INTRODUCTION

The gastrointestinal tract (GIT) microbiota plays an important role in host health due to it's invovlement in nutritional, immunolgic and physiological functions.Microbial imbalances have been associated with enhanced risk of specific diseases ***(Collado et al.,2009).***

Probiotics and prebiotics alone or together (synbiotics) can influence the intestinal microbiota and modulate the immune response. They may therefore be tools that can prevent or alleviate certain pathologies invovlving the gut immune system such as allergies for which no treatment is yet available ***(Gourbeyre et al., 2011*).** Prebiotics can also exert an influnce on the gut immune system via the stimulation of the autochthonous bacteria metabolism ***(Gourbeyre et al., 2011)***.

Prebiotics are selectively fermented non-digesteable food ingerdients that were identified and named by Marcel Roberfroid.They allow specific changes both in the composition and/or activity of the gastrointestinal microflora that confers benefits upon host well-being and health. Roberfroid stated that only the particular fructo-oligosaccharides fully meet this definition are the oligofructose and inulin.Other authorities also classify galactooligosaccharides (GOS) as prebiotics.Mannan loigo-saccharides (MOS) have been termed as immuno- saccharides ***(Roberfroid, 1995).***

Prebiotics are natural energy source for the growth of healthy bacteria in the gut.This growth suppots the infant's natural defences by increasing the levels of healthy bacteria and decreasing the levels of potentially harmful bacteria found in the intestine,positively influencing the entire microbiota **.** Prebiotics are specifically formulated in milk formulas to mimic the effect of oligosaccharides naturally present in breast milk,the effect being similar to seen in infants who are breast fed.Prebiotics are therefore important for any infant who is not breast fed,as they have a natural,positive effect on the entire gut microbiota,helping to support the infant's immune system and have an effect on gasrointestinal motility ***(Haarman, 2005).***

CD4 is a glycoprotein that recognize MHC class 2 antigens on antigen presenting calls. Whereas CD8 is a glycoprotein on cytotoxic T-cells which recognize class 1 antigens on target cells. CD4 is present on cell surface of T-helper calls and monocytes and it makes up tp 60% of circulating T-cell population.And C8 represents about 35% of circulating T-cell population ***(McMahon, 2004).***

AIM OF THE WORK

The study was done to evaluate the efficacy, safety and tolerability of prebiotics in infant formula to infants as well as to study it's immunological impact on CD4 and CD8.

EFFECTS OF PREBIOTICS ON HUMAN HEALTH

Human milk is considered to be the ideal nutrition for term infants because it provides all necessary nutrients for normal growth and development. The quantity and quality of nutrients are adapted to the functional maturation of the gastrointestinal tract as well as the metabolic state of the infant, so that relatively low concentrations of nutrients fulfill the requirements of the infant. In addition, human milk contains components that survive-partially or completely-intestinal digestion and provide functional capacity ***(Oddy et al., 2002, Hamosh, 1996).***

Before birth, the infant's gut is sterile. During vaginal delivery, the natural colonization of the infant starts with bacteria mainly from the vaginal and intestinal microbiota of the mother. For the further development of the intestinal microbiota of the infant, the diet plays an important role ***(Orrhage et al., 1999).*** During breast-feeding, the composition of the gut microbiota develops within a short period and becomes dominated by bifidobacteria, whereas formula-fed infants without prebiotics develop a flora of a more adult type ***(Harmsen et al., 2000)***. Therefore because of the importance of the intestinal microbiota for the development of the gut physiology and the immune system, many attempts have been made to mimic the intestinal microbiota of breast-fed infants in bottle-fed infants.

