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Asian Journal of Animal and Veterinary Advances



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Prebiotics: An Emerging Nutritional Approach for Improving Gut Health of Livestock and Poultry

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ABSTRACT

The complex interactions between diet, intestinal mucosa and intestinal microbiota affect health status in livestock and poultry. Various approaches have been tried to exploit this relationship for achieving maximum health benefits and supplementation of prebiotics constitutes one of them. Now a days prebiotics have become news mainly as an alternative to probiotics, which are difficult to handle in foodstuffs, but whose benefits to gut health are well established. Commercially available prebiotics are mostly inulin, fructo-oligosaccharides, mannan-oligosaccharides, galacto-oligosaccharides, transgalacto-oligosaccharides, lactulose and oligochitosan, etc. Prebiotics selectively stimulate the growth of *Bifidobacterium* sp., *Lactobacillus* sp. and certain butyrate producing bacteria and suppress the growth of toxogenic *Escherichia coli*, *Clostridium perfringens*, *Streptococcus* sp., peptococci, bacilli, *Staphylococcus* sp., *Salmonella enteritidis*, *Campylobacter* sp., bacteriodaeceae, pseudomonad, yeast and mould. Dietary supplementation of prebiotics improve a plethora of gut health attributes like hindgut fermentation, gut mucosal integrity, lipid and glucose homeostasis, mineral bio-availability and immune response in livestock and poultry owing to which many feed and pharma industries have been established. Their inclusion in diet can also suppress boar taint, control faecal odour, prevent colon cancer and reduce infestation of several parasites such as *Ascaris* sp., *Trichuris* sp. and *Oesophagostomum dentatum*. Their satietogenic effect results from decline in ghrelin secretion which in turn regulates body weight gain in obese animals. Prebiotics may eventually replace the role of antibiotics as growth stimulants in fishery, poultry and animal husbandry sectors. Because of all these multitude of benefits, inclusion of prebiotics in basal diet should be taken into consideration while preparing formula feed for livestock and poultry.

Key words: Prebiotics, microbiota, hindgut fermentation, gut health, colon cancer, parasitic infestation

INTRODUCTION

Prebiotics, earlier defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of colonic bacteria (Gibson and Roberfroid, 1995) have now been defined as selectively fermented food ingredients that improve host health by targeting indigenous components thought to be positive (Walton *et al.*, 2013). The concept of gut health is complex and broadly has three major components, namely; diet, intestinal mucosa and intestinal microbiota. By definition, a prebiotic substrate is available to some groups of beneficial

bacteria e.g., bifidogenic bacteria (*Bifidobacterium* sp.), lactic acid bacteria (*Lactobacillus* sp.) and less available to potentially pathogenic bacteria such as toxin producing *Clostridium* sp., proteolytic bacteroides and toxogenic *Escherichia coli* (Manning and Gibson, 2004). The use of prebiotics is a promising approach for enhancing the role of endogenous beneficial microbiota in the gut. They can be used as potential alternatives to growth promoting antibiotics. For several decades, antibiotics and chemotherapeutics in prophylactic doses have been used in livestock and poultry feed to improve animal welfare and to obtain economic benefits in terms of improved performance and reduced medication costs. However, there are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistances in pathogenic bacteria in both humans and livestock linked to the therapeutic and sub-therapeutic use of antibiotics in livestock and poultry (Hajati and Rezaei, 2010).

