

Review Paper

Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora

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Abstract

In recent years, there has been a growing appreciation on the relevance of gastrointestinal microflora in both ruminants and non-ruminants owing to revelation of their role in several physiological functions including digestion, nutrient utilization, pathogen exclusion, gastrointestinal development, immunity system, gut gene expression and quality of animal products. The ban imposed on the use of antibiotics and hormones in feed has compelled animal researchers in finding an alternative which could overcome the issues of conventional feed additives. Though the concept of prebiotic was evolved keeping in mind the gastrointestinal flora of human beings, presently animal researchers are exploring the efficiency of prebiotic (inulin) for modulating the gut ecosystem of both ruminants and non-ruminants. It was revealed that prebiotic inulin is found to exhibit desirable changes in the gut of non-ruminants like poultry, swine, rabbit etc for augmenting gut health and improvement of product quality. Similarly, in ruminants the prebiotic reduces rumen ammonia nitrogen, methane production, increase microbial protein synthesis and live weight gains in calves. Unlike other feed additives, prebiotic exhibits its effect in multipronged ways for overall increase in the performances of the animals. In coming days, it is expected that prebiotics could be the part of diets in both ruminants and non-ruminants for enabling modulation of gut microflora *vis a vis* animals productivity in ecological ways.

Key words: prebiotic, inulin, mechanism of action, livestock.

Introduction

The world has witnessed immense development in nutritional science during the last century which gave birth to the concept of balanced diet following identification of the essential nutrients for supporting growth, development, production in addition to the prevention of deficiency symptoms. Research efforts during the past decades resulted in the development of feeding standards for different species, breeds, and production levels across the world to maximize productivity as well as to ensure higher returns from livestock enterprises. During the 20th century, one of the major contributions of nutritional science is the concept of the “balanced diet” which may be defined (applicable to ruminants) as “an appropriate mixture of roughage and concentrate in the form of mash or block capable to fulfill the requirements of nutrients needed to support maintenance, growth and production of animals”. In the case of

non-ruminants it may be considered as “an appropriate mixture of feed ingredients that essentially fulfills the nutrient requirements for carrying out cell function without exhibiting deficiency disorders”. At the turn of the 21st century, the ‘balanced ration’ and the ‘nutrient requirements’ remained the key concepts. However, with changing lifestyle in the present century, the consumer demands and requirement has drastically changed. Recently, prebiotics has been introduced as a new concept under functional food science owing to concern over residues of antibiotic or hormones, consumer awareness and safety features. Keeping in view the above circumstances, presently animal nutrition research draws more attention on feed safety, quality, designer animal products, healthy animal products, ecological treatment for digestive disorder and organic animal production. It seems prebiotics specifically inulin has potential to address these issues and capable to bring out the desired effects on animal performances.

The addition of prebiotics in the diets of animals is a relatively recent endeavor and preliminary studies are very encouraging. Although the effect of most functional foods target only one or a limited number of functions, the prebiotics target a range of different physiological functions starting from bowel performance and colonic microflora activities to mineral absorption, lipid homeostasis and immunity (Gibson *et al.*, 2004; Samanta *et al.*, 2011).

Gastrointestinal microflora

Based on the occurrence of microbial habitat in the gastrointestinal tract (Figure 1), domestic animals may be classified into two categories viz.; mono gut fermentor - non-ruminants (namely poultry, swine, horse, rabbit, donkey etc) and twine gut fermentors - ruminants and pseudo-ruminants (namely cattle, buffalo, sheep, goat, mithun, camel etc). The gastrointestinal tract of ruminant is featured with the provision of two microbial habitats *i.e.* foregut (rumen, reticulum and omasum) and hindgut (caecum). On the other hand non-ruminants are featured with the presence of single microbial habitats *i.e.* hindgut only. The presence of foregut with enormous capacity to house diverse categories of microflora provide the edge to ruminant animals over the non-ruminants for consumption and dependence on ligno-cellulosic biomass to obtain their energy requirements through volatile fatty acids (*viz.*; acetate, propionate, butyrate etc) generated from the degradation and anaerobic fermentation of fibrous materials. The single fermentation site *i.e.* at the hindgut of non-ruminant animal permeates partial dependence over the volatile fatty acids for energy requirements. It is estimated that rumen fluids contain (number/mL) 10^{10} to 10^{12} bacteria, 10^4 to 10^6 protozoa, 10^8 to 10^9 archaea, 10^2 to 10^4 fungi in addition to yeast, phage etc. (Hobson *et al.*, 1988; Hungate, 1966; Samanta *et al.*, 2003).

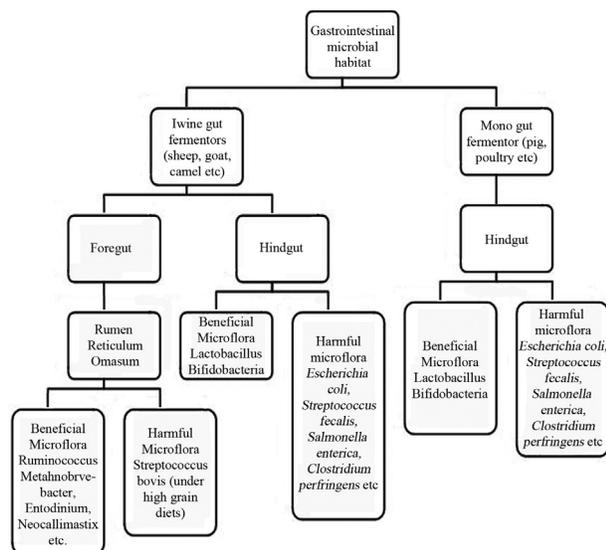


Figure 1 - Classification of livestock based on microbial habitats.

Similarly, the hindgut of non-ruminants is also act as habitat for millions of bacteria, archaea, fungi and yeasts. By virtue of unique environment available in terms of nutrients, pH, interaction, temperature, crosstalk in the gastrointestinal tract, both beneficial and harmful microflora get niche for their growth and multiplication. Inability to explain certain features following completion of human genome project enforces researchers to relook into the gut microflora for better understanding and answering the unresolved issues. In this regard, gut microflora are considered to evolve along with the human evolution and humans have been proposed to be “metaorganisms”; consisting of 10-fold greater numbers of bacteria than animal cells (Gill *et al.*, 2006; Neish, 2009; Xu *et al.*, 2007). The human “metaorganisms” include approximately 10^{14} prokaryotic organism having a biomass of > 1 kg and often considered as hidden organ having the capacity equivalent to liver. The complete development of gastrointestinal tract and immunological system takes place only after the establishment of gut microflora thus signifying the absence of signals within human genome to carry out complete development of either gastrointestinal tract or immune system. From the above analogy, one could guess the contribution and significance of microflora inhabiting at foregut and hindgut of ruminants and hindgut of non-ruminants.