Since the composition of the intestinal microbiota can be influenced either by administration of living health-promoting bacteria that survive the gastrointestinal tract, exert their biological activity by interaction with the surface of the small intestine, and colonize the colon ***(Fuller, 1989)*** or by application of dietary ingredients that are non-digestible which are prebiotics during the passage through the small intestine, reach the colon, and stimulate selectively health promoting colonic bacteria ***(Gibson et al., 1995; Gibson et al., 2004).***

Definition:

As prebiotics are a more recent concept, they were first defined about ten years ago ***(Gibson and Roberfroid, 1995).*** But here, the selective growth of indigenous gut bacteria is required. Prebiotics are then defined as indigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a number of health-promoting colon bacteria and thus improve host health ***(Gibson and Roberfroid, 1995)***. Prebiotics have become known mainly as an alternative to probiotics, which are difficult to handle in foodstuffs, but whose benefits to health in terms of prevention of diarrhea and immunomodulation are increasingly well established and because prebiotics currently in use, especially inulin and its derivates as well as galactooligosaccharides, are cheap to manufacture or extract and, in addition to having probable health beneficial effects on the gut flora, are also valuable functional ingredients in foods ***(Macfarlane et al., 2006).***

Gibson and Colleagues (2004) have recently reviewed their original prebiotic concept in the light of much research that has been published in the past decade, and in particular the three key aspects of their definition:

1) Resistance to digestion:

2) Fermentation by the large intestinal microflora

3) A selective effect on the flora that promote health.

So their new definition now is that:

"A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/ or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health". The key words in both definitions are "selective" and "benefit/ improve…host …health".

Therefore, a prebiotic substrate must be particularly readily available to some groups of bacteria (of which lactobacilli and bifidobacteria are considered indicator organisms) that are beneficial to intestinal health but less available to potentially pathogenic bacteria, such as toxin-producing *Clostridia*, proteolytic *Bacteroides* and toxogenic *Escherichia coli* ***(Manning & Gibson, 2004).***

In this manner, a "healthier" microbiota composition is obtained where by the bifidobacteria and/or lactobacilli become predominant in the intestine and exert possible health promoting effects.

An exclusively breastfed baby has flora dominated by lactobacilli and bifidobacteria, which are part of the baby's defence against pathogens and which is an important primer for the immune system ***(Newburg, 2005; De Morais & Jacob, 2006***). These flora are nurtured by the oligosaccharides of breast milk, which is considered to be the original prebiotics. While some peptides, proteins and certain lipids are potential prebiotics, non-digestible carbohydrates, in particular non -digestible oligosaccharides, have received most attention ***(Ziemer & Gibson, 1998***).

Any dietary component that reaches the colon intact is a potential prebiotic. However, the prebiotic property has been demonstrated adequately for only a few food ingredients. According to Gibson *et al*. (2004) and Roberfroid (2005) to date only the inulin-type fructans, galactooligosaccharides and lactulose are proven prebiotics. However, the latter is considered a therapeutic ingredient rather than a food ingredient.

Charaterstics of prebiotics:

Prebiotics should have the following properties:

1. Capable of passing to large intestine without being digested and absorbed in the upper part of gastrointestinal tract ***(Kolida et al., 2002; Gibson, 2004).***
2. Capable of being digested in the large intestine by beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* ***(Kolida et al., 2002; Gibson, 2004).***

3. Capable of enhancing the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, but not pathogens causing gastrointestinal diseases such as *Clostridium perfringens* ***(Gibson et al., 1995; Gibson and Roberfroid, 1995; Kolida et al., 2002).*** Gibson *et al.* (1995) fed 100 volunteers with 5-20 g/d of fructooligosaccharide and inulin for 9 weeks and found an increase in *Bifidobacterium* in their intestinal tracts. Prebiotics must be able to withstand acid hydrolysis in the stomach, able to move to large intestine without changes or being absorbed in small intestine so that they can be utilized by the indigenous microflora in the large intestine to enhance their growth ***(Gibson, 2004).***

This benefits the host by improving the absorption of elements such as Ca, Mg, and Fe, or preventing cancer of the large intestine ***(Van Loo et al., 1999).*** Oligosaccharides that are considered prebiotics are lactose, lactulose, raffinose, stachiose, and fructooligosaccharide.

There are other compounds that can be fermented by *Bifidobacterium* and *Lactobacillus* in the large intestine, e.g., resistant starch, non-starch polysaccharides, which include plant constituents such as pectin, cellulose, hemicelluloses, and xylan.

