva, A. 2010. Gastrointestinal microbiota in irritable bowel syndrome: Present state and perspectives. *Microbiology*, 156:3205-33215.
- Samona, A. & Robinson, R.K. 1994. Effect of yoghurt cultures on the survival of bifidobacteria in fermented milks. *Journal society of dairy technologies*, 47:58.
- Sanders, M. 2007. How do we know when something called a “probiotic” is really a probiotic? A guideline for consumers and health care professionals. *Functional food review*, 1:3-12.
- Sarlani, E., Grace, E.G., Reynolds, M.A. & Greenspan, J.D. 2004. Sex differences in temporal summation of pain and after sensations following repetitive noxious mechanical stimulation. *Pain*, 109:115-123.
- Sartor, R.B. 2004. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: Antibiotics, probiotics and prebiotics. *Gastroenterology*, 126: 1620-1633.
- Satoshi, N., Yoichi, I. & Hisakazu, I. 2007. Effects of Bifidobacterium lactis DN 173-010 on the intestinal transit time, the condition of defecation and intestinal microflora: A randomized, double-blind, placebo-controlled, cross-over study among healthy Japanese women. *Alimentary pharmacology and therapeutics*, 18: 2671-2683.
- Saunders, R.P., Evans, M.H. & Joshi, P. 2005. Developing a process-evaluation plan for assessing health promotion program implementation: A how to guide. *Health promotion practice*, 6(2):134-147.

- Schneider, M., Hall, W.J., Hernandez, A.E., Hindes, K., Montez, G., Pham, T., Rosen, L., Sleigh, A., Thompson, D., Volpe, S.L., Zeveloff, A. & Steckler, A. 2009. Rationale, design and methods for process evaluation in the HEALTHY study. *International journal of obesity*, 33:S60-S67.
- Scrimshaw, N.S. & Murray, E.B. 2001. The acceptability of milk and milk products in population with a high prevalence of lactose intolerance. *American journal of clinical nutrition*, 48: 1079-1159.
- Sen, S., Mullan, M.M., Parker, T.J., Woolner, J.T., Tarry, S.A. & Hunter, J.O. 2002. Effect of *Lactobacillus plantarum* 299v on colonic fermentation and symptoms of irritable bowel syndrome. *Digestive disease*, 53:2714-2718.
- Sgorbati, B., Biavati, D. & Palenzona, D. 1995. The genus *Bifidobacterium*. In: *The lactic acid bacteria, Volume 2*, B.J.B Wood, W.H. Holzapfel (Eds), Chapman and Hall, London, UK, 279-306.
- Shah, N., Lankaputhra, W.E.V., Britz, M. & Kyle, W.S.A. 1995. Survival of *Lacidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. *International dairy journal*, 5:515. 101:326-333.
- Sharara, A.I., Aoun, E., Abdul-Baki, H., Mounzer, R, Sidani, S. & Elhajj, I. 2006. A randomized double-blind placebo-controlled trial of rifaxamin in patients with abdominal bloating and flatulence. *American journal gastroenterology*, 101:326-333.
- Shaw, G., Srivastava, E.D. & Sadlier, M. 1991. Stress management for irritable bowel syndrome: a controlled trial. *Digestion*, 50:36-42.
- Simren. M., Abrahamsson, H. & Bjornsson. E.S. 2006. Lipid-induced colonic hypersensitivity in the irritable bowel syndrome: The role of bowel habit, sex, and psychologic factors. *Clinical journal of gastroenterology and hepatology*, 5:201-208.
- Snydman, D.R. 2008. The safety of probiotics. *Clinical infectious disease*, 46(2):S104-S111.
- Socol, C.R, Vandenberghe, L.P, Spier, M.R., Medeiros, A.B.P. & Yamaguishi, C.T. 2010. The potential of probiotics: A review. *Food technology and biotechnology*, 48: 413-434.
- Sondergaard, B.O., Johan, O., Ohlson, K., Svensson, U., Bytzer, P. & Ekesbo, R. 2011. Effects of probiotic fermented milk on symptoms and intestinal flora in patients with irritable bowel syndrome: A randomized, placebo-controlled trial. *Scandinavian journal of gastroenterology*, 46: 663-672.