Classification of gut microflora

The classical cultivation based methods adopted by researchers grouped the gut microflora based on strain, shape, biochemical features, nutrient utilization etc and nowadays it is presumed that only 10 to 20% of microflora from the habitat could be cultivable by following the standard cultivation techniques (Harmsen *et al.*, 2000). To address the issues of uncultivable microflora, presently gut ecologists study the microflora through 16S rRNA based protocols which take care of both cultivable and uncultivable microflora. Ignoring the specific substrate utilization pattern (cellulose, hemicellulose, protein, lipid, pectin etc) or biochemical features, microflora of the gastrointestinal tract could be broadly grouped into two main categories namely pathogenic or harmful or detrimental microflora and health promoting or friendly or beneficial or pathogen suppressive microflora. The class of pathogenic bacteria includes *Escherichia coli*, *Streptococcus faecalis*, *Salmonella enterica*, *Clostridium perfringens* etc. Lactobacillus and Bifidobacteria inhabited in the gastrointestinal tract are presumed to be the major members of beneficial or health promoting class. The significance of prebiotic consumption arises from their ability to selectively stimulate the growth and multiplication of indigenous Bifidobacteria and Lactobacilli in the hindgut, which in turn suppresses the activity of putrefactive or harmful bacteria for reducing the concentration of toxic fermentation products in the gastrointestinal tract (Samanta *et al.*, 2007, 2010; Tomomatsu, 1994).

Harmful fermentation metabolites

In non-ruminants (poultry, swine, horse, dog), the large intestine especially the caecum is the primary site for millions of microflora belonging to diverse groups. In the ruminants (cattle, buffalo, sheep, goat), the ingested materials are exposed to extensive action by diverse group of microflora and their enormous array of hydrolyzing enzymes before they are subjected to utilization by animal's own gastric or pancreatic enzymes. The gastrointestinal motility permeates the delivery of digested materials at large intestine and hence once again the ingested materials are subjected to the action of microbial enzymes. As a result, several biochemical reactions take place at the site of microbial habitats *i.e.* either at rumen or caecum, resulting in the generation of enormous metabolites possessing beneficial or harmful action.

A wide number of toxic metabolites are generated following microbial fermentation of feed at large intestine. The major toxic metabolites of hindgut include ammonia, amines, nitrosamines, phenols, cresols, indole, skatole, estrogens, aglycone, secondary bile acid etc. The adverse effects of these metabolites are hepatotoxicity (ammonia, amines etc.), carcinogenicity (nitrosamines, phenols, cresols etc), mutagenic (aglycone) etc. Microflora involved in the formation of various toxic metabolites are *Escherichia coli* and *Clostridium* spp. (ammonia, amines, nitrosamines, phenol, indole, aglycone, secondary bile acids), *Bacteroides* spp., *Streptococcus fecalis* (nitrosamines, aglycones, secondary bile acids), *Proteus* spp. (ammonia, amines and indoles) (Tomomatsu, 1994). Primary enzyme involved in the formation of carcinogens from pro-carcinogens in the gastrointestinal tracts is azoreductase. Highest order of azoreductase enzyme activity is noticed in *Clostridium* spp., followed by *Eubacterium* spp. and *Peptostreptococcus* spp. and no activity is detected in *Bifidobacteria* spp. (Saito *et al.*, 1992). In addition to the formation of toxic metabolites or secretion of enzymes involved in production of harmful products, several gastrointestinal disorders are also noticed due to breakdown of gut microbial homeostasis (Moxley and Duhamal, 1999; Pope and Cherry, 2000).

Prebiotics

Prebiotics are a group of bio-molecules grouped together by virtue of their capability to promote the growth and multiplication of specific beneficial gut microflora. Ban on the use of antibiotics and hormones as feed additives, consumer awareness, strict quality control measures are the driving factors for intense research and development in the areas of functional food, especially the prebiotic oligosaccharides. Although the concept of functional foods has been introduced a long time ago by great ancient Greek philosopher Hippocrates "Let food be thy medicine and medicine be thy food", scientific evidences started to sup-

port the above principle only recently through the application of prebiotics for modulating the gut microflora. The term "Prebiotics" came into light only recently and is coined by Gibson and Roberfroid (1995), who exchanged "pro" to "pre", which means "before" or "for" (Aida *et al.*, 2009). Prebiotics may be defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and or activity of one or a limited number of bacteria in the colon". As it matches with certain aspects of dietary fiber, the updated version of prebiotics encompasses "selectively fermented ingredients that allow specific changes, both in the composition and/ or activity in the gastrointestinal microflora that confers benefits upon host well being and health" (Gibson *et al.*, 2004). Recently, FAO (2007) defined the prebiotic as "A non-viable food component that confers a health benefit on the host associated with modulation of the microbes". Although the effect of most functional foods targets only one or a limited number of functions, but the prebiotic targets a range of different physiological functions including better gut health, higher mineral absorption, lowering of cholesterol, immune stimulation and pathogen exclusion (Raschka and Deniel, 2005; Roberfroid, 2007). Therefore, the prebiotics are characterized by their non-digestibility at gastric levels, selective stimulation to the beneficial gut microflora, biological origin and obviously without any residue problems.