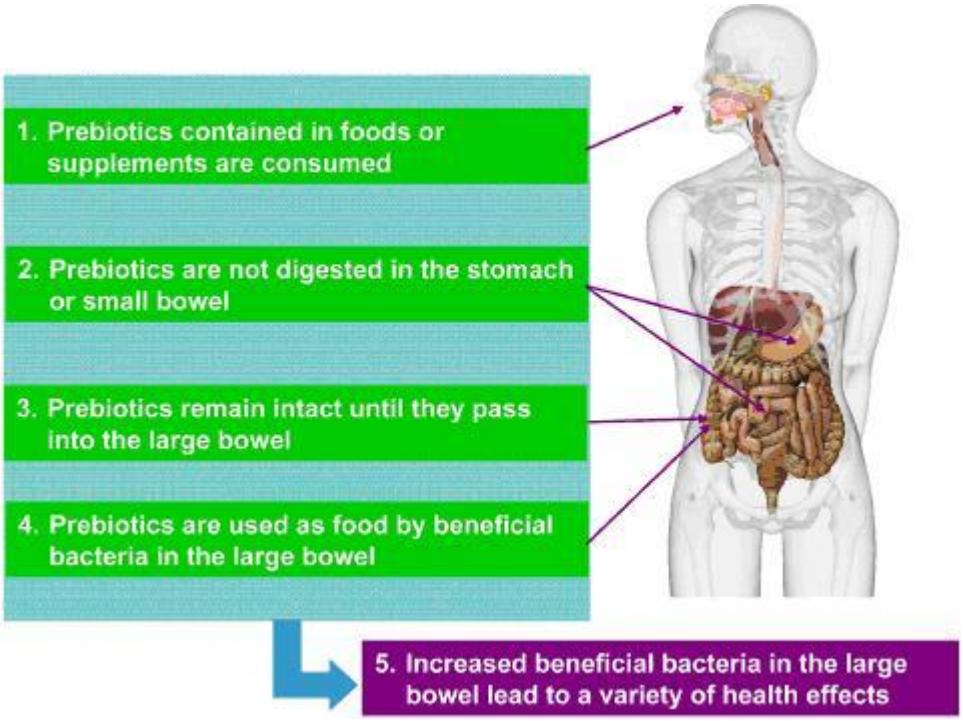

Fig. (1): How does prebiotics work.

Types of prebiotics:

- Inulin is a group of fructose polymers (or fructans) linked by ß (2-1) bonds, which limit their digestion by upper intestinal enzymes. Naturally occurring fructans exist with a varied degree of polymerisation (DP) from 2 to 60 ***(Duggan et al., 2002).***

- Oligofructose is defined as any fructose oligosaccharide containing 2 to a maximum of less than 10 monosaccharide residuals connected by glycosidic linkages ***(Roberfroid, 2007).*** Oligofructose is always present in inulin, a blend of fructose oligomers and polymers, and which differs from

inulin in regard to its DP (2 < DP < 10 for oligofructose and 2 < DP < 60 for inulin) ***(Watzl et al., 2005).*** Fructans are found in many plant species, including chicory (from which inulin is extracted) and to a lesser extent in onion, garlic, banana, asparagus, leek and Jerusalem artichoke. For cereal grains, wheat is the best source, providing on average about 2, 5 g fructans per 100 g raw bran and baked flour ***(van Loo et al., 1995)***. Wheat is the major food source of naturally occurring inulin and fructooligosaccharides, providing about 70% of these compounds in American diets ***(Moshfegh et al., 1999).***

Table (1): Synthetized prebiotics and their production methods.

Prebiotic	*Production Method*
• Inulin (Fructooligosaccharides)	• Hot water extraction from chicory root followed by enzymatic hydrolysis, or polymerization of fructose monomers.
• Galacatooligosaccharides (GOS)	• Enzymatic lactose transgalactosylation.
• Xylooligosaccharides (XOS)	• Enzymatic hydrolysis of plant xylans.
• Isomaltooligosaccharides (IMO)	• Transglucosylation of liquefied starch.
• Lactulose	• Isomerization of lactose.

(Vernazza et al., 2006).

