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Review Article

PREBIOTICS AND SALMONELLOSIS: ROLE OF FRUCTOOLIGOSACCHARIDES AND INULIN

Anindita Deb Pal

Department of Food Science & Nutrition Management, J.D. Birla Institute. 11, Lower Rawdon Street, Kolkata, India

**Corresponding author: deb_anindita@yahoo.com*

ABSTRACT

Salmonellosis is a worldwide concern emerging as one of the key causes of global diarrheal disorders. *Salmonella enterica* var Enteritidis and Typhimurium are the two most common serovars associated with potent foodborne illnesses followed by *S Enterica* Newport and Heidelberg, leading to major economic losses in the food industry. Numerous approaches have been employed for prevention and treatment of the same. Recent studies have highlighted the role of prebiotics in control of the disease. Fructose-based prebiotics including fructooligosaccharides and inulin have been shown to influence the occurrence, progression, severity as well as overall pathogenesis of Salmonellosis. These compounds protect host health via inhibition of pathogen colonization and translocation along with hastened elimination of the microbe. Moreover, these oligosaccharides also improve intestinal histology and alter inflammatory cascades. Indeed, these compounds have been implicated in TGF-β and PPAR-ϒ mediated downregulation of NF-κB dependent inflammation. The effectiveness of these prebiotics is enhanced by stimulation of probiotic dependent increase in short chain fatty acids, anti-microbial compounds as well as antioxidant enzymes. Administration of these fructose-based oligosaccharides have been shown to improve prognosis in human cells, poultry, swine, and certain mice models. Nonetheless, the deleterious effects of the above have also been reported in rodents. The combined efficacy of these prebiotics is therefore dependent of a variety of external and internal factors. A complete understanding of the above may help in generation of novel approaches for combating *Salmonella* infection.

Keywords: Fructooligosaccharides, Host, Inulin, Prebiotic, *Salmonella*

1. INTRODUCTION

Prebiotics are organic compounds that are selectively utilized by the gut microbiota to confer health benefits to the host. These nutrients resist digestion by the enzymes of the upper gastrointestinal (GI) tract and are hence acted upon by commensal microbes present in the lower tract [1]. Prebiotics, although not limited to carbohydrates are generally confined to oligosaccharides and commonly include fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), mannooligosaccharides (MOS) and xylooligosaccharides (XOS). Fructose based prebiotics also called fructans mainly comprise FOS and inulin. Fructooligosaccharides (FOS) constitute one of the major classes of prebiotics characterized by the presence of several units of fructose polymerized via (2-1) linkages with a terminal glucose unit. Inulin is another fructan possessing longer chain length and a cross-linked structure. Owing to this organization, they resist stomach acidity, hydrolysis by digestive enzymes as well as absorption in the GI tract. Selective fermentations of the above by intestinal microorganisms produce Short Chain Fatty Acids (SCFAs) which bring about numerous benefits to the host both directly and indirectly. These oligosaccharides also help in strengthening the gut epithelial barrier as well as stimulating the growth and activity of the probiotics, thereby positively contributing to host health [2]. An important aspect necessary for all prebiotics is their ability to activate only the beneficial microflora of the gut. FOS and inulin confer several effects such as prevention of pathogen attachment and their elimination, increasing bioavailability of nutrients, improving innate and adaptive immune responses, maintaining intestinal physiology and morphology, enhancing digestion as well as reducing the risk of common chronic disorders via their interaction with the favourable microbes [3]. Interestingly, these are now becoming popular as alternatives to antibiotic growth promoters not only because of their anti-pathogenic action but also due the problem of antibiotic resistance associated with the latter [4]. Prebiotics can be administered either alone or as synbiotic combinations

with suitable probiotics. Consumption of inulin as well FOS has been associated with prevention and treatment of several acute and chronic disorders. The role of these prebiotics in *Salmonella* pathogenesis has also been well documented.

