Effects of prebiotics on growth performance

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Abstract: Due to the threat and emergence of bacterial resistance against antibiotics, the use of in-feed antibioticsat therapeutic and subtherapeutic levels has been limited. Complete withdrawal of antibioticsas growth promoters (AGP) has led to poor gut health signs in chickens that include conditions like wet litter, intestinal bacteria overgrowth, poor growth performance, malabsorption and various diseases. Two of the most common alternatives to AGP are prebiotics and probiotics. Bothprebiotics and probiotics have become the potential feed additives that improve the gut health, immunesystem and microbiota by various mechanisms of action, and enhance growth performance of chickens. The review discusses the modes of action like antibacterial, competitive exclusion (CE), and immunomodulatory properties of prebiotics, particularly in poultry.
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Introduction:

Administration of antibiotics and anticoccidials has facilitated intensification of modern broiler farming. Asub-therapeutic dose of antibiotics in chicken feed is used to inhibit bacterial growth and improve feed conversion and meat production (**Stutz and Lawton, 1984; Gaskins et al., 2002)** Prebiotics are non-digestible carbohydrates with selective influences on intestinal bacteria and immunity of chickens (**Kim et al., 2011; Bozkurt et al., 2014**). Mannan oligosaccharide (MOS), a commercial prebiotic yeast extract ingredient, has been reported to prevent gram-negative pathogenic infection by competitive exclusion in chicken gastrointestinal (GI) tracts **(Baurhoo et al., 2007a).** Inclusion of MOS in chicken diets also may enhance immune function and improve the growth of the intestinal mucosa layer and intestinal microbiotadiversity **(Baurhoo et al., 2007b; Pourabedin et al., 2014).** Beta-glucan, another commercial prebioticactive ingredient, is also reported to benefit broilers by improving innate immunity and body growth **(Chae et al., 2006).** Benefits of the combined use of MOS and *β*-glucans on growth performance have been reported for aquatic animals **(Van Hai and Fotedar, 2009; Refstie et al., 2010).** However, effects of the combined use of MOS and *β*-glucans have not been reported for broilers.

Prebiotics

Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the activity of one or a limited number of bacteria in the colon **(Gibson and Roberfroid, 1995).** Prebiotics influence intestinal bacteria and immunity of chickens **(Bozkurt et al., 2014; Kim et al., 2011).** Prebiotics should have the characteristics such as: 1) being not absorbed in the upper gastrointestinal tract (GIT),2) being resistant to acidic pH, 3) stimulating the growth of beneficial bacteria, 4) modulate host defense system **(Patterson and Burkholder, 2003).** The predominant prebiotics tried in chickens include types of oligosaccharides like fructooligosaccharides (FOS), inulin, mannanoligosachharides (MOS) and xylooligosaccharides. 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 **(Roberfroid et al., 2015)** linked together by β-1,4 glycosidic bonds, found in cell wall of *Saccharomyces* yeast **(Pourabedin et al., 2014).** Xylooligosaccharides are oligomers consisting of xyloseunits linked through β-(1-4) linkages (**Aachary et al., 2008).** Other potential oligosaccharides used in chickens are galactooligosaccharides (GOS) **(Jung et al., 2008)** and lactose **(Hajati and Rezaei, 2010).** Several commercial prebiotics are prepared from yeast cells including cell walls and fermentation products **(Ding et al., 2014; Santin et al., 2001).** Other compounds that show prebiotics-like effects include *Saccharomycescerevisiae* fermentation products or yeast culture **(Roto et al., 2015).**

Mechanism of action of prebiotics

Major prebiotics mechanisms of action include modulation of gut microbiotaby selectively regulating beneficial groups of bacteria by providing food for them **(Hajati et al., 2010)** and by reducing undesired intestinal colonization of pathogenic bacteria, thus improving the integrity of gut mucosa **(Ijiand Tivey, 1998)**. Prebiotics are not digested or absorbed in the upper GIT and instead provide food source for host beneficial bacteria such as *Lactobacillus* (LAB) and *Bifidobacteria* in the lower GIT. This eventually excludes the attachment of pathogens including *Salmonella* and promotes microbiota in the gut. Some sugars are able to block the binding of pathogens to the mucosa. For example, MOS is able to bind to mannose-specific lectin of gram negative pathogens that express Type-1 fimbriae such as *E. coli* resulting in their excretion from the intestine **(Thomas et al., 2004).** MOS are commonly derived from yeast and the outer cell of yeast. MOS are found to modulate the immune system and eliminate pathogens from intestinal tract **(Fernandez et al., 2002).** GOS have been shown to increase certain beneficial bacteria such as LAB, *Bifidobacteria* or their fermentation products **(Macfarlane et al., 2008).** Production of short chain fatty acids (SCFA), mainly butyrate, propionate and acetate as a part of fermentation process, is one of the main mechanisms of prebiotics **(Pourabedin et al., 2014).** SCFA lower the pH of gut lumen and provide energy to epithelial cells. This modulates the inflammation and regulates the metabolic functions **(Pourabedin et al., 2014).**