Evolution of prebiotic concept

Basically the novel concept of prebiotic was forwarded keeping in view its application for human health and well being (Gibson and Roberfroid, 1995). Since last few decades there has been growing interest between gastrointestinal microflora and different physiological functions of human beings with much attention being thrust on the prebiotic carbohydrates which are not digested or utilized by the enzymes secreted by the eukaryotic own glandular system; but are completely accessible to the selective group of gastrointestinal microbial communities *i.e.* beneficial gut microflora. Carbohydrates are stored in the form of fructan in more than 36,000 species available in the world (Hendry, 1987). Owing to the improper preservation of plant materials by early ancestors, the physical proof for consumption of crops rich in prebiotics is virtually nonexistent (Leach *et al.*, 2006). However, it could definitely be presumed that today's prebiotics either inulin or oligofructose were consumed by the Pliocene and Pleistocene ancestors a million years ago through the diverse plant materials (Laden and Wrangham, 2005; Leach, 2007). Gradually the prebiotics rich foodstuffs became part of the human diets in some areas in earlier days and it is thought that the quantity of prebiotic consumption might exceed today's prebiotic level that is consumed by the modern population (Van Loo *et al.*, 1995). Off late, the scientific definition for prebiotics was forwarded during mid nineties of previous century (Gibson and Roberfroid, 1995) keeping in view the

significance of utilizing plant derived compounds for augmenting gut health and functionality. Because of immense importance, prebiotic is presently thought to be a forerunner amongst several nutraceuticals for application towards routine maintenance of health as well as for ecological treatment of disorders pertaining to gastrointestinal tract.

Characteristics of prebiotics

Originally, the concept of prebiotics aroused after keeping in mind the gut ecology of human beings. Therefore, criteria set to qualify a biomolecule as prebiotic may not be the same as it was proposed by earlier researchers. Hence criteria for a compound to become a prebiotic in livestock are as follows (Samanta *et al.*, 2007):

- Indigestibility by animal's own gastric or pancreatic enzymes.
- Selectively utilized by so called beneficial gut microflora (exception several rumen bacteria ferments prebiotics).
- Plant origin or produced by microbial enzymes.
- Non-absorption from the epithelial surface of gastrointestinal tract.
- Protects structural and functional integrity while passing through either acidic or alkaline pH of gastrointestinal tract.
- Exhibit its potentiality even at minute concentration.
- Remains intact while undergoing physical action of digestion process *i.e.* mastication, chewing, mixing with several fluids etc.
- Presence of chemical bonds that is inaccessible to harmful gut microflora.
- No residue problems in livestock or their products.
- Non-carcinogenic.
- Its fermentation should not lead to generate metabolite of toxic nature.
- Easy to mix with other feed ingredients or micro-nutrient mixture.

Inulin type prebiotics

Despite the fact that the original concept of prebiotic was forwarded after keeping in mind the structure and function of inulin on human gastrointestinal tract but with the passage of time, a number of prebiotic has come into light and are on the way to establish their niche in the competitive market of functional foods (Samanta *et al.*, 2012). Nevertheless inulin occupies top position in the list of prebiotics because of their availability from a wide resources with minimum cost involvement and includes a group of biomolecules viz.; inulin, oligofructose and fructooligosaccharides. Inulin and its different forms are present in a wide variety of plants as natural storage carbohydrates.

It was first discovered as a "peculiar substance" by German scientist Rose in the year 1804 from the hot water

extract of roots of plants (*Inula helenium*) belonged to Compositae family. Later on, the term "inulin" was coined by Thomson in 1818. Chemically it is fructan made up of fructose units linked by β -2, 1 linkage of which terminal sugar is obviously glucose. Inulin is represented by general formula GF_n , while oligofructose by F_m and/or GF_n ; 'G' is glucosyl unit, 'F' is fructosyl unit, 'n' is the number of fructose units linked to terminal glucose units and 'm' is the number of fructose units linked to each other in the carbohydrate chain (Franck, 2000; Ninness, 1999). In case of inulin, 'n' is greater than 2 and less than 60. Oligofructose, obtained by partial hydrolysis of inulin can be a mixture of both GF_n and F_m molecules with DP varying from 2 to 7 or 8 to 10 depending on the products' brand (Franck, 2000). Nonetheless only GF_n type oligofructose could be produced from inulin by enzymatic hydrolysis or by transfructosylation of sucrose molecule in the presence of fructosyl transferase (Sangeetha *et al.*, 2005). First elongation of sucrose with one fructose molecule leads to generation of fructooligosaccharides termed as 1-kestose and further elongation leads to production of 1-nystose and so on (Samanta *et al.*, 2010). The degree of polymerization (DP) refers to the number of repeating units (fructose) present in the prebiotic inulin or oligofructose and depends upon plant source, growing stages, climatic conditions, post harvest processing etc. Inulin may also contain minor amounts of F_m fructans (F_2), although GF_n fructans with DP from 2 to 60 are predominant.

Inulin is storage polysaccharides that encompasses all linear fructan (Roberfroid, 2007) and is widely distributed in nature. Spectrum of inulin occurrence is reported in more than 36,000 plant species (Carpita *et al.*, 1989; Van Loo *et al.*, 1995) and the list include roots of chicory (15-20%), burdock (3.5 -4%), salsify (4-11%), yacon (3-19%), murnong (8-13%), bulbs of onion (2-6%), garlic (9-16%), leek (3-10%), camas (12-22%), tubers of Jerusalem artichoke (16-20%), leaves of dandelion (12-15%), artichoke (3-10%) in addition to a number of cereals and fruits. Presently most of the inulin is produced from roots of chicory in which chain length ranges from 2 to 60, with an average degree of polymerization 10. Oligofructose is made by 2 to 8 molecules of fructose units, terminally linked with a glucose moiety. The presence of β -2,1- osidic bonds either inulin or oligofructose make the prebiotic indigestible by mammalian enzymes (Gibson *et al.*, 2004).

Effect of inulin in livestock

During the last one and a half decades there has been growing interest amongst the diverse field of researchers to ensure multidimensional application of prebiotics for well being of human society. As a result it finds its place for augmenting gut health and functionality, regularization of fecal output in older individuals, sweetener for diabetic patient, ecological treatment of gastrointestinal disorders especially

inflammatory bowel disease etc. (Gibson *et al.*, 2004; Saito *et al.*, 1992; Van Loo *et al.*, 1995). Although preliminary studies on prebiotic application in the diets of livestock are encouraging, the quantum of data pertaining to livestock are frugal. In the present review efforts have been made to assemble that sporadic information into meaningful directions.