SOURCE OF PREBIOTICS

Commercially available prebiotics are fructo-oligosaccharides (FOS), oligofructose, inulin, mannan-oligosaccharides (MOS), mannitol, galacto-oligosaccharides (GOS), transgalacto-oligosaccharides (TOS), lactulose, lactitol, gluco-oligosaccharides, glycol-oligosaccharides, maltodextrin, polydextrose, stachyose, raffinose, sorbitol, oligochitosan isomalto oligosaccharides (IMO), malto-oligosaccharides, arabinoxylo oligosaccharides (AXOS), xylo-oligosaccharides (XOS) and neoagaro-oligosaccharides (NAOS), etc (Femia *et al.*, 2010; Patel and Goyal, 2012). Lactose has prebiotic effect in chickens (Hajati and Rezaei, 2010). Inulin-rich chicory, burdock and dahlia (Milani *et al.*, 2011; Zubaidah and Akhadiana, 2013), FOS-rich Jerusalem Artichoke (JA) (Samal *et al.*, 2015) and yacon (Scheid *et al.*, 2014), fructan-rich agave (Garcia-Vieyra *et al.*, 2014), AXOS and XOS-rich wheat bran (Boll *et al.*, 2015), diosgenin-rich yam (Huang *et al.*, 2012) and resistant starch-rich whole grains (Birt *et al.*, 2013) are novel sources of prebiotics. Pectic oligosaccharides of bergamot peel (Mandalari *et al.*, 2006), lupin kernel fibre (Fechner *et al.*, 2013), MOS of yeast cell wall and palm kernel (Navidshad *et al.*, 2015), β -glucans of oat (Daou and Zhang, 2012) and mushrooms i.e., *Pleurotus ostreatus* and *P. eryngii* (Oloke and Adebayo, 2015), fibres and glycosylated anthocyanins of blueberry (Duenas *et al.*, 2015), inulin-type fructans of Indian mulberry (Patel and Goyal, 2012), oligosaccharides of white and red-flesh pitayas/dragon fruit (Rohin *et al.*, 2014), glycated pea proteins (Patel and Goyal, 2012), phenolic compounds of green tea (Cardona *et al.*, 2013), polysaccharides of seaweeds and agar (Ramnani *et al.*, 2012), sulphated heteropolysaccharide (ulvan) of green algae *Ulva rigida* (Patel and Goyal, 2012), D-fagomine sugar of buckwheat (Prestamo *et al.*, 2003), gum of flaxseed and fenugreek (Patel and Goyal, 2012) are also suggested to be valuable prebiotics.

CRITERIA FOR CLASSIFYING A SUBSTANCE AS PREBIOTIC

Prebiotic supplementation has become increasingly popular as the body of evidence supporting its use continues to grow. Based on ideal characteristics, a prebiotic should (i) Be neither hydrolyzed nor absorbed by host enzymes or tissues, (ii) Selectively increase the survival rate of probiotic strain(s) Commensal to caecum/colon by providing non-digestible fibres as substrates, (iii) Beneficially alter the intestinal microbiota and their activities, (iv) Improve luminal or systemic aspects of host defence system and (v) Have overall positive influence on hindgut health or well-being of the host (Wang, 2009).

FUNCTIONAL PROPERTIES

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 in

orexigenic peptide (ghrelin). These entero-endocrine derived peptides control the miscellaneous metabolic and physiological processes thereby creating a link between the gut and the brain (Wynne *et al.*, 2005). Dogs readily ingested the diet when supplemented with fructan compared to the control (Propst *et al.*, 2003). However, supplementation of 3% inulin in dog diet did not affect food and water intake (Verlinden *et al.*, 2006). But in another study, dogs fed FOS tended ($p = 0.13$) to have lower food intake than dogs fed sucrose (Swanson *et al.*, 2002). The intake of various organic and inorganic nutrients were reported to increase ($p < 0.01$) in dogs upon supplementation with 1% MOS (Kore *et al.*, 2009). In contrast, no influence on intake of nutrients and growth were apparent in dogs when supplemented with 1.5% MOS (Pawar *et al.*, 2008) or 3% JA (Samal *et al.*, 2012) in dogs. However, addition of FOS to milk replacer improved Average Daily Gain (ADG) in veal calves of 10 weeks of age (Kaufhold *et al.*, 2000).

Xu *et al.* (2003) found a dose-dependent effect of FOS on ADG in broilers; whereas, Juskiwicz *et al.* (2006) reported no impact on the performance or productivity of turkeys. However, on MOS supplementation, Sims *et al.* (2004) reported an improvement in live weight in turkeys. Piray *et al.* (2007) reported that relative weight of breast and thigh to body weight were significantly ($p < 0.01$) higher in Fermacto[®] (*Aspergillus oryzae* meal) fed broilers as compared to control. The NFOS improved broiler's gain by about 5-8% and Feed Conversion Ratio (FCR) by 2-6% (Yang *et al.*, 2009). Increased carcass weight and abdominal fat weight were observed with MOS and inulin supplementation (Samarasinghe *et al.*, 2003; Yusrizal and Chen, 2003).

