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Influence of fermentable carbohydrates on the intestinal bacteria and enteropathogens in broilers

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The gastrointestinal tract is a complex milieu as a result of interaction between dietary ingredients and the intestinal bacteria. Following the European ban on the use of in-feed antibiotics, research has focused mainly on the potentially beneficial activities of the intestinal microbiota. Fermentable carbohydrates, or 'prebiotics', such as non-digestible oligosaccharides, are considered to have beneficial effects on the composition and activity of the indigenous microbiota, which can enhance the resistance of the host against colonisation of pathogenic bacteria in the GIT. Only a limited number of prebiotics has been tested in broilers that include fructo-oligosaccharides, inulin, mannan-oligosaccharides, alpha gluco-oligosaccharides, isomalto-oligosaccharides, different kestoses and lactose along with its derivatives. This review provides an overview pertaining to the potential impact of prebiotics on the intestinal bacterial population in broilers and summarizes the data regarding the role of prebiotics in preventing the colonisation of enteropathogens especially *Salmonella spp.*, *Campylobacter spp*. and *Clostridium spp*. Moreover, the influence of prebiotics on the intestinal bacterial fermentation profile, particularly short chain fatty acids, ammonia and lactate, is also discussed. Prebiotics have been found to affect the intestinal bacterial population particularly elevating the caecal count of *Lactobacillus spp*. and *Bifidobacterium spp*. The effect of prebiotics on the intestinal bacteria is also evident in terms of change in the total concentration or relative proportion of short chain fatty acids. The ability of prebiotics in controlling the colonisation of different enterpathogens especially *Salmonella spp*., *Clostridium perfringens* or *Campylobacter spp*. showed inconsistent results depending upon the available literature.

Keywords: prebiotics; inulin; fructo-oligosaccharide; mannan-oligosaccharide; isomalto-oligosaccharide; kestoses; broilers; short chain fatty acids; gastrointestinal tract; bacteria; lactose

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Introduction

Intestinal bacteria play an important role in the nutritional, physiological, immunological, and protective functions of the host (Vispo and Karasov, 1997) and can be influenced by the diet (Rehman *et al.*, 2007). Research into possible alternatives for in-feed antibiotics has focused on the activities of potentially beneficial bacteria. The most promising approach is the addition of specific ingredients to the diets that can enhance the ability of the indigenous intestinal bacteria to protect the host against pathogenic infections, an effect known as 'colonisation resistance'. Changes in the dietary distribution or nutrient densities may have dramatic effects on the intestinal bacterial community (Gibson *et al.*, 1996; Reid and Hillman, 1999) that can influence the host's ability to digest and absorb dietary nutrients. For this purpose, fermentable carbohydrates are considered to have potentially beneficial effects on the composition and activity of the intestinal bacteria (Gibson and Roberfroid, 1995). Prebiotics are non-digestible food or feed ingredients that beneficially affect the host by selectively stimulating the growth, activity or both, of one or a limited number of bacterial species in the intestine of the host (Gibson *et al.*, 2004). For a substrate to be a candidate as prebiotic, it:

1. must be neither hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract,
2. must be selectively fermented by one or a limited number of potentially beneficial bacteria which are stimulated to grow and/or be metabolically activated,
3. must influence the composition of the hind gut microbiota towards a healthier composition,
4. must preferably induce effects that are beneficial to the host health. (Gibson and Roberfroid, 1995).

Normally, prebiotics consist of a carbohydrate fraction (oligosaccharides, non-starch polysaccharides and starch). Oligosaccharides are believed to stimulate the growth of certain bacteria, especially *Lactobacillus spp*. and *Bifidobacterium spp*. The inhibition of pathogenic bacteria like *Salmonella spp*., *Campylobacter spp*. or putrefactive bacteria like *Clostridium perfringens* by the oligosaccharides can in part be explained by their fermentation products in the intestine (Gibson and Wang, 1994). However, the acid production from these bacteria and an acidic pH are not the sole mechanisms of inhibition, as other mechanisms are also involved which are yet to be elucidated. For instance, some of lactic acid bacteria also produce antibacterial substances, like bacteriocine, that inhibit the growth of different enteropathogens (Lasagno *et al.*, 2002).