Swine

Swine experiences many stressors during their entire life, out of which weaning is most important. During this phase often there is breach in the gut microbial homeostasis as a result of changes in gut microflora composition. Presently the European Union has banned the use of antibiotics in feed and some countries are considering banning it as feed supplements. Under such circumstances, prebiotics either derived from plant or synthesized by microbial enzymes offers to occupy the vacuum of antibiotics for improving the productivity of livestock keeping in mind the demerits of antibiotics applications. Newborn piglets consuming oligofructose exhibited higher number (1.68×10^{10} vs. 4.85×10^9 CFU/g fecal contents) of Bifidobacteria on 6th day of consumption (Howard *et al.*, 1995). In an effort to see the potentiality of prebiotic inulin on recovery from induced diarrhoea by cholera enterotoxin at the age of 3rd weeks in piglets, the population of lactobacilli was significantly higher in both caecum (9.3 vs. 7.7 log₁₀ CFU per gram) and at colon (9.3 vs. 8.3 log₁₀ CFU per gram) following daily administration of 9.5 g of oligofructose (Oli *et al.*, 1998). At the same time, population of harmful bacteria *i.e.* Enterobacteria was reduced significantly at both caecum (6.2 vs. 7.4 log₁₀ CFU per gram) and colon (6.2 vs. 8.5 log₁₀ CFU per gram) in oligofructose piglets as compared to the piglets not receiving the prebiotics. There was significant increase in the population of total anaerobes, Bifidobacteria and lactobacilli with concomitant decrease in the number of Enterococci and Clostridium population following supplementation of prebiotic and probiotic (Nemcova *et al.*, 1999). Contrary to this, some researchers did not find positive influence of prebiotics in animals. Replacing cellulose fraction with oligofructose in the diets of weanling pigs, neither altered the ileal population of total anaerobes nor lactobacilli (Houdijk *et al.*, 1999). A non-significant increase in the population of Bifidobacteria was noticed at the ileal contents of weanling pigs by the same researchers. The pig waste is associated with malodor due to presence of microbial metabolites such as phenols, indoles, sulfides, fatty acids etc (Hobbs *et al.*, 1996). Supplementation of chicory inulin in the diets of pig resulted in significant decrease of ammonia concentration in both caecum and colon (Mul, 1997). Consumption of prebiotics also enhanced both dry matter and organic matter digestibility at ileum (Houdijk, 1999).

For studying the effect of prebiotics on gastrointestinal worms, four month old pigs were challenged with lar-

vae of *Oesophagostomum dentatum* (Petkevicius *et al.*, 1997). The burden of worm was significantly lower in pigs consuming inulin as feed additives; indicating potentiality of prebiotic in prevention of parasitic infection. Weaned piglets consuming regularly oligofructose along with milk replacer could counteract the detrimental effect of challenged *E. coli* and exhibited increased survival rate, higher fecal concentration of Bifidobacteria and lower concentration of fecal *E. coli* (Bunce *et al.*, 1995).

By virtue of prebiotic potentiality, inulin exhibited positive alteration of the gastrointestinal microflora which in turn demonstrated increased performance by livestock. On this aspect attempts were made to elucidate the effect of oligofructose on food intake and live weight gains in weaning pigs even before the introduction of prebiotic concept. No significant effect of either oligofructose or Jerusalem artichoke flour was noticed on feed intake, daily gains, and feed conversion efficiency in pig (Farmworth *et al.*, 1992); as a result of insufficient levels of prebiotics. Later on, further studies revealed reduced dry matter intake, increased daily weight gains and feed conversions efficiency in growing pigs following supplementation of either oligofructose or trans-galactooligosaccharides (Houdijk *et al.*, 1999).

Poultry

Like other animals, poultry also possesses a specialized organ (ceca) at the terminal part of the gastrointestinal tract to offer nutrient rich habitat for the millions of microflora (10^{11} CFU/g belonging to 200 or more strains) and to avail benefits of synergism from microbial fermentation (Timms, 1968). The microflora of the poultry caecum are broadly categorized into three: dominating, sub-dominating and temporary populations (Barnes, 1979). On the 1st day of chick's life, the major caecum microflora includes species of Enterobacteriaceae, Enterococcus and Lactobacillus. Bacteriodes and Eubacterium spp. got established after 2nd weeks of chick's age (Van der Wielen *et al.*, 2001). The microflora of the poultry gut is presumed to play multidimensional role including digestion, metabolism, pathogen exclusion, immune stimulation, vitamin synthesis etc. Under *in vitro* culture system, it has been noticed that *Salmonella* serotype could not grow in the presence of oligofructose as sole source of energy, while so called beneficial microflora namely *Lactobacillus lactis*, *Enterococcus faecium* and *Pediococcus* grow well by virtue of their ability to secrete enzymes competent to break down the osidic bonds present prebiotics (Oyarzabal and Conner, 1995). Therefore, it is noteworthy to incorporate inulin as prebiotic for overcoming *Salmonella* in chickens. Inclusions of oligofructose in the diets of chicks enabled substantially reduction in *Salmonella* colonization at the gastrointestinal tract (Bailey *et al.*, 1991; Fukata *et al.*, 1999).

Supplementation of inulin as prebiotics in the diet of layers and broiler birds improved growth performance (Verdonk and Van Leeuwen, 2004; Yusrizai and Chen,

2003). Contradictorily, some of the research endeavor was unable to get a positive response on growth performance following inulin supplementation in birds (Biggs *et al.*, 2007; Ortiz *et al.*, 2009). The variability in the effectiveness of inulin may be ascribed to several factors namely level of inclusion, type of diet, animal individuality, degree of hygiene etc (Verdonk *et al.*, 2005). The dietary inclusion of graded levels of inulin had no significant effect on the relative weight and relative length of the duodenum, jejunum, ileum and caecum of birds (Ortiz *et al.*, 2009; Yusrizai and Chen, 2003). The inability of inulin to influence effectively the gross intestinal morphology might be a result of its high water solubility and its minimal capacity to increase the viscosity of the digesta (Schneeman, 1999).

Supplementation of broiler diets with prebiotic inulin showed no effect on the weight and measurement of tibia but linearly improved the relative apparent retention of Ca, Zn and Cu (Ortiz *et al.*, 2009). It increased the ash contents and Ca contents of tibia in broilers receiving inulin and established role of prebiotics on improvement of mineral utilization and bone mineralization (Figure 2). Inulin, being a prebiotic, is not digested by the birds own gastric or pancreatic enzymes but utilized by the specific group of microflora namely Bifidobacteria and Lactobacilli at the large intestine for production of various short chain fatty acids and lactic acids (Samanta *et al.*, 2012). The production of fatty acids at large intestine leads to lowering of luminal pH. Acidic pH is found to increase mineral solubility and facilitates higher mineral absorption (Levrat *et al.*, 1991; Lopez *et al.*, 2000). In this respect butyrate is more effective than acetate for ensuring higher Ca absorption.