Supplementation of whey powder (lactose 75%) improved body weight, feed intake and FCR and decreased numbers of *E. coli* and cecal pH (Radfar and Farhoomand, 2008). Refstie *et al.* (2006) found that Atlantic salmon, supplemented with 7.5% inulin had increased relative mass of the gastrointestinal tract, but the absorptive capacity of the fish was not affected. In fish like carnivorous turbot, herbivorous sturgeon and omnivorous catfish, increased growth and reduced FCR were associated with longer intestine and modified intestinal fermentation (Mahious *et al.*, 2006). Prebiotic XOS enhanced the growth performance and digestive enzyme activities of the allogynogenetic crucian carp, *Carassius auratus gibelio* (Xu *et al.*, 2009).

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. For young chicks, amino acid digestibility and metabolizable energy decreased in response to prebiotic supplementation (Biggs *et al.*, 2007) whereas fibre digestibility was improved ($p < 0.05$) due to MOS (Pawar *et al.*, 2008; Kore *et al.*, 2009) and JA (Samal *et al.*, 2012) supplementation.

Prebiotics increase the production of Short Chain Fatty Acids (SCFA) which decrease 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 (Ohta *et al.*, 1998). 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 (Yasuda *et al.*, 2006). Agave fructans prevents bone loss and improves bone formation (Garcia-Vieyra *et al.*, 2014).

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 (Coudray *et al.*, 2003). It was observed that prebiotics stimulated Fe absorption (Samal *et al.*, 2015) 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 (Cashman, 2003). The absorption efficiency is better when the demand for calcium is high, i.e., in intact animals, in the rapid growing stage and in animals with impaired calcium absorption as a result of ovariectomy or gastrectomy (Scholz-Ahrens *et al.*, 2007).

Alleviation of constipation and diarrhoea: All carbohydrates that reach the hindgut have a laxative effect. The mechanism works via stimulation of microbial growth, increased bacterial cell mass and stimulation of peristalsis by the increased gut content. It has been shown that prebiotics that are fermented completely increase bowel frequency (Den Hond *et al.*, 2000) bringing relief from constipation. Soluble fibres have been shown to increase faecal bulk and faecal N. Therefore, prebiotics, which are readily fermentable substrates could be beneficial for the host animal.

Before weaning, dairy calves are mostly susceptible to diarrhoea. When given prebiotic supplement, Holstein calves showed lower faecal score (Nargeskhani *et al.*, 2010) improved rate of recovery from diet-induced scours and enhanced *Lactobacillus* sp. in their faeces (Heinrichs *et al.*, 2009). Antibiotics in milk replacers can be replaced with FOS and probiotics to obtain similar calf performance (Donovan *et al.*, 2002).

INTESTINAL 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 (CO₂, CH₄ and H₂). 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 NH₃ (and amines) to produce NH₄⁺. This NH₄⁺ is non-diffusible and so decreases the blood NH₃ 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 NH₃ 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 (Pawar *et al.*, 2008; Kore *et al.*, 2009) and NH₃ concentration but faecal lactate, propionate and butyrate concentrations tended to increase (Kore *et al.*, 2009). Similarly, there was a significant increase (p<0.05) in the faecal lactate and SCFA contents accompanying a reduction in the faecal NH₃ content in JA-supplemented dogs (Samal *et al.*, 2012). However, Zentek *et al.* (2002) found an increased faecal NH₃ concentration after lactulose supplementation.

Microbiological attributes: Prebiotics selectively stimulate the growth of *Bifidobacterium* sp., *Lactobacillus* sp. and certain butyrate producing bacteria (Prestamo *et al.*, 2003; Huang *et al.*, 2012; Ramnani *et al.*, 2012; Cardona *et al.*, 2013; Fechner *et al.*, 2013; Duenas *et al.*, 2015). At the same time, they suppress the growth of toxigenic *E. coli* and proteolytic bacteria like *Clostridium perfringens*, *Streptococcus* sp., peptococci, bacilli, *Staphylococcus* sp., bacteriodaeceae, pseudomonad, yeast and mould (Samarasinghe *et al.*, 2003; Cao *et al.*, 2005; Rohin *et al.*, 2014;