- Spiller, R.C, Jenkins, D. & Thornley, J.P. 2000. Increased rectal mucosal enterendocrine cells, T lymphocytes, and increased gut permeability following acute *Campylobacter* enteritis and in post-dysenteric IBS. *Gastroenterology*, 47:804-811.
- Spiller, R.C. 2004. Infection, immune function, and functional gut disorders. *Clinical gastroenterology and hepatology*, 2:445-455.
- Spiller, R. & Campbell, E. 2007. Post-infectious irritable bowel syndrome. *Current opinion of gastroenterology*, 22:13-17.
- Stanghellin, V., Barbara, G., De Giorgio, R., Cremon, C., Cottrell, G.S., Grady, E.F. & Collins, S.M. 2004. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology*, 126:693-702.
- Staudacher, H.M, Lomer, M.C. & Anderson, J.L. 2012. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *Journal of nutrition*, 142: 1510-1518.
- Stevenson, C. & Enerst, E. 2000. A pilot study of hypericum perforatum for the treatment of premenstrual syndrome. *British journal of obstetrics and gynaecology*, 107:870-876.
- Stevenson, C. & Blaauw, R. 2011. Probiotics, with special emphasis on their role in the management of irritable bowel syndrome. *South African journal of clinical nutrition*, 24(2):63-73.
- Steckler, A. & Linnan, L. 2002. Process evaluation for public health interventions and research. Jossey-bass: San Francisco.
- Story, M., Mays, R.W. & Bishop, D.B. 2000. 5-a-day power plus: process evaluation of a multicomponent elementary school program to increase fruit and vegetable consumption. *Health educational behaviour*, 27:187-2000.
- Streiner, D.L. & Norman, G.R. 1995. Devising the items. Health measurement scales. *A practical guide to their development*, 15-27.
- Szabo, S. 1995. The World Health Organization quality of life (WHOQOL) assessment instrument. *In quality of life and pharmacoeconomics clinical trials*, 355-362.
- Tache, Y., Martinez, V., Million, M. & Wang, L. 2001. Stress and the gastrointestinal tract III: Stress-related alterations of gut motor function-role of brain corticotrophin-releasing factor receptors. *American journal of physiology of gastrointestinal tract and liver*, 280:G173-G177.

- Taguchi, R. 1998. Effects of fermented-milk administration on defecation and faecal microflora of healthy volunteers. *Chonai saikingaku zasshi*, 11: 117-122.
- TALLEY, N.T. 2008. Functional gastrointestinal disorders as a public health problem. *Neurogastroenterology and motility*, 1: 121-129.
- Talley, N.J., Weaver, A.R., Zinsmeister lii, A.R. & Melton, L.J. 1992. Onset and disappearance of gastrointestinal symptoms and functional gastrointestinal disorders. *American journal of gastroenterology*, 33:161-177.
- Talley, N.J. & Spiller, R. 2002. Irritable bowel syndrome: A little understood bowel disease? *The lancet*, 360:555-564.
- Tamine, A.Y., Marshall, V.M.E. & Robinson, R.K. 1995. Microbiological and technological aspects of milks fermented by *bifidobacteria*. *Journal of dairy products*, 62:151-187.
- Tanaka, H., Doesburg, K. & Iwasaki, T. 1999. Screening of lactic acid bacteria for bile salt hydrolase activity. *Journal of dairy science*, 82:2530-2535.
- Tanaka, H., Hashiba, H. & Kok, J. 2000. Bile salt hydrolase of *Bifidobacterium longum*-biochemical and genetic characterization. *Enviromental microbiology*, 66:2502-2512.
- Tannock, G., Dashkevicz, M.P. & Feighner, S.D. 1989. Lactobacilli and bile salt hydrolase in the murine intestinal tract. *Enviromental microbiology*, 55:1848-1851.
- Tillisch, K., Labus, J.S., Naliboff, B.D., Bolus, R., Shetzline, M. & Mayer, E.A. 2005. Characterization of the alternating bowel habit subtype in patients with irritable bowel syndrome. *American journal of gastroenterology*, 100(4): 896-904.
- Theodorakopoulou, M., Perros, E., Giamarellos-Bourboulis, E.J. & Dimopoulos, G. 2013. Controversies in the management of the critically ill: The role of probiotics. *International journal of antimicrobial agents*, 42S: S41-S44.
- Thompson, W.G., Longstreth, G.F., Drossman, D.A., Heaton, K.W., Irvine, E.J. & Muller-Lissner, S.A. 1999. Functional bowel disorders and functional abdominal pain. *Gastroenterology*, 45(2):1143-1147.
- Turroni, F., Van Sinderen, D. & Ventura, M. 2011. Genomics and ecological overview of genus *bifidobacterium*, *International journal of microbiology*, 149:37-44.
- Two Feathers, J., Kieffer, E.C., Palmisano, G., Anderson, M., Janz, N., Spenser, M.S., Guzman, R. & James, S.A. 2007. The development, implementation, and process evaluation of the REACH Detroit partnership's diabetes lifestyle intervention. *American association of diabetes educators*, 33:509-520.