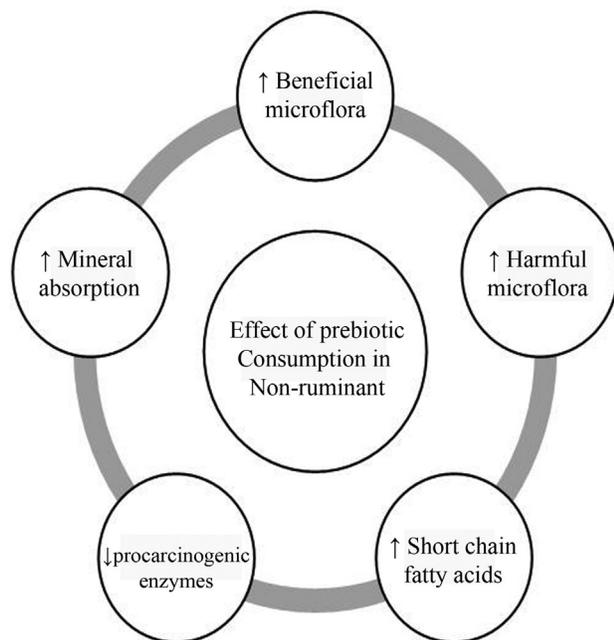


Figure 2 - Schematic diagram on effect of prebiotic consumption in non-ruminants.

Dog

Intake of oligofructose at a concentration of 1% of diet (w/w) for a period of around 50 days significantly reduced the population of total gut aerobes, facultative aerobes in duodenal fluid and mucosa of German shepherd dogs suffering from intestinal bacterial overgrowth (Williard *et al.*, 1994). It is presumed that supplemental oligofructose are preferentially fermented and utilized by Enterobacteria and Eubacteria strains which in turn prevent adhesion and survival of pathogenic bacteria in the colon of Beagles (Kearns *et al.*, 1998). While incorporating graded levels of oligofructose in the diets of female hounds, there is significant decrease in the fecal concentration of *Clostridium perfringens* and increased concentrations of total aerobes and population of Bifidobacteria (Flickinger *et al.*, 2002). *In vitro* fermentation of oligofructose in the presence of microflora of dog fecal matter leads to rapid production of short chain fatty acids including acetate, butyrate and propionate (Sunvold *et al.*, 1995). Short chain fatty acids are supposed to possess trophic effect on the colonic epithelium (Flickinger, *et al.*, 2003). Dogs consuming oligofructose had longer and heavier small intestine with greater available surface areas for absorption (Buddington *et al.*, 1999). Fecal concentration of ammonia, putrescine, cadaverine, total phenols are found to be reduced following supplementation of oligofructose (Flickinger *et al.*, 2002). These are the metabolites usually produced by the harmful bacteria inhabited at the gastrointestinal tract and supposed to be responsible for bad odor of feces or other detrimental consequences.

Consumption of inulin at a concentration of 7% of diet (w/w) exhibited no effect on apparent dry matter digestibility in dogs but digestibility of organic matter, crude protein, fat were lowered as compared to the control (Diez *et al.*, 1998a). Contrary to the above, no difference in apparent dry matter, organic matter, crude protein or fat digestibility was observed following supplementation of various levels of oligofructose to the healthy dogs (Flickinger *et al.*, 2002). Plasma cholesterol concentration of dogs reduced significantly following regular consumption of oligofructose for a period of 6 weeks (Diez *et al.*, 1998b).

Rabbit

In case of rabbit, caecum is the primary site of fermentation which is carried out by several anaerobic genera (Flickinger *et al.*, 2003). To have higher performances from rabbit, its diets are devised to be rich in protein and starch but low in fiber. Therefore, it seems the diet of rabbit should be enriched with sufficient quantities of fermentable carbohydrates (prebiotic) to maintain the homeostasis of gut microflora *vis a vis* its functionality. Thus oligofructose is routinely added at the levels of 1 to 3 g/kg in European feeds in order to improve gut health and reduce mortality (Mul, 1997; Mul and Perry 1994). Fewer rabbits exhibited

clinical symptoms of enteritis during *E. coli* induced diarrhoea following consumption of oligofructose unlike control animals (Morisse *et al.*, 1993). Moreover rabbits consuming prebiotic oligosaccharides have higher body weights with higher average daily gains. Lower level of oligofructose in the diet of rabbits is capable of reducing the gastrointestinal pH as a result of production of short chain fatty acids from fermentation of prebiotics.

Ruminant livestock

The foregut and hindgut houses millions of diverse groups of microflora namely bacteria, fungi, yeasts, phage particles, archaea etc with the exception that protozoa are supposed to be present only at foregut and absent at hindgut. Prebiotics are fermented by number of rumen bacteria for its utilization as source of energy (Cota and Whitefield 1998; Samanta *et al.*, 2012). The encouraging results of prebiotics on human health especially the gut microbial ecology have prompted ruminant researchers to explore its potentiality on different livestock species like cattle, buffalo, sheep etc. Ruminants are exposed to different kind of stress on different occasions like weaning, transportation, which adversely affect the health of the livestock, resulting in diarrhoea, off feed, depression of growth, impaired intestinal morphology etc (Fraser *et al.*, 1998; Nabuurs, 1998). Under such situations, ecological treatment through prebiotic may be potential alternatives to overcome the gut associated problems of livestock.