The impact of prebiotics on the intestinal bacteria is still relatively poorly understood in broilers. This paper describes the potential impact of different prebiotics described in literature in broilers (*Table 1*) on the intestinal bacteria and colonisation resistance against different enteropathogens especially *Salmonella spp*., *Campylobacter spp*. and *Clostridium spp*. Overviews from studies on the influence of dietary prebiotics on the caecal microbial population (*Table 2*) and on bacterial fermentation metabolites in different segments of the intestine (*Table 3*) are also given.

Mannan-oligosaccharides

Mannan-oligosacharides (MOS), a glucomannoprotein complex derived from the cell wall of the yeasts, provides a mannose rich source for the attachment of some bacteria that would otherwise attach to the gut wall. The competition between MOS and the gut wall for the binding of bacteria involves the presence of lectins in the

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bacterial surface that are specific for some sugars. Lectins specific for mannose predominate in the intestinal pathogens (*Escherichia coli, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium* etc). These lectins, associated with pili or fimbriae of bacteria (Type-1 fimbriae) attach to mannose-containing cells in the intestinal tract (Oyofo *et al.*, 1989a; 1989b; Spring *et al.*, 2000). MOS can be hydrolyzed by different species of *Lactobacillus* and some *Bifidobacterium*, however, it seems to be less fermentable by the intestinal bacteria than fructo-oligosaccharides (Zentek *et al.*, 2002). It has been found that dietary inclusion of MOS enhanced the growth of caecal *Lactobacillus spp*. and *Bifidobacterium spp.,* while numbers of coliforms and *Clostridium perfringens* were reduced in broilers (Shao *et al.*, 2000; Denev *et al.*, 2005; Baurhoo *et al.*, 2007).

On the other hand, dietary MOS failed to alter the caecal concentrations of anaerobes, lactic acid bacteria, coliforms, lactose-negative enterobacteria and *Clostridium perfringens* except that a greater population of coliforms has been found in the duodenal and ileal digesta of birds fed a diet supplemented with MOS compared to control birds (Yang *et al.*, 2007). In laying hens, dietary MOS reduced the caecal counts of enterobacteria with a concomitant increase in *Enterococcus spp*. without affecting anaerobes, *Clostridium spp*., *Lactobacillus spp*., *Eubacterium spp*., coliforms, *Fusobacterium spp*. and *Bacteroides spp*. (Fernandez *et al.*, 2002). Spring *et al.* (2000) could not find any difference in the caecal population of anaerobes, coliforms, *Lactobacillus spp*. and *Enterococcus spp*. in salmonella-infected broilers supplemented with MOS. The authors suggested that this lack of effect of MOS on *Lactobacillus spp*. and *Enterococcus spp*. was due to the absence of type-1 fimbriae on the bacterial surface. In the same study, MOS failed to alter the caecal concentrations of short chain fatty acids (SCFA) and lactate. However, in one study, caecal lactate was higher in MOS-fed broilers experimentally infected with *Campylobacter jejuni* and *Campylobacter coli* (Anderson *et al.*, 2005). Dietary addition of MOS did not cause any major change in the SCFA profile of caecal digesta of broilers. However, the relative proportion of acetate was higher in MOS-fed broilers (Yang *et al.*, 2007; *Table 3*) on day 14 but not on day 35.