Salmonellosis is a disease of zoonotic origin commonly associated with consumption of contaminated meat, meat products, vegetables, and eggs. Apart from being a major health concern, it is also responsible for severe economic losses in the food industry. This pathogen is recognized for infection in human beings and other mammalian species. *Salmonella enterica* var Enteritidis and *Salmonella enterica* var Typhimurium are the two most common serovars associated with potent food borne illnesses followed by *S Enterica* Newport and Heidelberg [5]. These are gram negative intracellular pathogens that typically result in gastroenteritis manifested by symptoms of diarrhea, nausea, fever and vomiting for a period of 4-7 days. However, symptoms may get aggravated to severe and fatal conditions in immunocompromised individuals [6]. Transmission generally occurs either through the fecal-oral route or through direct contact with infected animals. *Salmonella* infected poultry are one of the major carriers of the disease. The infection usually occurs in two stages viz., invasion and systemic phases. Once ingested, this pathogen colonizes the intestinal epithet-lium indicating the invasion phase. This is followed by a systemic phase where it penetrates the intestinal mucus membrane followed by lamina propria to enter the bloodstream via translocation through M cells of the ileal Peyer's patches [7]. *Salmonella* resists action by humoral immune response and are instead phagocytosed by macrophages and transported throughout the body where they gain access to various internal organs. The disease is normally treated with oral rehydration therapy without usage of anti-microbials. However, advanced cases do require extensive medical care, therapy, and antibiotics [8]. Fructan prebiotics are now being researched as alternative and safe strategies to combat *Salmonella* infection in humans as well as animals [9]. These compounds have been shown to decrease the intensity of *Salmonella* infection not only via inhibition of pathogen colonization and invasion but also by generation of anti-microbial substances, strengthening of host immunity and generation of SCFAs. The present review summarizes the influence of fructooligosaccharides on the establishment, progression, and prevention of *Salmonella* infection in a variety of host microenvironments including human cells, poultry, swine, and rodents.

2. EFFECT OF FOS AND INULIN ON SALMONELLOSIS IN HUMAN CELLS

Fructooligosaccharides and inulin have been known to benefit the host by preliminarily modulating and stabilizing the gut flora. These prebiotcs have been shown to selectively stimulate and activate probiotic microorganisms including *Lactobacillus* and *Bifidobacteria* in the intestinal cells. Microbes play an important role in intestinal wellbeing. However, pathogen infection alters the homeostatic balance causing the development of either acute or chronic inflammatory diseases. Supplementation with FOS increases the percentage of *Bifidobacteria*, which thereby stimulates the Dendritic Cells (DCs) to eliminate pathogens via immune-modulation as manifested by the change in expression of the pro-inflammatory cytokines [10]. Moreover, inulin in association with *Lactobacillus acidophilus* has been shown to reduce the incidences of inflammation in human caco-2 cells. Upon ingestion, *Salmonella* establishes infection in the gut and penetrates the intestinal cells through Type 3 Secretion System (T3SS) thereby establishing a systemic infection. The Lipopolysaccharide (LPS) present in the pathogen cell wall is known to be one of the major triggers of inflammation. *Salmonella* infection in mammalian cells has been portrayed to generate physiological as well as immunological stress, leading to the activation of the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κβ) dependent inflammatory signalling pathway. Activation of this cascade further up-regulates the expression of inflammatory mediators allowing establishment of the symptoms of the disease. Previous studies have correlated the manifestation of inflammation with increased competitive advantage of this pathogen over other bacterial counterparts. Transforming Growth Factor (TGF)-β has been identified to decrease NF-κB activation during inflammation [11]. TGF-β activates SMAD2/3 leading to stimulation of SMAD4 dependent induction of IκBα, an inhibitor of NF-κB, hence diminishing the inflammatory effect. Interestingly, fructose containing synbiotic mixtures have been documented to reduce activation of NF-κβ pathway and decrease the expression of downstream inflammatory mediators including Tumor Necrosis Factor (TNF-α), (Interleukin) IL-8 and IL-12. Moreover, these have also been associated with activation of

