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Prebiotics: The gut ecology modifiers

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Abstract
Antibiotic resistant microbes are a global public health concern in human or animals out of which gram negative bacteria pose greater threat due to presence of mobile genes present on plasmid. Moreover currently India does not have any prohibitory regulations regarding use of antimicrobials in livestock feed as growth promoters. These resistant microbes developed in animals find their way in humans through direct or indirect contact with animal or environment. Though European Union has phased out the antibiotic growth promoters in animal feed from 2006 and soon in due future we will be expecting it in India as well. This has paved a wave to find new alternatives sources which can replace antibiotics as growth promoters but without compromising the benefits which we get in terms of growth. Among many alternatives like probiotics, acidifiers' etc prebiotics have emerged as one of the most promising source with consistent results. In the review detailed description of prebiotic including its definition, methods of extraction and its effect *in vivo* are discussed in detail.

Keywords: Antibiotic, resistance, prebiotics

Introduction
Antibiotic resistance is a global public health threat, but nowhere is it as stark as in India. A decade ago, concern for resistance was centered on gram positive bacteria, like *Staphylococcus aureus* and *Enterococcus* spp. However, nowadays concern is more about multidrug resistant gram negative bacteria, posing greatest risk to public health. Antimicrobial resistance spreads faster in gram negative bacteria due to presence of mobile genes on plasmids which spread readily through bacterial populations, besides there are fewer developmental antibiotics active against them. Widespread resistance may hold more consequence for India than for other countries because of India's high bacterial disease burden. At present, India have no prohibitory regulations regarding use of antimicrobials in livestock feed. Europian Union phased out the antibiotic growth promoters in the feed from 2005 and it is not too early to speculate that a ban on antibiotic growth promoters is imminent in India as well. Organization for Economic Co-operation and Development report indicates that the costs of withdrawing antimicrobial growth promoters in India would be roughly US$ 1.1 billion [1].
In view of severe restriction of total ban on the use of antibiotics as growth promoters and therapeutic agents in animal feed, the search for alternatives to replace antibiotics has gained increasing interest in animal nutrition. As a result, last decade has seen intensive search for alternatives to antibiotic growth promoters such as probiotics, prebiotics, symbiotic, organic acids, herbal drugs, plant extracts, phytobiotics and antimicrobial peptides. Out of these possible alternatives, prebiotics have emerged as a promising alternative to antimicrobial growth promoters with consistent results.

What is a Prebiotic
Prebiotic may be defined a substrate that is selectively utilized by host microorganisms conferring a health benefit [2]. Prebiotic is defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of colonic bacteria [3]. For a dietary substrate to be classed as a prebiotic, at least three criteria are required:
(1) The substrate must not be hydrolyzed or absorbed in the stomach or small intestine,
(2) It must be selective for beneficial commensal bacteria in the large intestine such as the lactobacillus, bifidobacteria etc.
(3) Fermentation of the substrate should induce beneficial luminal/systemic effects within the host.
The mechanism by which prebiotics exert these features is increase in the number of

lactobacillus in the gut which may aid in competitive exclusion of pathogens from the gastrointestinal tract. The increased production of SCFAs with administration of prebiotics results in increased intestinal acidity also contributes to the suppression of pathogens in the gut. Prebiotics have also been reported to enhance the immune response, resulting in rapid clearance of pathogens from the gut due to direct interaction between prebiotics and gut immune cells.

Commercially available prebiotics are fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS), xylo-oligosaccharides (XOS), oligofroctose, inulin, mannitol, galacto-oligosaccharides (GOS), trans galacto oligosaccharides (TOS), lactulose, lactitol, gluco-oligosaccharides, glycol- oligosaccharides, maltodextrin, polydextrose, stachyose, raffinose, sorbitol, oligochitosan isomalto oligosaccharides (IMO), malto-oligosaccharides, arabinoxylooligosaccharides (AXOS) and neoagaro-oligosaccharides [4]. FOS are linear polymers of β-(2-1)-linked fructosyl units, terminated by one glucose residue and are not digested in the upper gut of avian species. Inulin is the longer chain version of FOS. MOS are mannose-based oligomers linked together by β-1,4 glycosidic bonds, found in cell wall of *Saccharomyces* yeast.