Navidshad *et al.*, 2015). In poultry, *Salmonella* sp. and *Campylobacter* sp. counts are decreased (Yusrizal and Chen, 2003). The symbiotic microbiota inhibit 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 (Pawar *et al.*, 2008; Kore *et al.*, 2009). 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 (Samal *et al.*, 2015). 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 (Samal *et al.*, 2012). In growing pigs, TOS increased significantly the faecal *Bifidobacterium* sp. and *Lactobacillus* sp. counts (Smiricky-Tjardes *et al.*, 2003). Modesto *et al.* (2009) reported that sugar beet FOS at 4% level tended to increase the endogenous *Bifidobacterium* sp. The intestinal Total Viable Count (TVC) and *E. coli* were lower ($p < 0.01$) in birds receiving diet containing MOS, whereas *Staphylococcus* sp., yeast and mould counts remained unaltered. The TVC in breast and thigh meat was also low ($p < 0.05$) in broilers fed diets containing 0.2% MOS (Elangovan *et al.*, 2005). 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 (Munj *et al.*, 2010). Kleessen *et al.* (2003) described decreased *C. perfringens* number and a reduction in bacterial endotoxin levels by adding 0.5% JA syrup in drinking water of broilers. Thitaram *et al.* (2005), with different amounts of IMO, showed increased *Bifidobacterium* sp. and significant 2-log reduction in the level of inoculated *S. enterica* serovar typhimurium present in the ceca of young broiler chickens. Likewise, GOS showed a significant increase in the intestinal *Bifidobacterium* sp. population (Jung *et al.*, 2008). Concentrations of intestinal and faecal yeast increased in response to FOS and TOS supplementation (Mikkelsen *et al.*, 2003). Herfel *et al.* (2011) 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. and bacteroides in Wistar-ST rats (Watanabe *et al.*, 2008). Treatment of chicken macrophage cell line with β -1, 4-mannobiose increased dose-dependently both phagocytic activity and *Salmonella*-killing activity of macrophages *in vivo* in chickens (Ibuki *et al.*, 2011). Brown *Ascophyllum nodosum* algae showed prebiotic potential in weaned piglet feed material for improving the gut microbiota (Dierick *et al.*, 2010). Huang *et al.* (2012) investigated the effect of diosgenin of yam on the growth of enteric *Lactobacillus* sp. Oral administration of diosgenin on murine model restored the density of faecal *Lactobacillus* sp. associated with food allergic reactions.

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 (Gee *et al.*, 1996). Delzenne *et al.* (2000) 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 (Yang *et al.*, 2005). 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 (Shim, 2005). Howard *et al.* (1995) observed that distal colonic 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 (Yusrizal and Chen, 2003), villous height, crypt depth (Rehman *et al.*, 2007), villous height: crypt depth (Zikic *et al.*, 2008), microvillus height and numbers of goblet cells per villus (Xu *et al.*, 2003). Caecal crypt depth was significantly increased in JA-fed rats (Samal *et al.*, 2015).

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 (Schley and Field, 2002). Prebiotics can modulate the type and function of cells from Gut Associated Lymphoid Tissue (GALT) especially Peyer'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 γ -globulins (Reema *et al.*, 2005). In dry dairy cows, MOS supplementation increased serum rotavirus neutralization titres, protein and IgA concentrations (Franklin *et al.*, 2005). Cell death markers, mast cells, eosinophils, IgA and IgG decreased in the lamina propria in inulin-fed pigs (Krag *et al.*, 2006). Under a *Salmonella typhimurium* challenge, puppies consuming either FOS or inulin experienced decreased enterocyte sloughing and maintenance of Na^+ -dependent glucose transport (Apanavicius *et al.*, 2007). When fed to primiparous beagles, FOS increased IgM concentrations in colostrum, milk and blood (Adogony *et al.*, 2007). Dietary supplementation of MOS (Pawar *et al.*, 2008) or chicory (Kore *et al.*, 2009) 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 (Pawar *et al.*, 2008) or JA supplementation (Samal *et al.*, 2012). In trials with Jian carp (Zhou and Li, 2004), common carp (Staykov *et al.*, 2005) and rainbow trout (Staykov *et al.*, 2007), the non-specific immune system was positively affected when MOS was used. Torrecillas *et al.* (2007) 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. Vos *et al.* (2010) observed immune-modulatory effect of GOS, FOS and pectin-derived acidic oligosaccharides during the early phase of a murine model. The potential immunotherapeutic implications of oyster mushroom are enormous (Oloke and Adebayo, 2015).