the TGF-β pathway, hence confirming their anti-inflammatory roles. SMAD7 has been associated with inhibition of the TGF-β receptor, consequently attenuating the negative regulation of the former on the NF-κB mediated inflammatory response. However, TGF-β induces microRNA-21 (MIR21) expression, which targets 3'- Untranslated (UTR) of SMAD7 and enhances TGF-β signalling. Fructoligosaccharides have indeed been studied to decrease the expression of SMAD7 with subsequent activation of IκBα resulting in reduced pathological aggravation [12]. Additionally, administration of prebiotic blend containing FOS, inulin and GOS has been observed to downregulate several pro-inflammatory cytokines such as IL-1β, IL-8 and TNF-α in Caco-2 cells [13]. Experiments by Zehnom et al have shown FOS to exhibit the anti-inflammatory action via induction and activation of Peptidoglycan recognition protein 3 (PGlyRP). The PGlyRP 3 in turn modulates the activity of Peroxisome proliferator-activated receptor gamma (PPAR-ϒ) mediated anti-inflammatory response. Inhibition of PPARϒ has been shown to abolish this anti-inflammatory effect of FOS, although silencing of PGlyRP 3 retained this action. Therefore, dietary oligosaccharides such as FOS also influence the expression and secretion of the inflammatory cytokines via induction of PGlyRP 3 activated PPAR-ϒ transcription factor [14]. FOS and inulin can hence be useful for improving prognosis by virtue of their ability to modulate NF- κB, TGF-β and PPAR-ϒ dependent inflammatory pathways. The fructose containing prebiotics have also been found to be effective in cases of paediatric diarrhea. Formulations containing 900mg inulin has been observed to improve the prognosis of *Salmonella* mediated diarrhea in children by decreasing the duration of the disease by an average of 31 hours compared to placebo controls [15]. Moreover, administration of this prebiotic mixture reduced the frequency of diarrheal stools in the above study group. Therefore, fructooligosaccharides may be employed for not only prevention but also treatment of *Salmonella* induced acute gastroenteritis in humans.

3. EFFECT OF FRUCTAN PREBIOTICS ON SALMONELLOSIS IN POULTRY AND SWINE

Salmonella, especially *S enterica var* Enteritidis and Typhimurium are the most common pathogens associated with gastrointestinal disorders in the poultry. Alarmingly, majority of infection with the above remain asymptomatic, hence increasing the chances of translocation to the humans via the food chain. However, Salmonellosis has nonetheless been associated with decreased productivity and altered feed conversions in poultry [16]. Prebiotics have become an attractive option in the poultry industry for maintenance of health and increasing production. Several poultry industries are now attempting to provide dietary fibre-based prebiotic and synbiotic feeds instead of antibiotics owing to the chronic problem of antibiotic resistance. Fructooligosaccharides and inulin have been demonstrated to strengthen the intestinal barrier, induce beneficial structural modifications in the intestinal membrane and promote probiotic bacteria present in the poultry gut. These changes have been studied to enable the host to resist enteric pathogens including *Salmonella enterica* [17]. Broilers treated with fructose oligosaccharides have been documented to possess good histological ambience of the intestine manifested by longer intestinal villi, shallower crypts, improved villi crypt ratio and thickened duodenum epithelial membranes even after *Salmonella* infection. Moreover, administration of FOS based preparations has been shown to increase the growth and proliferation of several beneficial microbes of the Lactic Acid Bacteria (LAB) cluster and lower the numbers of enteric pathogens in addition to decreasing the symptoms of the disease. Furthermore, inulin has been portrayed to decrease the intensity of intestinal lesions as observed by lowered intestinal inflammation, mucosal degradation, lymphocyte infiltration and villi congestion. However, inulin and FOS has been shown to have minimum impact on body weight and feed intake [18].The crop, gizzard and duodenum of the chicken gastrointestinal tract is known to contain 99% microbial species belonging to the genus *Lactobacillus*. Nevertheless, the caecum has been observed to possess a greater diversity of commensal microbes compared to the upper GIT with the abundance of *Clostridium*, *Lactobacillus* and *Ruminococcus*. This microenvironment has been documented to undergo variations in response to several physiological and pathological stresses. Prebiotics have been attributed towards maintenance of the intestinal microbial homeostasis thereby aiding in heightened immunity and improved pathogen evasion by the host. Administration of fructan prebiotics has been observed to increase the diversity as well as preserve the integrity of the avian intestinal tract owing to the availability of the former as nutrients for the probiotic microbes including *Lactobacillus*[19]. Furthermore, these oligosaccharides can themselves induce immunological changes