Presently frugal information is available on the effects of prebiotic in ruminant animals. All the rumen hemicellulolytic bacteria are capable to utilize xylooligosaccharides as growth substrate (Cota and Whitefield, 1998). These are *Butyrovibrio fibrisolvens*, *Eubacterium ruminantium*, *Ruminococcus albus* etc. The rumen pH remained unchanged (6.7), when prebiotic is given to Holstein cows maintained on orchard grass silage or alfalfa silage (Santoso *et al.*, 2003). In ruminant species, the above pH (6.6 to 6.8) is ideal for growth and multiplication of useful plant biomolecules degrading bacteria (Samanta *et al.*, 2003). Some of the researchers did not notice any significant changes of rumen pH in steers supplemented with prebiotics; however, they recorded significantly higher oxidation reduction potential (Mwenya *et al.*, 2004). The rumen ammonia nitrogen concentration was slightly lower in prebiotics supplemented Holstein cows and steers (Figure 3), which might be due to the utilization of ammonia for microbial protein synthesis in the rumen (Mwenya *et al.*, 2005; Santoso *et al.*, 2003). Lower rumen ammonia nitrogen concentration in sheep was also observed as a result of prebiotic administration, which may be due to the suppression of ammonia producing bacteria (Mwenya *et al.*, 2004). Dry matter intake, nutrient digestibility is as such not affected by prebiotic supplementation but showed higher nitrogen retention owing to increased microbial protein synthesis in rumen (Santoso

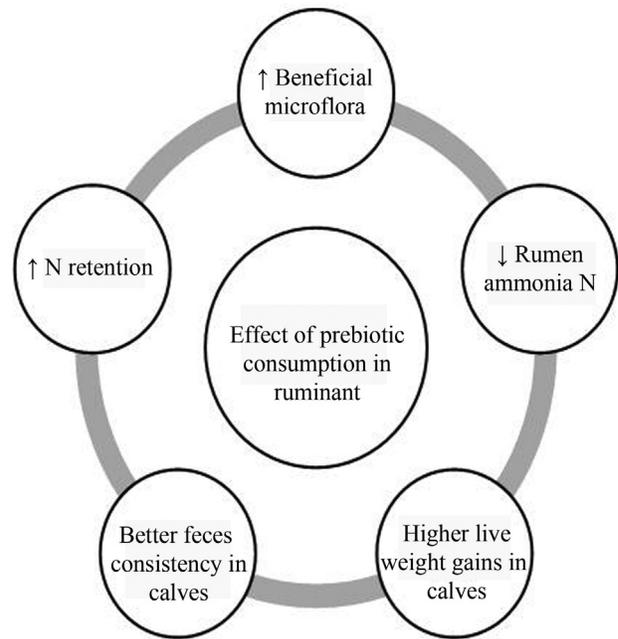


Figure 3 - Schematic diagram on effect of prebiotic consumption in ruminant.

et al., 2003). Inclusion of inulin in the milk replacer of pre-ruminant calves leads to significantly higher live weight gains, better feces consistency (Kaufhold *et al.*, 2000; Verdonk and Van Leeuwen, 2004). It is postulated that increase in body weight might be ascribed due to increased fermentation at the small intestine followed by increased flow of microbial nitrogen at large intestine, stable microflora composition at rumen, small and large intestine of calves (Verdonk *et al.*, 1998).

The fermentation of inulin is faster at pH 6.0 than at neutral pH by rumen inoculums obtained from sheep maintained on sole forage diets (Flickinger *et al.*, 2003). The diet of calves supplemented with oligofructose resulted in decreased population of fecal *E. coli* and total anaerobic microflora while Bifidobacteria population exhibited increasing trends (Bunce *et al.*, 1995a). This might be attributed by beneficial effects brought out through the consumption of prebiotics followed by their fermentation at hindgut of calves. Incorporation of oligofructose in the milk replacer of calves resulted in improved body weight gains, feed conversion efficiency with reduction in the incidence of diarrhoea and firmer feces (Mul, 1997). Incorporation of fructooligosaccharides at a concentration of 0.5% to 1% of total mixed ration (w/w) significantly improved the organic matter and dry matter digestibility of total mixed ration by virtue of modulation of rumen metabolic profile (Samanta *et al.*, 2012).

Mechanism of action of prebiotics

While forwarding the concept of prebiotic, Gibson and Roberfroid (1995) put the conditions for notifying a

compound as a prebiotic; it must be indigestible by gastrointestinal enzymes but categorically utilized by selective group of gut beneficial microflora. Unlike other functional foods, prebiotics exhibit multi-dimensional activities beginning from modulation of gut microflora to mineral absorption, pathogen exclusion etc (Roberfroid, 2002; Rycroft *et al.*, 2001; Samanta *et al.*, 2010). Therefore, to bring out its positive influence on the livestock system, the channel of activities is supposed to be widely distributed amongst several systems including gastrointestinal, immune regulatory, skeletal organs etc. From the literature it is evidenced that prebiotics are getting its niche for routine consumption for gut health as well as management of gastrointestinal disorders. Obviously most of the studies towards unfolding the mechanism of prebiotic actions are carried out in laboratory animals *i.e.* mice or rats with subsequent support through investigating under *in vitro* or human volunteers. Under such circumstances mechanism of prebiotic function are discussed under the following aspects.

Trophic effect

To elucidate the trophic action of oligofructose, piglets were raised with a diet ensuring regular consumption of 1.4 g oligofructose (Howard *et al.*, 1995). There was significant increase of caecum mucosal cell density (40.4 *vs.* 44.7 cells/crypt) along with significantly higher number of labeled cells (8.2 *vs.* 9.6 cells/crypt). Proximal colonic mucosal crypt depth, labeled cells, proliferation zone, labeling index were exhibited to increase as a result of oligofructose supplementation as compared to control piglets. Similarly, at the distal colon, intake of oligofructose permeate to have increased mucosal crypt depth, cell density, labeling index, number of labeled cells and proliferation zone. It seems consumption of prebiotic oligofructose enhances indices of epithelial cell proliferation throughout the full length of large intestine in piglets (Flickinger *et al.*, 2003). The direct trophic effect on colonic mucosa was later on substantiated on rat model by supplementing its diet with chicory inulin and oligofructose or pectin (Poldbeltsev *et al.*, 2006). There was increase in number of epithelial cells and its secretion capacities, length and width of colonic crypts followed by increased areas of nutrient absorption including micronutrients and minerals. Further studies in rat caecum and colonic mucosa noticed that 2.2 times increase in macroscopic areas, 2.4 times increase in tissue wall weight following intake of prebiotic containing FOS and inulin (Raschka and Deniel 2005). Significant increase in crypt depth ($p < 0.001$) and goblet cell numbers ($p < 0.001$) at caecum epithelium were recorded in male Sprawley Dawley rats while administering prebiotics (Mineo *et al.*, 2006). Studies on supplementation of yacon flour in the diet of male wistar rats showed increased number of bifurcating crypts for enlargement of absorbing surface in the large intestine of the animals (Lobo *et al.*, 2007). This

might be resulted from elevated epithelial cell proliferation and crypt fission (Mandir *et al.*, 2008).