MOS AND COLONISATION RESISTANCE

There is a growing interest to investigate the specific and selective properties of some oligosaccharides and to describe how they can affect the colonisation of some pathogenic bacteria like *Salmonella spp*., *Escherichia coli* and *Clostridium spp*. in the intestine of broilers. Dietary MOS given to young chicks challenged with *Salmonella typhimurium* reduced caecal count of *Salmonella typhimurium* (Spring *et al.*, 2000). Similarly, in a second series of trials with *Salmonella dublin* as challenge organism, the number of young broilers that tested salmonella positive in the caeca was less when MOS was part of the diet. Fernandez *et al.* (2000) studied the protection against the caecal colonisation of *Salmonella enterica* serovar *Enteritidis* in broilers when birds were inoculated with the caecal contents from hens fed a MOS-supplemented diet. Results showed that chicks were better protected with both a MOS-supplemented diet plus inoculation with the caecal culture obtained from hens that were also fed a MOS-supplemented diet. In another trial (Fernandez *et al.*, 2002), the number of birds infected with *Salmonella enterica* serovar *Enteritidis* was lowered in MOS-fed birds compared to birds fed a control diet. MOS reduced the caecal count of *Campylobacter spp*. in broilers infected with ten different strains of *Campylobacter jejuni* and *Campylobacter coli* (Anderson *et al.*, 2005). The effect of treatments was increased over the time as colonisation was reduced by 45.49% (or log 1.92) at 5 weeks. There is a paucity of information regarding the effect of MOS on *Clostridium spp*. in broilers. It has been

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reported that MOS effectively reduced the mortality and sub-clinical effects of *Clostridium perfringens* infection in broilers (Hofacre *et al.*, 2003).

Fructo-oligosaccharides

Although indigestible oligosaccharides composed of fructose and glucose units are often referred to as fructo-oligosaccharides (FOS), this term refers especially to short chains (~3-6 units) of fructose units bounded by a ß(2-1) linkage, that are attached to a terminal glucose unit. FOS has been shown to enhance the growth of *Bifidobacterium spp*. and *Lactobacillus spp*. but to inhibit the growth of *Escherichia coli* and *Salmonella spp*. in the intestine (Hidaka *et al.*, 1986; Choi *et al.*, 1994; Roberfroid *et al.*, 1998; Fukata *et al.*, 1999). *Lactobacillus lactis, Enterococcus faecium* and *Pedicoccus spp*. grew in media with pure FOS as the energy source, but not the tested *Salmonella* serotypes. All *Salmonella* serotypes that have been tested utilised an impure form of FOS (Oyarzbal and Conner, 1995). *In vitro* competitive experiments demonstrated a non-significant difference in the concentrations of *Clostridium spp*. when grown in FOS-containing media, either co-cultured with *Bifidobacterium spp*. or alone (Kullen *et al.*, 1998). Supplementation of fructan-rich Jerusalem artichoke or topinambur syrup, containing inulin (degree of polymerization (DP) > 10), FOS (DP 5-10), oligofructose (DP 2-4), saccharose, fructose, glucose, minerals and vitamins in broilers reduced the caecal total aerobes, *Clostridium perfringens* and *Enterobacteriaceae* with a concomitant increase in the population of *Bdellovibrio bacteriovorus* (Kleessen *et al.*, 2003; *Table 2*). A number of studies showed that dietary FOS influenced the intestinal bacterial community by increasing the population of *Bifidobacterium spp*., *Lactobacillus spp*. and *Eubacterium spp*. while decreasing the concentrations of *Clostridium spp*. and *Escherichia coli* (Wang and Shan, 2002; Cao *et al.*, 2003; Xu *et al.*, 2003; Zhan *et al.*, 2003; Cao *et al.*, 2005; *Table 2*). On the other hand, it has been shown that dietary FOS did not alter the caecal populations of *Bifidobacterium spp*., *Bacteroides spp*., *Lactobacillus spp*. and *Escherichia coli* in broilers (Fukata *et al.*, 1999; Yusrizal and Chen, 2003). However, numbers of *Lactobacillus spp*. were elevated only in female broilers, without altering the numbers of *Escherichia coli* and total aerobes in the small intestine (Yusrizal and Chen, 2003). Adding oligofructose to a diet for broilers reduced the faecal volatile ammonia at four weeks of age, but later concentrations returned to normal (Yusrizal and Chen, 2003). Supplementation of FOS in broiler diets has been reported to decrease the concentrations of caecal phenols and indole without affecting total SCFA (Cao *et al.*, 2005; *Table 3*) and to enhance the production of caecal SCFA (Zhan *et al.*, 2003) by elevating the concentration of butyrate (Zhan *et al.*, 2003) and valerate (Cao *et al.*, 2005).