in the host, inhibit pathogen attachment and produce favourable structural changes thereby enabling the host to eliminate the pathogen. Fermentation of these complex carbohydrates generates a plethora of Volatile Fatty Acids (VFAs) that further aid in reducing *Salmonella* content by lowering the pH and enhancing the activity of protective commensals. Oral consumption of FOS has been shown to not only increase *Lactobacillus* diversity but also decrease IL-1β associated inflammation and the content of live *Salmonella enteritidis* in the chicken intestines [20]. Additionally, *in ovo* addition of inulin has been associated with a decreased expression on IL-4, IL-6, IL-8, IL-12p40, and IL-18 in the cecal tonsils [21].

The beneficial effect of FOS and inulin towards *Salmonella* infection in hens has also been studied. A diet supplemented with FOS has been shown to increase the body weight as well as egg production between the 19th and 23rd week of age. This effect has been attributed to an increase in nutrient availability and absorption as propagated by the prebiotic [22]. FOS administration has been observed to accelerate the proliferation and activity of predominant beneficial microbes in the gut including *Lactobacillus* and *Pediococcus*. Moreover, these oligosaccharides decrease the viability of *Salmonella* in the cecal tissues of the hen post pathogen exposure. These prebiotics have also been strongly correlated with enhancement of immunity in hens. *Salmonella* infection immediately triggers innate response manifested by proliferation and activation of macrophages, DC and heterophils followed by establishment of adaptive immune response accompanied by activation of T lymphocytes and generation of IgG and IgA [23]. T regulatory cells have been shown to modulate this response by supressing the immune mediated inflammation through generation of anti-inflammatory cytokines. *Salmonella* has been shown to intelligently usurp the above machinery and decrease the pathogen-directed immune response by generating Single Nucleotide Polymorphism (SNPs) in anti-inflammatory cytokines, especially IL-10. This phenomenon has been known to be induced by the *Salmonella* LPS. Administration of FOS containing mixtures have been associated with an elevated antibody response specifically IgA, hence facilitating an early clearance of the pathogen [24]. Fructans have also proven to be beneficial during molting of hens. Molting exposes the host towards extreme physiological as well as immunological stress thereby facilitating infection by several pathogens including *Salmonella*. FOS supplementation has been documented to inhibit the translocation and colonization of this bacteria in the internal organs including liver, spleen, ovary, and lymph nodes compared to non-supplemented diets. Additionally, the fermentative properties of the prebiotic further increase the propensity of commensal *Lactobacillus* in the hen GIT through various mechanisms including production of SCFAs. Indeed, FOS has been observed to heighten the iso-butyric acid concentration in the hen intestine. Furthermore, addition of fructose-based prebiotics has been observed to elevate the lactic acid content leading to increased acidity and pathogen elimination [25].

Fructooligosaccharides have also been documented to relieve incidences of *Salmonella* mediated infection in pigs. Supplementation of drinking water with FOS has been observed to reduce the excretion of *S* Typhimurium in the fecal contents of pigs. Moreover, consumption of this oligosaccharide has also been associated with a selective increase in gram positive microorganisms including *Lactobacillus* in the swine gut. The prebiotic mediated enrichment of commensal microflora has been associated with reduced *Salmonella* load due to competitive exclusion, inhibition of adhesion, immune modulation and production of antibacterial substances by the former [26]. Therefore, FOS and inulin may benefit the host health during incidences of *Salmonella* exposure with their efficacies dependent on the dosage, type of host, immunological health as well as environmental influences.