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 (Kelly, 2009). 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 (Kok *et al.*, 1998) 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 (Kaufhold *et al.*, 2000). The plasma glucose levels reduced ($p < 0.05$) in rats due to supplementation of pulverized JA tuber powder (Samal *et al.*, 2015). The AXOS have the potential of improving glucose tolerance and suggested mechanisms are improved insulin sensitivity and increased gut fermentation (Boll *et al.*, 2015).

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 (Kore *et al.*, 2009). The oat β -glucan can attenuate blood postprandial glycemic and insulinemic responses, lower blood total cholesterol and LDL-cholesterol and improve High Density Lipoprotein (HDL) cholesterol and blood lipid profiles as well as to maintain body weight (Daou and Zhang, 2012).

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 , interferon- 1β and TNF- α . Among SCFA, butyrate has the highest anti-carcinogenic activity and it prevents cell differentiation. The growth and proliferation of tumour cells depends on glucose availability but inulin-type fructans decreases the serum glucose level and might deprive cancer cells of their essential substrate.

Several microbial enzymes help in the transformation of procarcinogens to carcinogens. Azoreductase (found in *E. coli* and *Clostridium* sp.) hydrolyses the azo bond in azo dyes, nitroreductase (found in *Clostridium* sp. and Bacteroides) reduces nitro groups and both generate substituted aromatic amines. β -glucuronidase (found in *E. coli*) can hydrolyse the glucuronides to

potent mutagenic aglycones. The 7- α -dehydroxylase (found in *Clostridium* sp.) helps in conversion of primary bile acids into secondary bile acids i.e., deoxycholic acid (DCA), the promoters of colon cancer. Deoxycholic acid causes cyclo-oxygenase-2 (COX-2) expression and induces DNA damage. These enzymes are very less in *Lactobacillus* sp. and *Bifidobacterium* sp. These beneficial bacteria express bile salt hydrolases that helps in detoxification of faecal bile acids. Butyrate induces the glutathione-S-transferase enzyme which decreases the expression of COX-2 in primary colon cells. In experimental rats, tumours are induced by azoxymethane or dimethylhydrazine which causes DNA damage and forms Aberrant Crypt Foci (ACF). Inulin-type fructans have been shown to increase caecal butyrate concentration, decrease ACF and reduce tumour incidence (Verghese *et al.*, 2003). The prebiotic epilactose inhibit the conversion of primary bile acids to secondary bile acids (Watanabe *et al.*, 2008). Gavaging rats with CAM30 (Cassis Anthomix 30; blackcurrant extract powder) and FL (First Leaf; blackcurrant extract powder, lactoferrin and lutein) significantly increased the numbers of *Bifidobacterium* sp. and *Lactobacillus* sp. and decreased the numbers of bacteroides and *Clostridium* sp. It also exhibited reduction in the activity of β -glucuronidase and increment in the activity of β -glucosidase (Molan *et al.*, 2010). Consumption of AXOS-enriched diet has been reported to reduce the occurrence of preneoplastic lesions in the colon of rats treated with the carcinogen (Femia *et al.*, 2010).

Anti-obesity: Endocrine L-cells are distributed all along the intestinal tract and are also present in caeco-colon. These endocrine cells secrete GLP-1 which is the modulator of appetite. Prebiotics decrease the secretion of ghrelin thereby regulating the body weight gain. Leptin (increases the feed intake) level is also decreased in serum. Moreover, triglyceride level is decreased due to prebiotic supplementation. It was speculated that chronic resistant starch feeding decreases the size of adipocyte cells and reduces whole body weight gain relative to digestible starch feeding (Birt *et al.*, 2013). Fermentable dietary fibres as short-chain FOS can be supplemented in foods to induce satiety and thus prevent obesity (Hess *et al.*, 2011). Everard *et al.* (2011) investigated that in obese mice, prebiotic feeding decreased firmicutes abundance with simultaneous improvement in glucose tolerance, reduced fat accumulation, oxidative stress and inflammation.