FOS AND COLONISATION RESISTANCE

Attempts to control the colonisation of *Salmonella spp*. with dietary FOS have shown mixed results. Feeding FOS to broilers had little influence on the caecal colonisation of *Salmonella spp*. When FOS was fed in combination with competitive exclusion (CE) intestinal bacteria, there was a reduction in both number of salmonella positive-birds and caecal population of *Salmonella spp*. (Bailey *et al.*, 1991). FOS has been found to reduce the population and number of salmonella positive-birds when broilers were subjected to feed withdrawal (Bailey *et al.*, 1991), although this effect was not evident in broilers fed with FOS plus CE bacteria (Oyarzbal and Conner, 1996). It has been shown that there was a reduction in the caecal colonisation of *Salmonella spp*. in broilers fed either FOS alone (Choi *et al.*, 1994; Chamber *et al.*, 1997; Fukata *et al.*, 1999) or in combination with CE bacteria (Fukata *et al.*, 1999).

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Inulin

Inulin, extracted from chicory (*Cichorium intybus*), is a heterogeneous compound with respect to polymer chain length, containing molecules ranging from DP 3 to 60. The sugars moieties and the glycosidic linkages are similar to those of FOS, but with an average DP of 10. Inulin is believed to enhance the growth of health-promoting bacteria and suppresses the growth of potential pathogenic bacteria including *Clostridium perfringens* (Zentek *et al.*, 2003). *In vitro* fermentability of oligofructose and inulin was compared with a range of reference carbohydrates by measuring bacterial end-product formation in a batch culture. SCFA and gas formation indicated that these substrates were utilised by mixed populations of intestinal bacteria. The results showed that inulin exerted a preferential stimulatory effect on the genus *Bifidobacterium*, whilst maintaining populations of potential pathogens (*Escherichia coli, Clostridium spp*.) at relatively low levels (Wang and Gibson, 1993).

In broilers, dietary inulin failed to alter the intestinal bacterial community, either determined by traditional culture-based technique (Biggs and Parsons, 2005) technique or a culture-independent molecular fingerprinting technique (Rehman *et al.*, 2008b). Using traditional culture methods, it has been reported that dietary inulin did not affect the numbers of total aerobes and *Lactobacillus spp*. (Yusrizal and Chen, 2003) or *Clostridium perfringens* (H. Rehman, unpublished data) except for lowering concentrations of *Escherichia coli*/coliforms in the small intestine and caecal digesta of female, but not male, broilers (Yusrizal and Chen, 2003). The reason for this difference is not clear and needs further consideration.

It seems that response of broilers to prebiotics may also depend upon gender, as it has been reported that bacterial community of the jejunum of broilers is affected by gender (Lumpkins *et al.*, 2008). An increased concentration of caecal *Bifidobacterium spp*. has been found in inulin-fed one-week-old laying hens (Rada *et al.*, 2001). It has been shown that inulin did not clearly affect the colonisation of *Salmonella spp*. and *Campylobacter spp*. in the digesta of the small intestine and caeca, except for lower caecal salmonella count in female broilers (Yusrizal and Chen, 2003).

Dietary inulin reduced faecal pH and volatile ammonia levels in broilers during the second week of age, although later it returned to normal (Yusrizal and Chen, 2003). Rehman *et al.* (2008a; 2008b) studied the intestinal microbial fermentation spectrum in inulin-fed broilers. Lactate was higher in the jejunal digesta of inulin-supplemented birds, but this effect could not be observed in the crop and gizzard digesta. Inulin did not affect the total concentrations of caecal SCFA; however, the proportion of butyrate was higher in supplemented birds (*Table 3*). Caecal ammonia was decreased in supplemented-birds without affecting the jejunal ammonia. Total biogenic amines in the jejunal and caecal digesta remained unaffected by the dietary supplementation of inulin, but putrescine was elevated in the caecal and jejunal digesta of inulin-supplemented birds compared to the control group.