4. EFFECT OF FOS AND INULIN ON SALMONELLOSIS IN RODENTS

Influence of fructose-based prebiotics on the status of *Salmonella* pathogenesis in mice have been reported. Infection by this microorganism often leads to a plethora of effects including inflammation of the digestive tract culminating in the development of Inflammatory Bowel Disease (IBD). Previous research by Chen et al has shown a prebiotic blend containing FOS to reduce the levels of TNF-α inflammatory cytokine in colon tissues of C57BL/6 mice. Additionally, the above prebiotic concoction was found to elevate the levels of IL-10 anti-inflammatory cytokines in mice [27]. This mixture was also shown to reduce the symptoms of the disease including loss of body weight, intestinal hyperplasia as well as tissue damage in these animals. Salmonellosis is generally associated with loss of barrier function, mainly due to interruptions of tight junctions in the intestinal cells. FOS containing prebiotic supplements have been

shown to prevent the destruction of tight junction protein Occludin in mice compared to untreated controls experiencing IBD. Moreover, the above mixtures were also observed to favour the microbial diversity towards beneficial commensal members including *Clostridium* XIVa and *Lactobacillus sp* in the rodent gut [28]. The capability of FOS and inulin to decrease the intensity of inflammation and other histo-pathological abnormalities of the disease has been attributed to the activation of the PPAR-ϒ signalling pathway. Enteropathogenic infection has been shown to decrease the content of PPAR-ϒ which can then be enhanced both at the mRNA and protein levels by the action of FOS and inulin [29]. Previous experiments have also portrayed inulin either alone or in symbiotic combination with *Lactobacillus sp* to reduce the intensity of *Salmonella* associated liver damage in mice models. Infection with intestinal pathogens often disrupt the gut mucosal barrier thereby facilitating transmission to internal organs. However, the translocation and colonization of the liver with *Salmonella* was found to be attenuated if treated with fructooligosaccharides containing prebiotic mixture. Moreover, FOS has also been found to be capable of reducing the degree of liver hyperplasia and portal vein damage in experimental mice. The ability of the prebiotic to establish gut homeostasis along with inhibition of pathogen attachment to the intestinal membrane and re-enforcement of systemic immunity together contributes to these effects. Aspartate transaminase (AST) and Alanine Transaminase (ALT) are the two major indicators of liver damage. Inulin treatment has been documented to decrease the levels of these enzymes in mice during *S typhimurium* infestation, thereby further highlighting its liver protective action. *Salmonella* challenge generally disrupts the antioxidant enzymes especially Superoxide Dismutase (SOD) leading to an elevation of hydroxyl radicals (OH^-). An increased load of OH^- results in damage of mucosal layers, hence weakening the barrier. Interestingly, treatment with fructose-based prebiotics reduces the levels of free radicals such as lipid peroxides with a concomitant increase in the antioxidant systems including glutathione and SOD in mice [30]. This effect may hence be responsible for prebiotic mediated alleviation of inflammation. Furthermore, inulin treatment has been shown to downregulate the expression of Nitric Oxide (NO) that is normally associated with an aggravated patho-physiological condition. Elevated NO has also been correlated to an increase in inflammatory mediator TNF-α. Consumption of inulin as prebiotic as well as synbiotic formulations has been proved to be effective in decreasing pathogen load, infection associated histological and physiological abnormalities as well as the general symptoms of *Salmonella* infection. Moreover, inulin and inulin oligo-fructose diets have been shown to protect mice from *S typhimurium* induced mortality [31].

Notwithstanding the positive effect of FOS and inulin towards improved prognosis of *Salmonellosis* in mice, several research groups have also indicated them to increase the severity and intensity of the disease. BALB/c mice fed with FOS diets have been displayed to exhibit increased *S enterica* Typhimurium SLI344 in the liver, spleen and mesenteric lymph nodes post pathogen challenge compared to those fed with a control corn-based diet. Moreover, pathogen numbers were also found to be elevated in the ileal and caecal tissues as well as feces of these mice despite the activation of innate and T cell mediated immunity. Additionally, the above diet was shown to heighten the expression of serum acute phase protein Haptoglobin thereby highlighting increased severity and translocation. These studies have also demonstrated the capability of *S enterica* Typhimurium to ferment FOS [32]. Moreover, it has also been noticed that mice fed with a high fibre diet displayed an increase in colonization, mortality and morbidity with several enteric pathogens [33]. Therefore, the effect of fructose-based prebiotics on *Salmonella* infections in mice may not be a direct correlation but influenced by a combination of external as well as internal factors.