Nutritional and associated health properties of prebiotics:

The nutritional properties of prebiotics are related directly to the physiological changes they induce in the host. Bacterial metabolites are probably the main effectors of most observed effects. The most important metabolites are the short-chain fatty acids (SCFA) acetate, propionate and butyrate ***(Cummings et al., 2001).*** Prebiotic consumption can double the pool of SCFA in the gastrointestinal tract. These SCFA acidify the colon environment, which is beneficial for the development of bacteria such as bifidobacteria and lactobacilli, and detrimental to the growth of potential pathogenic species ***(Brandt, 2001; Blaut, 2002).*** All the SCFA are rapidly absorbed from the colon and then metabolised by various tissues: butyrate by the colonic epithelium, propionate and acetate (partly) by the liver and acetate (partly) by muscle and other peripheral tissues ***(Gibson and Roberfroid, 1995; Blaut, 2002).*** Acetate is the principal SCFA in the colon, and after absorption it has been shown to increase cholesterol synthesis ***(Jenkins et al., 1991; Wolever et al., 1991)***. However, propionate, a gluconeogenerator, has been shown to inhibit cholesterol synthesis in vitro ***(Chen et al., 1984; Venter et al., 1991).***

Promoting health effects of prebiotics:

Anyhow in the past decade, a large number of studies investigated the health-promoting effects of prebiotics. Although some of the postulated effects have not been fully demonstrated,

the data suggest clinically significant effects that warrant further study and explanation.Demonstrating a direct clinical or health benefit of prebiotics is proving difficult. Small changes in lipid metabolism, calcium absorption or immune function may not give rise to evident improvements in health for many years. However, resistance to pathogen invasion through increased colonisation resistance of the gut microbiota, brought about by stimulation of bifidobacteria and lactobacilli, should in principle be easier to show *in vivo* ***(Cummings and Macfarlane, 2002).*** The postulated beneficial effects of prebiotics are summarised below.

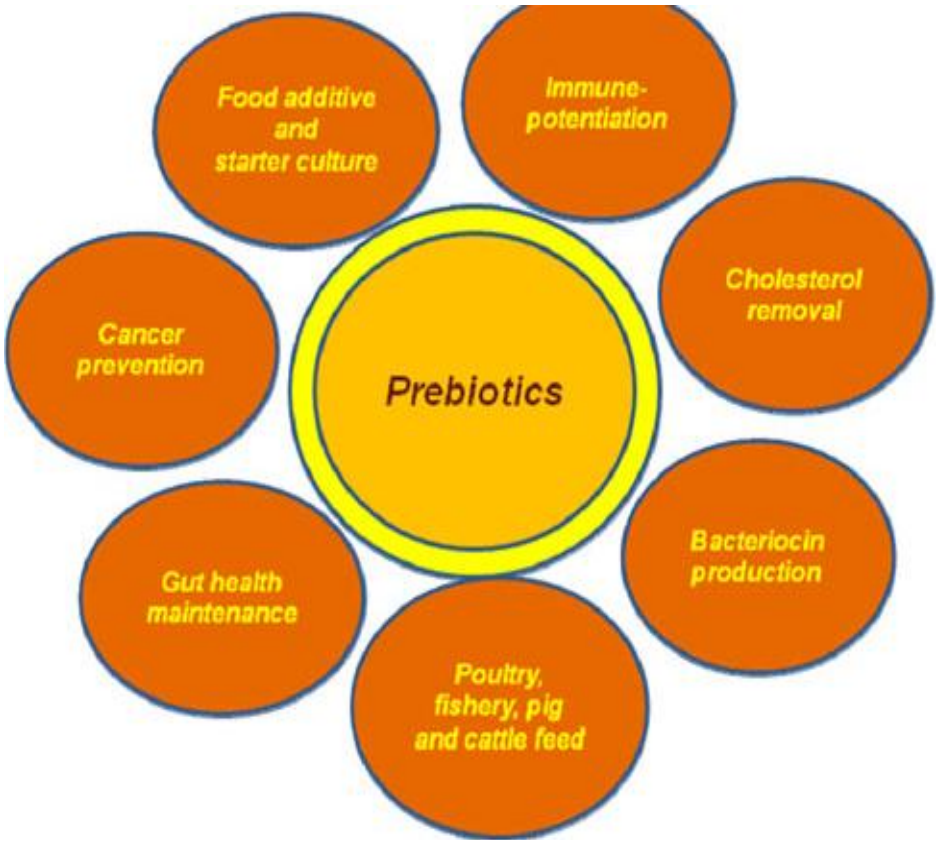

Fig. (2): Potential applications of prebiotics.