Prebiotics in chickens (effects on growth performance, immune response, microbiota, intestinal morphology and pathogenic bacteria)

Growth performance is the general and direct indicator in poultry as it involves feed utilization and overall effectiveness of poultry production **(Ajuwon, 2015).** Some of the major prebiotics that have shown beneficial effects in performance and gut health are given in Table 1. Replacement of antibiotics as growth promoters (AGP) with prebiotics or probiotics to observe the effect mainly in growth is the major reason for the researches.

Supplementation of MOS and FOS in broilers is found to be associated with improved body weight gain (BWG), feed conversion ratio (FCR) and carcass weight **(Baurhoo et al., 2007; Sims et al., 2004; Xu et al., 2003).** Improving broiler performance by dietary beta-glucans and MOS has been found to be associated with the improvement of innate immune function **(Bozkurt et al., 2012).** Also, production of SCFA is the reason behind better growth performance as this increases the partition of nutrients into other tissues of body **(Lu et al., 2012; Ajuwon, 2015).** The improvement of growth performance in chickens by prebiotics is affected by many factors. Prebiotics may increase SCFAs which are directly absorbed in the hind gut and used as an energy source in tissues **(Chapman et al., 1994).** Performance, egg cholesterol and gut microflora were improved by addition of inulin in laying hens diet **(Shang et al., 2010).** Improvement in egg shell and bone quality that increased the overall mineral metabolism due to inulin or oligofructose was also observed **(Swiatkiewicz and Arczewska-Wlosek, 2012).**

Prebiotics like MOS, FOS and inulin were found to modulate the immune responses in the gut-associated lymphoid tissue (GALT) of chickens like cecal tonsil, enhanced antibody titers of plasma IgM and IgG, cecum IgA levels, mucin mRNA expression and also enhanced intestinal immune functions **(Janardhana et al., 2009 b; Huang et al., 2015)**. Prebiotic treated group (both MOS and FOS) had similar performance to an AGP treated group with better GALT immunity in chickens **(Janardhana et al., 2009 b).** Prebiotic-mediated immunological changes may in part be due to direct interaction between prebiotics and gut immune cells as well as due to an indirect action of prebiotics via preferential colonization of beneficial microbes and microbial products that interact with immune cells **(Janardhana et al., 2009a).** In a study by **Huang et al. (2015),** dietary inulin supplemented at 5–10 g/kg had better effects on a starter phase (0–21 d) in both feed intake (FI) and intestinal IL-6, IgA, CD8, CD4 lymphocytes, and did not have any effect on d 42 broiler chicks.

Length of time for adaptation and the exposure of GIT microbes to the supplemented FOS plays major role in producing positive effect due to FOS. When FOS was added for a longer duration, it produced better results with villi height and crypt depth of intestine **(Hanning et al., 2012)**. It is presumed that increased villi height is associated with the increased absorption of feed due to increased surface area transporting more feed nutrients **(Amat et al., 1996).** Feeding MOS and lignin in poultry has resulted in low pH, high production of SCFA like butyric acids and healthy gut, particularly increased villi height **(Baurhoo et al., 2007).** A study with MOS showed improved intestinal development as well as a healthy microbial community in broilers **(Baurhoo et al., 2009).**