Faecal odour reduction: In odour control, the emphasis is focused on N as it is the breakdown of endogenous and undigested nitrogenous materials like amino acids in colon that appears to produce most of the offensive volatiles like phenols, indoles and skatole. More importantly, these protein catabolites may have negative influences on gut health. Therefore, a decrease in their hindgut concentrations could be beneficial to host health. Prebiotics may decrease the concentration of these compounds by providing gut microbiota with an additional source of energy supply (carbohydrate). Accordingly, making N in feedstuffs more available to host and less available to its hindgut microbiota will result in production of fewer odorants. Prebiotics prevent the protein fermentation by the pathogens by preventing their colonization in the gut epithelium and decreasing the NH₃ concentration in the faeces.

Adding lactosucrose to a dog food decreased the faecal concentrations of NH₃, phenol, ethyl-phenol, indole and skatole (Swanson and Fahey, 2006). The FOS supplementation lowers faecal indole and phenol concentrations in dogs (Swanson *et al.*, 2002). Adding 8% of oligofructose and 2% of beet pulp to the diet of dogs reduced the apparent protein digestibility (Diez *et al.*, 1997), thereby reducing fecal odour components. When supplemented with lactosucrose and FOS, decreased caecal phenol and indole concentrations were observed

(Terada *et al.*, 1994; Cao *et al.*, 2005). However, Hesta *et al.* (2005) did not observe any effect on odour components due to FOS supplementation in cats.

Other effects: Another application of prebiotics in porcine nutrition is suppression of boar taint i.e., off-flavour of pork which is due to accumulation of skatole and androsterone in adipose tissue. Feeding male pigs an inulin preparation suppresses proteolytic conversion of tryptophan into skatole, which within 3-5 d reduces the typical odour that makes the meat of intact pigs unsuitable for human consumption (Jensen *et al.*, 1997). Feeding inulin suppresses parasites such as *Ascaris* sp. (Petkevicius *et al.*, 1997) and *Trichuris* sp. (Thomsen *et al.*, 2005). Lowering of intestinal pH, which is unfavourable for the embryonic development has been suggested as a possible mechanism. When challenged with *Oesophagostomum dentatum*, pigs supplemented with inulin experienced decreased intestinal *O. dentatum* eggs and worms (Petkevicius *et al.*, 2003). Fish blood profile like total protein, albumin, creatinine, compleman 3, compleman 4 and IgM were significantly higher in fish fed 3% GroBiotic-A, a commercial prebiotic (Yousefian *et al.*, 2012).

CONCLUSION AND FUTURE PERSPECTIVES

Dietary supplementation of prebiotics is a valuable approach in improving gut health attributes like bifidogenecity, hindgut fermentation, gut mucosal integrity, mineral bioavailability, lipid and glucose homeostasis and immune response of livestock and poultry. They have added benefits in terms of easy accessibility, easier integration into feeding system and exploitation of non-viable dietary components. Moreover, they can be fortified in wide range of feeds. However, to obtain optimal results, standardization concerning specific prebiotic chosen, dietary inclusion level, adaptation period, chemical structure (degree of polymerization, linear or branched, type of linkages between monometric sugars), origin of prebiotic, way of administration, animal characteristics (species, age, stage of production and health status) and housing conditions is required. In this perspective it would be possible to compare data from different experiments and to provide the basis for more refined clinical trials.

Future research should focus on determining the mechanism of action, evaluating prebiotic and probiotic interaction and elucidating how the genetic and bacterial profiles of the host can influence treatment responsiveness. Moreover, as better information on structure to function information accrues as well as individual metabolic profiles of target bacteria are compiled, it may be easier to tailor prebiotics for specific health attributes. With the new generation of molecular microbiological tools now becoming available, it will be possible to gain definitive information on the species rather than genera that are influenced by the test prebiotic. The more we identify and characterize the bacterial genera, species and even strains that compose the intestinal microbiota, the more we can understand both qualitatively and quantitatively, changes in that composition, their physiological roles and mechanisms of effects.

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