Mannanolig-osaccharides contain protein which has relatively high proportion of serine, threonine, aspartic and glutamic acids and a paucity of methionine. MOS adsorbs bacteria containing type-1 fimbriae inhibiting them from binding to the carbohydrate moieties of the intestinal lining or by agglutination with type-1 fimbriae to aggregate or clump [5], bringing them out of the solution. Xylooligosaccharides are oligomers consisting of xylose units linked through β-(1-4) linkages.

Methods of extraction

There are three main manufacturing processes for prebiotic from plants i.e direct extraction from plants; controlled enzymatic hydrolysis of high-DP polysaccharides to lower DP oligosaccharides and enzymatic-catalyzed synthesis via microbial action on simple sugars. However, the production of prebiotic from carbohydrates is summarized in the table below:

Oligosaccharides	Structure	Linkages	Process	Origin
Xylo oligosaccharides	(Glu)n	-1,4	Hydrolysis	Cereals
Lactulose	Gal-Fru	-1,4	Isomerization	Lactose
Isomalto-oligosaccharides	(Glu)n	-1,6	Hydrolysis	Algae
Gluco-oligosaccharides	(Glu)n	-1,2 and "-1,6	Synthesis	Sucrose
Galacto-oligosaccharides	(Gal)n-Glu	-1,2 and "-1,6	Synthesis	Lactose
Fructo-oligosaccharides	(Fru)n-Glu	(-2,1)-"-1,2	Synthesis	Sucrose
Oligofructose	(Fru) n-(Fru)n-Glu	(-2,1)	Hydrolysis	Inulin

Biological role of prebiotics

There are several beneficial effects of prebiotics on host health like optimization of colonic functions as well as metabolism such as increase or change in the composition of short chain fatty acids, increased mineral absorption, immune stimulation and decreased colonic pH, nitrogenous end products, pro carcinogenic enzymes.

Voluntary food intake, satiety and growth performance

The satietogenic effect of prebiotics is due to overproduction of anorexigenic gut peptide (glucagon like peptide-1) and decline inorexigenic peptide (ghrelin). These entero-endocrine derived peptides control the miscellaneous metabolic and physiological processes thereby creating a link between the gut and the brain. The intake of various organic and inorganic nutrients were reported to increase ($p<0.01$) in dogs upon supplementation with 1% MOS [6]. However, addition of FOS to milk replacer improved Average Daily Gain (ADG) in veal calves of 10 weeks of age [7] and in broilers. There are reports that relative weight of breast and thigh to body weight were significantly ($p<0.01$) higher in *Aspergillus oryzae* fed broilers as compared to control. Increased carcass weight and decreased abdominal fat weight were observed with MOS and inulin supplementation. Supplementation of prebiotics improved body weight, feed intake, FCR and decreased numbers of *E. coli* and cecal pH [8]. In fish increased growth and reduced FCR were associated with longer intestine and modified intestinal fermentation.

Antioxidant activity

Lactobacillus GG was found to scavenge superoxide anion radicals, inhibit lipid peroxidation and chelate Fe *in vitro*. Fe-chelating property may account for antioxidant activity. Other LAB including strains of L. acidophilus, L. bulgaricus, B. longum and Str. Thermophilus have demonstrated antioxidative ability. Mechanisms include chelation of metal ions (Fe, Cu), scavanging of reactive oxygen radicals and reducing activity.

Nutrient bio-availability

Protein digestibility is negatively impacted by prebiotics consumption. Decreased protein metabolism and increased intestinal fermentation lead to more N being fixed with bacterial biomass (faecal N) which relieves the N burden on kidney. This suggests an increased faecal mass shifting the excretion of blood urea from urine to faeces. These bacteria also convert neutral ammonia to charged NH_4 thus blocks its absorption. So, it is excreted through faeces than through kidney.