- Vahedi, H., Merat, S., Rashidioon, A., Ghoddoosi, A. & Ramalezadeh, A. 2005. Effect of fluoxetine in patients with pain and constipation predominant IBS: A double-blind randomized study. *Alimentary pharmacology & therapeutics*, 2: 381-385.
- Van Der Waaij, D. 1991. The microflora of the gut: Recent findings and implications. *Digestive diseases*, 9:36-48.
- Van Eeden, T. & Gericke, G. 1996. Effect of acculturation on habitual food intake and dietary patterns of rural black home economics students. *South African journal of food science nutrition*, 8: 85-94.
- Van-Teijlingen, E.R. & Hundley, V. 2001. The importance of pilot studies. *Soc Res Update*, 35:341-347.
- Vollaard, E.J. & Clasener, H.A.L. 1994. Colonization resistance. *Anti-microbiology agents and chemotherapy*, 38:409-414.
- Wagner, T.H., Patrick, D.L., Bavendam, T.G., Martin, M.L. & Buesching, D.P. 1996. Quality of life of persons with urinary incontinence: Development of a measure. *Urology*, 47(1):67-72.
- Wandersman, A. 2009. Four keys to success (Theory, implementation, evaluation and resource/system support): High hopes and challenges in participation. *American journal of community psychology*, 43:3-21.
- Waring, W.S., Chui, M. & Japp, A. 2004. Autonomic cardiovascular responses are impaired in women with irritable bowel syndrome. *Journal of clinical gastroenterology*, 38:658-663.
- Wassenaar, T.M. & Klein, G. 2009. Safety aspects and implications of regulation of probiotic bacteria in food and food supplements. *Journal of food and protein*, 71(8):1734-1741.
- WHO. 2007. *International statistical classification of diseases and related health problems*, 10<sup>th</sup> edition, Geneva, Switzerland.
- Whorwell, P.J., Altringer, L. & Morel, J. 2006. Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. *American journal of gastroenterology*, 101: 1581-1590.
- Wight, D. & Obasi, A. 2002. Unpacking the "black box": the important of process data to explain outcomes. *Experimental evaluation*, 20:151-166.
- Wild, D., Patrick, D.L., Johnson, E., Berzon, R. & Wald, A. 1995. Measuring health related quality of life in persons with herpes. *Quality of life research*, 4(6):532-539.

Williams, E.A., Stimpson, J., Wang, D., Plummer, S, Gariova, I. & Barker, M.E. 2008. Clinical trial: A multistrain probiotic preparation significantly reduced symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Alimentary pharmacology and therapeutics*, 29: 97-103.

Yeo, A., Boyd, P. & Lumsden, S. 2004. Association between functional polymorphisms in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women, *Gastroenterology*, 53:1452-1458.

Zijdenbos, I.L, De Wit, N.J., Van Der Heijden, G.J., Rubin, G. & Quartero, A.O. 2009. Psychological treatment for the management of irritable bowel syndrome. *Cochrane database systematic review*, CD006442.

Zhu, D.J., Che, X.W., Wu, J.H., Ju, Y.L., Feng, J., Ren, B.J. & Li, Y. 2012. Effect of perioperative intestinal probiotics on intestinal flora and immune function in patients with colorectal cancer. *Journal of South Medicine University*, 32:1190-1193.

Zuckerman, M.J. 2006. The role of fibre in the treatment of irritable bowel syndrome: therapeutic recommendations. *American journal of gastroenterology*, 40:104-108.