By virtue of hydrolysis and utilization of prebiotic at lower gut of animals, a variable quantities of short chain fatty acids (acetate, butyrate and propionate) along with acid lactic are produced and subsequently reduces the lumen pH. Amongst those fatty acids, butyric acid is reported to possess strong trophic actions for stimulation of mucosal proliferation (Blottiere *et al.*, 2003). On the other hand lactic acid could stimulate mitosis in the rat caecum epithelium (Ichikawa and Sakata 1997). Above theory of trophic functions are further confirmed when germfree animals failed to exhibit trophic effects at large intestine following consumption of prebiotics; substantiating the microbial generation of fatty acids from prebiotics are utmost to demonstrate the above functionality. The permeability of paracellular pathway is dependent on tight junctions that linked between adjacent epithelial cells and large multi-protein complex (Steed and Macfarlane 2009). Application of non-digestible oligosaccharides in the caco-2 intestinal cell line model, there was rapid increase in paracellular ion transport because prebiotics may increase the permeability of paracellular pathways.

Mucus

The goblet cells present at the gut epithelial lining secretes cysteine rich glycoprotein (high molecular weight), known as mucus. The major function of the mucus is to form a slimy layer along the entire mucosal surface and often considered as first line of defense of the intestine. Although thickness of mucus varies along the length of the gastrointestinal tract, it is thickest at the distal part of the intestine. In spite of being a rich source of nitrogen and energy, very few gastrointestinal microfloras is capable to secrete necessary enzymes for the degradation of mucus. The bacterial flora plays a significant role in the distribution of different types of mucus. In the small intestine, concentration of neutral and sulphomucin is higher, whereas sialomucin is lower. Conversely, concentration of sialomucin is higher in the caecum and colonic mucosa, whereas concentration of sulphated mucin is decreased significantly in caecum and colonic mucosa (Meslin *et al.*, 1999). Administration of prebiotics caused thickening of mucus layer as well as increased secretion of goblet cells (Steed and Macfarlane, 2009).

Hydrolysis of phytic acid

Phosphorus is present in plants either in phytate or phytic acid form. Usually phytate or phytic acid of plant are subject to microbial degradation for further release of phosphorus in the reticulo-rumen of ruminant species unlike mono-gastric livestock. In the latter animals, phytate binds with minerals like Zn, Ca and Fe for making these unavailable for absorption. Similarly, phytic acid is also capable of reducing Fe absorption as well as its availability at gastroin-

testinal tract. Consumption of prebiotics is reported to show stimulatory effects on iron absorption by increasing soluble fractions of iron at large intestine. Addition of fructooligosaccharides restores zinc absorption by enhancing zinc bioavailability in human and rats. Supplementation of prebiotic in the diet of rats substantially reduces the phytate concentration of feces as it is hydrolyzed in greater concentration through increased and altered gut microflora. Short chain fatty acids produced by gut microflora forms soluble ligands with cation and prevents the formation of insoluble mineral phytate complex (Steed and Macfarlane, 2009).

Modulation of gut microflora

By virtue of its definition, prebiotics consumption leads to modulation of gut microflora composition towards higher growth and multiplication of beneficial category namely Bifidobacteria and Lactobacilli (Gibson *et al.*, 2004; Samanta *et al.*, 2011). These bacteria are often considered as friendly bacteria as they are capable of fermenting non-digestible carbohydrates, reduces gut pH, produces short chain fatty acids, help in competitive exclusion of pathogens, stimulates immunoglobulin production etc. Prebiotics are being fermented at large intestine into short chain fatty acids like acetate and butyrate; energy source for the host. Although Bifidobacteria is not able to produce butyrate through its fermentative metabolism but it stimulates other butyrate producing bacteria like Eubacteria, inhabited at the gastrointestinal tract (Belenguer *et al.*, 2006). The short chain fatty acids have multidimensional role including growth and cellular differentiation, colonic epithelial cell transport, lipid metabolism, carbohydrate metabolism etc. On the other hand, prebiotics are able to reduce the population of harmful gut microflora namely Clostridium, Bacteroides, Enterococcus, Enterobacteriaceae etc. Obviously concentration of toxic metabolites (ammonia, indoles, phenols, thiols, etc.) generated through action of those harmful bacteria are substantially reduced following consumption of prebiotics. Some of these toxic metabolites are also carcinogenic. Therefore prebiotic consumption reduces population of harmful bacteria in the gastrointestinal tract followed by reduced production of toxic microbial metabolites *vis a vis* reduced incidence of colorectal cancer. As each strain of beneficial gut microflora has specificity on substrate utilization, all the prebiotics do not equally stimulate the growth and multiplication of complete set of beneficial gut microflora. Prebiotic potentiality depends upon several factors namely degree of polymerization, composition, availability of other carbohydrates, quantity of consumption and so on. Consumption of inulin and fructooligosaccharides increased mucosal population of Bifidobacteria and Eubacteria (Langlands *et al.*, 2004). Similarly, under *in vitro* system, fructooligosaccharides derived from chicory roots were found to increase significant growth of *Lactobacillus plantarum* and *Enterococcus faecalis* (Samanta *et al.*, 2012). Recently, it has come to notice that prebiotics are also de-

graded by other gut commensal microflora such as *Faecalibacterium prausnitzii* and the end product of fermentation is butyrate (Ramirez-Farias *et al.*, 2009).

Immune modulation

By modulating the composition and functionality of microflora, dietary inclusion of prebiotics modulates the immune system and host defense system in addition to its potentiality to exclude the gut pathogens through competitive exclusion (Lomax and Calder, 2008). Mode of this action is still not clear and it is doubtful whether it is direct or indirect effect aroused from stimulation of immunomodulating microflora or production of short chain fatty acids. Short chain fatty acids stimulate the goblet cells for increasing secretion of mucin and decreasing translocation by binding to specific receptors like G-protein coupled receptor 41 (Gpr41) of immune cells located at the gut lymphoid associated tissue (GALT) (Brown *et al.*, 2003). Besides, there has been increased mucosal immunoglobulin production and altered cytokine formation in the spleen and intestinal mucosa (Schley and Field, 2002).