Prebiotics increase the production of Short Chain Fatty Acids (SCFA) which decreases the pH of lumen, thereby increasing the solubilisation of minerals. Butyrate acts as an energy source for intestinal epithelial cells and improves their absorptive capacity. Both butyrate and polyamines help in increasing cell proliferation. Other researchers have observed an increased capacity of calcium transporters (calbindin) in the colon [9]. Generally, prebiotics increase the absorption of Ca, Mg, Fe, Zn and Cu. They also increase the absorption of Na^+ and colonic water. Iron bioavailability in corn and soybean meal was increased due to prebiotic supplementation [10]. Fructans prevents bone loss and improves bone formation. In poultry, prebiotic supplementation leads to increased feed efficiency, mineralization of tibia and egg production due to increased skeletal and plasma calcium level, egg shell strength and decreased yolk cholesterol concentration without affecting yolk weight. In addition to inulin, FOS and GOS; resistant starches and sugar alcohols have also been shown to increase mineral absorption and bone mineral content. It was observed that prebiotics stimulated Fe absorption and of bone-relevant minerals such as Ca, Mg and Zn in short-term

experiments and improved bone mineral concentration in long-term studies in rats.

Lipid profile

The SCFA like acetate and propionate play important role in lipid metabolism. They enter the portal blood stream where they are utilized by the liver. Acetate is converted to acetyl-CoA in the liver and acts as a lipogenic substrate for *de novo* lipogenesis, whereas propionate inhibits lipid synthesis. Prebiotics decrease Low Density Lipoprotein (LDL) cholesterol, triglycerides and total cholesterol by decreasing the activity of all lipogenic enzymes like acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, ATP citrate lyase and glucose-6-phosphate dehydrogenase. They also decrease Very Low Density Lipoprotein (VLDL) cholesterol secretion

from liver and increase GIP secretion which increases activity of lipoprotein lipase. They increase bile acid deconjugation thereby increasing bile acid excretion and reducing cholesterol. The cholesterol content of the yolk was reduced in the prebiotic-fed laying hens. Serum cholesterol levels and fat tissue deposits were significantly reduced in broilers. FOS decreases *de novo* synthesis of triglycerides by liver. Inulin can also modulate insulin-induced inhibition of triglyceride synthesis. Supplementation of MOS reduced plasma triglyceride of dogs [11].

Glucose homeostasis

Prebiotics have a clinically relevant benefit in terms of improving metabolic control of blood glucose concentration particularly in hyperglycaemic animals and it is unlikely to have a positive effect in normoglycemic animals. In hyperglycaemic animals, prebiotics particularly fructans increase gut hormones like glucose dependent insulinotropic polypeptide (GIP) from small intestine and Glucagon Like Peptide-1 (GLP-1) from terminal ileum and colon which increase the insulin concentration. Moreover, fructans are converted to fructose rather than glucose so can be well tolerated by diabetic patients. In veal calves, FOS increased insulin concentrations and decrease post-prandial glucose concentrations. The plasma glucose levels reduced ($p<0.05$) in rats due to supplementation of pulverized JA tuber powder [10]. The XOS have the potential of improving glucose tolerance and suggested mechanisms are improved insulin sensitivity and increased gut fermentation.

Anti-carcinogenic activity

It is due to immune-modulation by the action of *Bifidobacterium* sp. and *Lactobacillus* sp. which induces the production of cytokines, interferon-γ and interferon-1. Prebiotics like XOX, FOS, GOS, MOS etc have established effect to selectively supplement the growth of these bacteria *in vivo*. Among SCFA produced by prebiotic fermentation, butyrate has the highest anti-carcinogenic activity and it prevents cell differentiation. The growth and proliferation of tumour cells depends on glucose availability butinulin type fructans decreases the serum glucose level and might deprive cancer cells of their essential substrate [11]. Butyrate induces the glutathione-S-transferase enzyme which decreases the expression of Deoxycholic acid causes cyclo-oxygenase-2 (COX-2) expression and induces DNA damage COX-2 in primary colon cells, promoters of colon cancer. Inulin-type fructans have been shown to increase caecal butyrate concentration, decrease ACF and reduce tumour incidence.