Prebiotics beneficially interact with animal's physiology by selectively stimulating favorable microbiota in the intestinal system **(Macfarlane et al., 2008).** Abundance of LAB and *Bifidobacteria* in chicken gut have been associated with the prebiotics supplementation, mainly MOS, FOS and inulin type fructans **(Geier et al., 2009; Kim et al., 2011; Baurhoo et al., 2007)**. Microbial flora such as LAB and *Bifidobacterium* sps. support the defense system of animal against invading pathogens by stimulating GIT immune response **(Mead, 2000).** According to **Seifert and Watzl (2007)**, prebiotics such as inulin and oligofructans can modulate immunesystem directly. However, it is not clear if prebiotics directly affect the pathogen or host in a microbiota-independent manner. Oligosaccharides like beta-glucans stimulate the performance by enhancing phagocytosis and proliferating monocytes and macrophages **(Novak and Vetvicka, 2008).** Prebiotics compete for the sugar receptors thus preventing adhesion of pathogens like *Salmonella* and *E. coli* **(Iji and Tivey, 1998)**. MOS have receptor properties for fimbriae of *E. coli* and *Salmonella* that leads to elimination of such pathogens with the flow of digest a instead binding mucosal receptor **(Fernandez et al., 2002).**

Studies have showed an increase in *Bifidobacteria* and LAB count and decrease in *Salmonella, E. coli* and *Clostridium perfringes* numbers in broilers fed MOS, FOS, fructan and lignin supplemented diets **(Baurhoo et al., 2007; Macfarlane et al.,2008; Zhao et al., 2013; Cao et al., 2005; Fernandez et al., 2002; Spring et al., 2000)** (Table 1). The population of *Clostridium* and *E. coli* decreased with 0.25% FOS and0.05% MOS supplementation whereas LAB diversity increased in ileum by these two prebiotics **(Kim et al., 2011).** MOS have been reported to promote LAB growth contributing to overall microbial diversity in the contents of chicken cecum **(Pourabedin et al., 2014).** Feeding lignin or MOS increased cecal population of LAB and *Bifidobacteria* whereas reduced *E. coli* in cecum of broilers **(Baurhoo et al., 2007).** The reason behind this might be the competitive exclusion (CE) where LAB and *Bifidobacteria* competed against *E. coli*. On the other hand, bacteriocin produced by LAB and organic acids produced by *Bifidobacteria* might suppress the colonization of pathogenic bacteria. The increase in intestinal microbial diversity is believed to have positive effects on gut and overall host health **(Janczyk et al., 2009).** Due to the low pH created by SCFAs, pathogens like *Salmonella* and *Campylobacter* are reduced from the gut. Fermentation products such as SCFA increased after prebiotic supplementation as a result of oligosaccharide fermentation by resident microbiota **(Macfarlane et al., 2008).** SCFA such as acetate, propionate, butyrate etc. modify the bacterial ecosystem by lowering the pH that becomes intolerant to pathogens. Due to low pH of the cecum, prebiotics have been shown to inhibit pathogens growth and stimulate the growth of beneficial bacteria like *Bifidobacterium* and LAB, and the process is the most effective in cecum **(Cummings et al., 2001).** The overall integrity of gut is also improved due to the production of SCFA **(Alloui Mohamed et al., 2013).** Stimulation of immune system includes increase in antibodieslike secretory IgA and activation of phagocytic cells **(Macfarlane et al., 2008).** Thus, production of SCFA and reduction of gut pH are key mechanisms of prebiotics in order to limit pathogen colonization and maintain optimal growth performance and health in poultry.

The use of prebiotics as possible alternative to antimicrobial growth promoters, has given contradictory results, while their use in the modulation of the gut microbial population has been promising. Oligosaccharides, esp. raffinose series that are naturally present in feed ingredients have shown imprecise results with respect to broilers performance **(Iji and Tivey, 1998)**. Broiler growth performance was negatively affected when FOS was supplemented at higher level (8 g/kg) **(Xu et al., 2003).** Feed intake and FCR both were increased upon either *in ovo* or water administration of prebiotics like GOS **(Bednarczyk et al., 2016).** Another study that used GOS as a prebiotic source found neither positive nor negative effects on growth performance but observed increased intestinal anaerobic bacteria and LAB **(Jung et al., 2008).**

Biggs et al. (2007) supplemented GOS at 4 g/kg and did not observe any significant growth performance in broiler chicks. The authors have also found depressed growth performance and a negative impact on amino acid digestibility as well as metabolizable energy when supplemented with higher level of inulin (8 g/kg). Promotion of *Bifidobacterium* without any effect on BW, FI and FCR has been observed in studies that used GOS in broilers. Addition of 0.025% beta-glucan did not improve broiler performance including FI, FCR and BWG in a starter period **(Józefiak et al., 2008).** Supplementation of inulin had no effect on villus height and crypt depth of jejunum **(Rebolé et al., 2010).**

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