Lipid metabolism

Consumption of prebiotics is reported to lower the concentration of both cholesterol and triglycerides; indicating a greater significance for their lipidemia and cardiovascular benefits in both animals and human (Jackson *et al.*, 1987, 1999). Oligofructose supplementation in rats resulted in significant reduction of serum phospholipids and triglycerols especially very low density lipoprotein (VLDL). This is mediated through the reduced activities of hepatic lipogenic enzymes namely FAS, malic enzymes, ATP citrate lyase, acetyl-coA carboxylase, glucose -6 -phosphate 1-dehydrogenase, fatty acid synthase mRNA (Fiordaliso *et al.*, 1995; Kok *et al.*, 1996). It is suggested that prebiotics may modify gene expression of lipogenic enzymes. In rat maintained on high fat diet, prebiotics regulates the plasma free cholesterol levels; implicating its extra-hepatic regulation of lipid metabolism. It seems that prebiotic effect might link with insulin, which potentiates the gene expression effects. Consumption of prebiotics resulted in short chain fatty acids (acetate, propionate, butyrate etc.) production at gut lumen followed by their increased concentration. Propionate is found to inhibit synthesis of fatty acids *in vivo*. Butyrate is the preferable source of energy for enterocyte of gut epithelium. Following absorption of acetate, it enters into the hepatocyte for taking part in lipogenesis. Propionate competes with the protein that regulates acetate entry into the hepatocyte cells. Therefore prebiotic regulates the acetate and propionate ratio in the cells and exhibits its control over lipid metabolism.

Mineral metabolism

Prebiotic shows its positive influence on livestock by increasing absorption of Ca, Mg, Fe, Zn at gastrointestinal

tract and bone mineralization. Both in broilers and layers, inulin is found to exhibit positive effect on mineral balance especially Ca, P, Zn (Swiatkiewicz *et al.*, 2010; Ortiz *et al.*, 2009). Solubility of Ca is higher in acidic pH (Dupuis *et al.*, 1978). Production of short chain fatty acids by beneficial gut microflora from degradation of prebiotics reduces the luminal pH at lower part of large intestine and this might be the reason for higher Ca retention following prebiotic supplementation in broilers. Acidic pH brought out by fermentation of prebiotic at lower part of large intestine leads to increased solubility of Ca as well as increased gradient across the epithelium; which further promotes passive uptake of the element. However, some school of thought believed in divalent ion transport mechanism for prebiotic induced higher Ca retention (Raschka and Deniel, 2005). Addition of short chain fatty acids at rat mucosal epithelium increases the permeability and decrease the electrical resistance. Therefore, it is hypothesized that prebiotic interact with tight epithelial junctions and increase the permeability for minerals entry. The mechanism of the beneficial influence of inulin on mineral utilization is complex and might happened as a result of increased solubility of minerals owing to the increased production of short chain fatty acids from degradation of inulin. Additionally higher mineral absorption is attributed also by alteration of intestinal mucosa, increased absorption surface areas by means of beneficial effects of bacterial fermentation products on the proliferation of enterocytes, increased expression of calcium binding proteins, release of bone modulating factors, degradation of phytates and overall improvement of gut health (Swiatkiewicz *et al.*, 2010).

Another hypothesis for higher mineral absorption considers that colonic uptake of Ca take place along with uptake of short chain fatty acids. Moreover, prebiotic fermentation regulates the gene expression of proteins that are linked to sequestration and mucosal ion binding. It is noteworthy to say that chain length or type of branching of prebiotic fructan did not influence the degree of increased mineral absorption. Lactate pool of gut in combination with lumen pH significantly affects the absorption of Mg. Other school of thought endorsed the role of short chain fatty acids for increased Mg absorption through activation of $Mg^{2+}/2H^{+}$ antiport. Among the various short chain fatty acids, butyrate is the potent stimulant for the magnesium flux from the mucosal to serosal direction (Kashimura *et al.*, 1996).

Anti-carcinogenesis

The typical lifespan of livestock and birds does not offer enough scope for the development of colon cancer. However, in some instances other forms of tumor are often noticed in domestic animals. Research findings on gut ecology revealed production of several mutagenic products from the microbial fermentation of plant biomolecules in the gastrointestinal tract in addition to secretion of enzymes

responsible for conversion of pro-carcinogens to carcinogens. A few of the gut microbial metabolites possessing carcinogenic properties are nitrosamine, phenols, cresols, indole, skatole, estrogens, secondary bile acids, aglycones etc (Bingham, 1988; Flickinger *et al.*, 2003). Gut microflora actively participate in the formation of those carcinogenic metabolites are *E. coli*, *Clostridium* spp., *Bacteroides* spp., *Streptococcus fecalis*, *Proteus* spp. (Tomomatsu, 1994). The administration of prebiotics in the diet of animals modulate the gut microflora towards higher population of Bifidobacteria as well as lactobacilli, which in turn reduces the population of those harmful bacteria as well as creates a microenvironment unsuitable for growth and multiplication of harmful bacteria.

Conclusion

For several decades, antibiotics and chemotherapeutic agents in prophylactic dose were used in livestock feed to improve the productivity and to ensure higher economic returns. Following a ban over the application of antibiotics in the diet of livestock coupled with growing concerns of residues, livestock managers are now looking towards ecological feed additives. The application of prebiotics in the diets of livestock is a relatively recent endeavor and many issues are still unresolved even though the available data are promising. At the same time increasing consumer awareness for health and nutritional issues makes the emerging market for prebiotics very promising. However, till date the product cost of prebiotics is very high and beyond the reach of common livestock farmers. At the same time, there is urgent need of scientific research to establish the efficacy of prebiotics in routine diets of livestock and to develop cost effective processes for prebiotic production at industrial scale.

The gut of newly born livestock or newly hatched chick is sterile and colonization with diverse groups of microflora takes place following contact between newborn and its environment, mode of delivery/ hatching conditions, hygiene levels, medication, type of feeding etc. It has often been commented that more than 90% of the total cells of healthy individual belongs to bacterial cells, largely present in the gastrointestinal tract. In terms of perspectives, the future research on prebiotics as functional food ingredients relies on the following: (i) development of process for cost effective production of prebiotics, (ii) unraveling the mechanism of action and (iii) generation of scientific evidences for elucidating the prebiotic potentiality on animal systems especially the production quality and quantity. Prebiotics might be the prime choice from the basket of functional foods as it exhibits its significance through multifarious means. Future research should substantiate prebiotic effect on microflora with application of advanced techniques like next generation sequencing (NGS). Additionally future research of prebiotic application in livestock should consider immunological aspects,

livestock product quality, changes at gut epithelial tissues, therapeutic aspects with more emphasis on common gastrointestinal disorders.

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