Gut health

Fermentative attributes: Prebiotics are fermented in the hindgut by beneficial bacteria resulting in the production of organic acids (lactic acid and SCFA like acetate, propionate and butyrate) and gases (CO_2, CH_4 and H_2). The former are extensively absorbed and this presents a process by which the host recovers a part of the chemical energy from these non-viable carbohydrates. Acid production causes the protonation of potentially toxic NH_3 (and amines) to produce NH_4+. This NH_4+ is non-diffusible and so decreases the blood NH_3 level. Decrease in the caecal pH due to lactic acid has been shown to favour the growth of *Lactobacillus* and *Bifidobacterium*. These bacteria are capable of using NH_3 as their N source and decrease its concentration both in the intestinal contents and in the blood. Supplementation of MOS in dogs did not show any significant impact on fecal pH and NH_3 concentration but faecal lactate, propionate and butyrate concentrations tended to increase [6].

Microbiological attributes: Prebiotics selectively stimulate the growth of *Bifidobacterium* sp., *Lactobacillus* sp. and certain butyrate producing bacteria. At the same time, they suppress the growth of toxogenic *E. coli* and proteolytic bacteria like *Clostridium perfringens*, *Streptococcus* sp., peptococci, bacilli, *Staphylococcus* sp., bacteriodaeceae, pseudomonad, yeast and mould [12]. In poultry, *Salmonella* sp. and *Campylobacter* sp. counts are decreased. The symbiotic microbiota inhibits pathogens through a variety of mechanisms which depends upon the microbial species and the ecosystem within which they are residing. Possible mechanisms are competitive exclusion and colonization resistance, production of toxic metabolites or antimicrobials (low pH, fermentation acids, bacteriocins, etc.) against pathogenic bacteria, competitions for nutrients and for receptors on epithelial surfaces, stimulation of the immune system and increased osmotic value in the intestinal lumen.

Addition of MOS reduced faecal *E. coli* with an associated elevation in *Lactobacillus* sp. counts [13]. The *Lactobacillus* sp. and *Bifidobacterium* sp. counts were increased significantly ($p<0.05$) in caecal digesta of JA-fed rats indicating better fermentation in cecum. Similarly, the faecal counts of *Bifidobacterium* sp. and *Lactobacillus* sp. were significantly higher in JA-fed dogs but that of faecal *E. coli* and *Clostridium* sp. counts were not altered. In growing pigs, TOS increased significantly the faecal *Bifidobacterium* sp. and *Lactobacillus* sp. counts. Use of Bio-MOS in Vencob broilers improved carcass quality by reducing liver weight, heart weight and abdominal fat percentage besides reducing the *E. coli* count. GOS showed a significant increase in the intestinal *Bifidobacterium* sp. population. Concentrations of intestinal and faecal yeast increased in response to FOS and TOS supplementation. Herfel [14] observed increased ileal *Lactobacillus* sp. population, enhanced propionic and lactic acid concentrations and decreased pH while evaluating the prebiotic effect of polydextrose enrichment in cow milk-based infant formula using 1 day old piglets for 18 days. Cellobiose 2-epimerase from *Ruminococcus albus* effectively converts lactose to epilactose. Dietary supplementation with epilactose increases cecal contents, decreases its pH, enhances *Lactobacillus* sp. and *Bifidobacterium* sp. populations and suppresses *Clostridium* sp.