## Appendix 1 Letter of ethical approval



Private Bag X6001, Potchefstroom  
South Africa 2520

Tel: +2718 299-1111/2222

Web: <http://www.nwu.ac.za>

Ethics Committee  
Tel: +2718 2999 4237

Email: [este.vorster@nwu.ac.za](mailto:este.vorster@nwu.ac.za)

20 August 2012

To whom it may concern

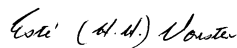
Dear Prof./Dr./Mr./Mrs./Miss

Ethics Application: NWU-00069-12-S1

### **"Effectiveness of probiotic *Bifidobacterium animalis* DN-173010 in the management of constipation-predominant IBS-C in Black South African women"**

This application meets with all the necessary ethical requirements. Ethical approval is granted.

Yours sincerely



Prof. H.H. Vorster

Chair

## Appendix 2 Informed written consent form

### TITLE OF PROJECT

Effectiveness of probiotic Bifidobacterium animalis DN-173 010 in the management of constipation-predominant irritable bowel syndrome in black South African women.

Ethics number: NWU-00069-12-S1

Participants ID NO:.....

Study no:.....

Name of researcher: Yvonne Rammbwa

### INTRODUCTION

You are invited to be participants in the research study to determine if a probiotic added to yoghurt has any effect on constipation predominant - Irritable Bowel Syndrome sufferers. The study is a parallel double blind and randomized which will run for 4 weeks and all participants are requested to visit the study unit weekly with completed questionnaires. You will be allocated to either the active product or placebo. In either group you will be required to consume two yoghurts daily, at breakfast and at dinner for 4 weeks. Yoghurt will be provided to all participants and their family members throughout the study.

Please tick

- I confirm that I understand the procedure of the above-mentioned study.
- I have had the opportunity to ask questions and have had these answered satisfactorily.
- I understand that my participation is voluntary and I am free to withdraw without my medical care or legal right being affected.
- I agree to my medical practitioner being informed of my participation in the study
- I agree to take part in the above research study