GIT development:

The two most dramatic periods of postnatal development of the GIT occur at birth with the onset of feeding and colonization with bacteria and again at weaning when dietary inputs changing from milk to the adult diet, with corresponding changes in GIT characteristics and assemblages of bacteria.

At birth the sterile GIT of neonates is colonized by bacteria originating from the maternal GIT, vagina, skin, and the surrounding environment ***(Mackie et al., 1999)***. Since the species composition and the relative abundances of the colonizers is not consistent. After the initial period of colonization, the densities, diversity and distribution of species within the GIT ecosystems are determined by a combination of dietary inputs, GIT functions, and inter-bacterial interactions, including the processes of competition and facilitation.

An example is the concept of ‘‘metabolic cross-feeding’’ whereby metabolites produced by one group of bacteria are used or converted by other groups of bacteria ***Flint et al., (2007).*** One of the better examples is the conversion of lactate produced by bifidobacteria into butyrate and other SCFA by anaerobic members of the GIT bacteria.

The interactions between dietary inputs, the resident bacteria, and health are particularly evident during infancy **(*Gil and Rueda, 2000*)** and can have profound health consequences.

Necrotizing enterocolitis (NEC) is an inflammatory reaction that is the most common GIT disorder of neonates, and particularly those born premature **(*Schnabl et al., 2008*).**

Dietary inputs can and do influence the successional changes that occur in the developing bacterial assemblages. By doing so, they influence postnatal GIT development as well as neonatal and infant health (***Amarri et al., 2000; Moreau and Gaboriau-Routhiau, 2001; Walker, 2000***). Of particular interest are the responses of the GIT bacteria to diet and the associated influences on the developing immune system of infants **(*Cebra, 1999; Gaskins et al., 2008; Haverson et al., 2007).*** This includes the relations with development of tolerance and allergy (***Kukkonen et al., 2007, 2008*).** Coinciding with these findings, there is understandable interest in increasing the proportion of beneficial bacteria in the GIT of infants to improve health and disease resistance.

Approaches were done to improve health and disease resistance one of the main approaches is to provide prebiotics to encourage the growth of beneficial bacteria. Human breast milk, which is high in oligosaccharides, encourages higher densities of health promoting lactobacilli and bifidobacteria. The addition of prebiotics to formulas has similarly increased the densities of lactobacilli and bifidobacteria **(*Veereman et al., 2007),*** thereby providing health benefits ***(Arslanoglu et al., 2007, 2008*).**

Alleviation of constipation

All carbohydrates that reach the large intestine have a laxative effect on bowel habit. The mechanism works via stimulation of microbial growth, increase in bacterial cell mass and thus stimulation of peristalsis by the increased bowel content ***(Cummings, 1994***). It can be predicted, therefore, that prebiotics will be laxative , as prebiotic carbohydrates are dietary fibers, as they are not digested by human enzymes but fermented by the flora of the large intestine. Thus, they increase biomass, feces weight and feces frequency, have a positive effect on constipation and on the health of the mucosa of the large intestine (***Cherbur, 2002; Nyman, 2002).***

Impact on the Intestinal Flora

Positive effects of pre- and synbiotics on the intestinal flora, i.e. growth-promotion of potentially protective bacteria (bifidobacteria and in part also lactobacilli) and/or the inhibition of potentially pathogenic microorganisms, as well as stabilization of the intestinal environment by lowering the pH and release of short-chain organic acids, have been investigated and confirmed frequently in in vitro and in vivo trials. Inulin, oligofructose, or TOS as well as their synbiotic combination with probiotic bacteria (strains of *L. plantarum*, *L.paracasei*, or *B. bifidum*) increased bifidobacteria and lactobacilli or inhibited various human- and animal pathogenic bacteria strains (*Clostridium* spec., *E. coli,*