Although FOS and inulin have been researched for their protective effects against *Salmonella* infection, studies carried out on rats display contradictory results till date. Rats fed with a diet rich in either fructooligosaccharides or inulin were found to increase the colonization of *Salmonella enterica* serovar Enteritidis in the caecum. Moreover, prebiotic administration was also associated with an increased rate of pathogen translocation to internal organs in the above studies as manifested by an elevated NO concentration in the same [34]. The fructose oligosaccharides were observed to decrease the pH due to saccharolytic fermentation. Nonetheless, this pH reduction was accompanied by a gradual increase of not only probiotic *Lactobacillus sp* but also enteric bacteria including *Salmonella*. The production of SCFA due to FOS fermentation has been attributed towards induction of mucosal damage and compromised barrier integrity in these cases. These oligosaccharides have also

been associated with secretion of mucus in feces thereby increasing the cytotoxicity of the fecal water. The timing and form of prebiotic administration may be related to the above effects. Some research opinions indicate overnight fasting prior to prebiotic administration to initiate an acid tolerance response in the *Salmonella,* hence leading to their stimulation via organic acids along with probiotic bacteria. Interestingly, the above effect was found to be attenuated by the addition of calcium. The calcium phosphate is believed to form an insoluble complex, leading to a buffering action, hence helping in prevention of membrane damage as well as pathogen invasion and translocation. Furthermore, calcium has been hypothesized to precipitate the cytotoxic compounds in the intestine thereby exerting a protective effect [35]. Infection with *Salmonella* has been associated with an alteration of gene expression patterns in rats. This pathogen has been observed to initially affect the expression of genes involved in transport, detoxification and anti-microbial activity thereby helping it to establish infection in the host. During the later course of infection, the bacteria further dismantles the balance of genes associated with immunity, oxidative stress, proteolysis and inflammation as observed by an increase in the severity of inflammation induced damage and connective tissue remodelling in several instances [36]. *Salmonella* mediated diseases have been correlated with an upregulation of IFN-1α, IFN-1β and IFN-ϒ as well as downregulation of antioxidant enzymes. Indeed, certain studies have actually portrayed FOS to increase the ability of *Salmonella enterica* to cause altered transcription and translation of the genes involved in the above processes, hence increasing pathogen virulence and decreasing host resistance [37]. Additionally, dietary FOS has been observed to exhibit internalization of tight junction proteins and increase inflammation leading to increase in intestinal membrane permeability followed by pathogen annexation in rats. These results have been further related to the increased *Salmonella* load and NO excretion in the above animals. Therefore, FOS based oligosaccharides have not been found to be useful for eliminating *Salmonella* in rats under certain experimental conditions. The protective effects of this prebiotic may hence be directed by a combination of factors and influenced by physiological parameters of the host.

5. CONCLUSION

Fructooligosaccharides and inulin are fructose-based prebiotics that have been widely studied for their role in *Salmonella* pathogenesis. Studies conducted in human cells, chicken, hen as well as pigs have portrayed a positive influence of the above prebiotics, manifested by inhibition of pathogen attachment and colonization, maintenance of intestinal membrane integrity, immune modulation, production of anti-microbial compounds and stimulation of probiotic microbes. Administration of these prebiotics has also been associated with downregulation of inflammatory pathways such as NF-κB with concomitant up-reguation of anti-inflammatory signalling cascades including TGF-β and PPAR-ϒ. FOS and inulin have also been correlated with production of favourable histo-morphological alterations in the poultry gut exhibited by increased villi height and improved crypt depth, thereby resisting *Salmonella* infection. Furthermore, these oligosaccharides increase the propensity of antioxidant enzymes in these model organisms. Additionally, dietary incorporation of the above has been implicated in facilitating growth and activity of commensal microbes of the LAB cluster in poultry, swine, and human cells. Protective effects of fructan prebiotics have also been documented in mice models as observed by decreased inflammation and increased expression of tight junction proteins. However, certain study groups have reported the deleterious effect of FOS and inulin in rodents. Therefore, the overall effectiveness of prebiotics against *Salmonella* infection may be dependent of the combined action of different factors including type of host, immune status, diet, dosage as well as environmental influences.

Conflict of interest

There is no conflict of interest in submission of this manuscript.

6. REFERENCES

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