.....  
Name of Participant

.....  
Date

.....  
Signature

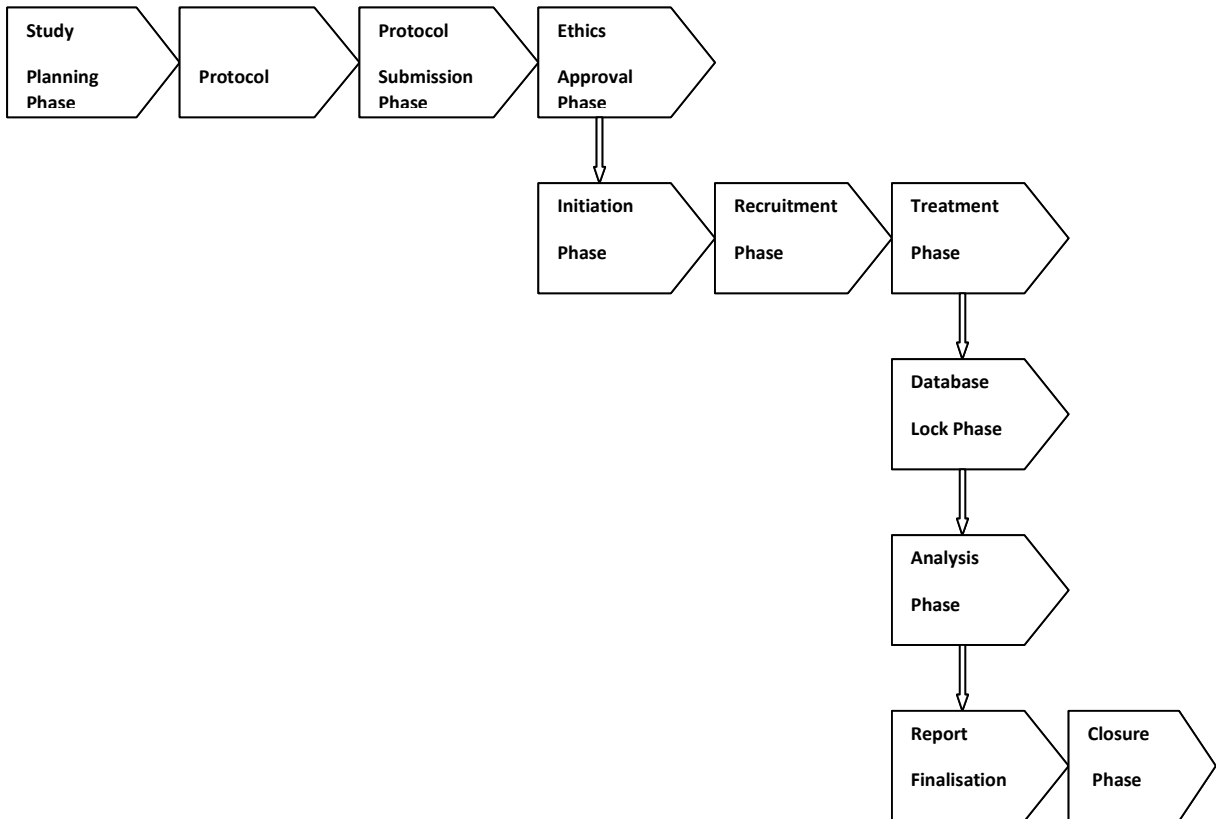
.....  
Name of Researcher

.....  
Date

.....  
Signature

## Appendix 3 Study process

### CLINICAL STUDY PROCESS



## Appendix 4 Procedure Flow Chart

### PROCEDURE FLOW CHART Schedule of study events

Study week	0	1	2	3	4	
Visit	1	2	3	4	5	
Study Period	Randomization	TREATMENT PERIOD				Data collected by
Written informed Consent	X					Researcher
Demographic Profile	X					Researcher & Assistant
Inclusion /Exclusion	X					Researcher
IBS SSS	X	X	X	X	X	Participants
IBS QOL	X				X	Participants & Researcher
Daily Record Form		X	X	X	X	Participants
Satisfactory Relief Question		X	X	X	X	Participants
Reminder SMSes for Appointment and Yoghurt consumption		2 X	2 X	2 X	2 X	Researcher & Assistant

## Appendix 5 Demographic questionnaire

### Demographic Questionnaire

Surname: ..... Initials: ..... Study no: .....

DOB: ..... Age: ..... Height (m): ..... Weight (kg): ..... BMI:

Tel no: ..... Cell no: .....

Physical address: .....

Postal code: .....

Employment status: .....

Household size (family members that sleeps at least for 4 nights in the house per week):

Referring Dr: .....

IBS Diagnosis (subtype specified): .....

Other diagnosis: .....

Source of recruitment: Out-patient  Hospital patient

Medical history: .....

Bowel cleansing habit:

Yes	No
-----	----

If yes how many times do you perform bowel cleansing in a week/month?

Mark only one:

1 every day

1/week

2/week

1/month

2/month

Method of bowel cleansing: .....

Other notes: .....

## Appendix 6 IBS-C screening

### IBS SCREENING MODULE

IBS Module		
1. In the past 3 months, how often did you have discomfort or pain anywhere in your abdomen?	(0) Never..... (1) Less than 1 day a month (2) 1 day a month (3) 2-3 days a month (4) 1 day a week (5) More than 1 day a week (6) Every day	Skip remaining questions
2. Did the discomfort or pain occur only during menstrual bleeding and not any other times?	(0) No (1) Yes (2) Does not apply because I have the change in life (Menopause)	
3. Have you had this discomfort or pain 6 months or longer?	(0) No (1) Yes	
4. How often did this discomfort or pain get better or stop after you had a bowel movement?	(0) Never or rarely (1) Sometimes (2) Often (3) Most of the time (4) Always	
5. When did this discomfort or pain started, did you have more frequent bowel movements?	(0) Never or rarely (1) Sometimes (2) Often (3) Most of the time (4) Always	
6. When this discomfort or pain started, did you have less frequent bowel movements?	(0) Never/rarely (1) Sometimes (2) Often (3) Most of the time (4) Always	
7. When this discomfort or pain started, were your stools (bowel movements) looser?	(0) Never/rarely (1) Sometimes (2) Often (3) Most of the time (4) Always	
8. When this discomfort or pain started, how often did you have harder stools?	(0) Never/rarely (1) Sometimes (2) Often (3) Most of the time (4) Always	
9. In the last 3 months, how often did you have hard or lumpy stools?	(0) Never/rarely (1) Sometimes (2) Often (3) Most of the time (4) Always	
10. In the last 3 months, how often did you have loose stools? Mushy or watery stools?	(0) Never/rarely (1) Sometimes (2) Often (3) Most of the time (4) Always	

**Alternative scale:** (0) Never/rarely (1) About 25% of the time (2) About 50% of the time (3) About 75% of the time (4) Always, 100% of the time