Intestinal architecture: Prebiotics alter favourably the morphology, structure and functions of the intestinal mucosa i.e., villous height, crypt depth, crypt density, epithelium

thickness, epithelial cell count, mitotic cell count, mucin-containing cell count and goblet cell count. These alterations are indicative of improved nutrient absorption and utilization. Several possible mechanisms have been proposed to explain these changes. Enteroglucagon might be responsible for the stimulation of crypt cell proliferation of small intestine and its level in the blood is affected by fermentable carbohydrates [15]. It was suggested that polyamines may be synthesized from prebiotics which is essential for small intestinal and colonic mucosal growth and development. The high digestive enzyme activities possibly caused the well growth and high turnover rate of intestinal mucosa in rats fed with prebiotics along with probiotics. The SCFA may also stimulate large and small intestinal cell proliferation and consequently may increase brush border digestion and nutrient absorption through increased villus height and crypt depth. Another mechanism may be the acidification of the cells induced by the fermentation of prebiotics. To recover the intracellular pH, multiple processes like Na+/H+ exchange and H+/SCFA co-transport induce cell swelling.

Pigs fed 0.25% FOS had longer villi (24%) and increased ratio of villous height: crypt depth in the proximal small intestine than control [16]. Distalcolonic crypt depth was greatest ($p<0.05$) in rats fed FOS, intermediate in those fed gum arabic and smallest in those fed XOS. Feeding inulin or FOS to broilers resulted in significantly improved zoo-technical performance because of significant increase in absorptive capacity due to increased gut length, villous height, crypt depth, villous height: crypt depth, microvillus height and numbers of goblet cells per villus.

Immunity: The effect of prebiotics on immunological function is not fully understood. Possible mechanisms underlying the immune-modulating effects may include: (a) Direct contact of lactic acid bacteria or bacterial products with immune cells in the intestine, (b) Production of SCFA from fermentation which increase T-cell numbers in the gastro-intestinal system and (c) Modulation of mucin production through increase in the number of goblet cells [17]. Prebiotics can modulate the type and function of cells from Gut Associated Lymphoid Tissue (GALT) especially payer's patches, secondary lymphoid tissues and peripheral circulation. Increase in *Bifidobacterium* sp. is associated with increased IgA levels in the small intestine.

Daily supplementation of MOS to the diet of buffalo calves improved their immune status by increasing ($p<0.01$) total protein, total immunoglobulins, circulating immune complexes and globulins. In dry dairy cows, MOS supplementation increased serum rotavirus neutralization titres, protein and IgA concentrations. Cell death markers, mast cells, eosinophils, IgA and IgG decreased in the lamina propria in inulin-fed pigs [18]. Under a *Salmonella typhimurium* challenge, puppies consuming either FOS or inulin experienced decreased enterocyte sloughing and maintenance of Na+-dependent glucose transport. When fed to primiparous beagles, FOS increased IgM concentrations in colostrum, milk and blood. Dietary supplementation of MOS or chicory has been shown to have a positive influence on humoral immunity and immunoglobulin status in dogs. Enhanced cell-mediated immunity in terms of delayed type hypersensitivity response to intra-dermal inoculation of phytohaemagglutinin-p and population of CD4+ lymphocyte subsets was observed due to MOS or JA supplementation. Torrecillas [19] reported dietary incorporation of 0.4% MOS activated sea bass immune system and increased its resistance to a bacterial infection directly inoculated in the gut. Many studies have observed immune-modulatory effect of GOS, FOS and pectin-derived acidic oligosaccharides during the early phase of a murine model.

Conclusion

Withdrawal of antibiotics from food animals created need for alternative solutions which would influence improvement of health and production. Prebiotics effect the gut micro flora and interact with digestive physiology improving growth in many complex ways, which can be further influenced or even determined by many other factors such as the compatibility between the diet, hygiene standards and animal husbandry practices. There possibly remain many questions to be answered or barriers to be overcome so that the alternatives can be applied more successfully in the industry in future. Further studies should be focus on evaluation of impact of prebiotics on tissue level (host tissues and microbial community) and intestinal digestive process which will improve the utilization of these compounds. Nutrigenomics and metabolomics approaches may help elucidating the mechanisms of prebiotics and interaction between the host and microbiome.

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