Isomalto-oligosaccharides

Isomalto-oligosaccharide (IMO), produced from glucose by enzymatic transgalactosylation (Hayashi *et al.*, 1994; Vetere *et al.*, 2000), is a mixture containing isomaltose, panose, isomaltotriose and several branched oligosaccharides composed of four or five glucose moieties. It can also be produced through the use of a modified *Leuconostoc mesenteroides* fermentation (Chung and Day, 2002). IMO can be hydrolyzed by enzymes in the brush border of the small intestinal mucosal cells

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(Hunziker *et al.*, 1986; Hertel *et al.*, 2000). Despite this, it has been tested as a prebiotic similar to other non-digestible oligosaccharides in broilers (Xia *et al*., 2001; Zhang, 2000; Zhang *et al.*, 2003; Chung and Day, 2004). Results from a pure culture technique indicated that IMO stimulated the growth of *Bifidobacterium bifidum, Bifidobacterium longum* and *Lactobacillus johnsonii* but not *Salmonella typhimurium* and *Escherichia coli* (Chung and Day, 2002). Similarly, using IMO as the only carbohydrate source, data from competitive strain studies showed a higher viable count and faster growth of caecal isolates of chickens compared to salmonella populations when IMO was provided to caecal bacteria as a sole carbon source (Chung and Day, 2004).

There are a few reports describing the influence of IMO on the intestinal bacteria in broilers. No differences were found in the population of total aerobes, *Lactobacillus spp*. or *Escherichia coli* in the digesta of crop and caecum of IMO-supplemented broilers (Zhang *et al.*, 2003; *Table 2*). On the other hand, IMO selectively increased the caecal *Bifidobacterium spp*., but not total lactic acid bacteria or total anaerobic bacteria in broilers experimentally infected with *Salmonella typhimurium* (Thitaram *et al.*, 2005; *Table 2*). Feeding IMO (1%) reduced the caecal population of *Salmonella typhimurium*, but this effect was lost when the supplementation level was increased to 2% or 4% (Thitaram *et al.*, 2005). This example indicates that the dosage of prebiotic carbohydrates needs to be carefully adjusted to achieve the desired effects. The explanation is not well founded, but there might be negative interference with the digestive processes, perhaps due to a depression of ileal nutrient digestibility and subsequent higher substrate availability for pathogenic bacteria.

The addition of IMO at different dosages in a diet of broilers did not influence the concentrations of ileal and caecal SCFA; however, the feeding of 0.3% IMO elevated of the concentrations of n-butyrate and iso-butyrate in the jejunal digesta (Zhang *et al.*, 2003).

Kestoses

Manley-Harris and Richards (1991) produced a series of kestose oligosaccharides by pyrolysis of sucrose containing 1-kestose, 6-kestose and neo-kestose and their alpha fructofuranosyl anomers (iso-1-kestose, iso-6-kestose and iso-neokestose)**.** Thermal treatment of amorphous anhydrous acidified sucrose produces sucrose thermal oligosaccharide caramel (STOC), a complex mixture containing fructose rich oligosaccharides and di-fructose di-anhydrides (Manley-Harris and Richards, 1994). Pure culture of *Escherichia coli*, *Bacteroides thetaiotaomicron, Bifidobacterium bifidum* and *Clostridium perfringens* did not grow on the thermal kestoses, although it stimulated the growth of *Bacteroides fragilis*, *Bifidobacterium breve*, *Klebsiella pneumoniae* and *Clostridium butyricum*. There was a slight growth of *Lactobacillus acidophilus*, *Lactobacillus salivarius* and *Enterococcus faecalis* (Patterson *et al.*, 1997). Inclusion of thermal kestoses in the diet of broilers increased the caecal population of *Bifidobacterium spp*. and *Lactobacillus spp*. without affecting the concentrations of total anaerobes and *Clostridium spp*. (Patterson *et al.*, 1972; *Table 2*). Orban *et al.* (1997) studied the effects of different levels of STOC, dietary vitamin-mineral levels and brooding temperatures on the caecal bacterial population in broilers and received inconsistent results.

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Lactose

Lactose can not be considered as a "true" prebiotic by definition, as enterocytes contain lactase. The activity of lactase is low in poultry (Siddons and Coates, 1972), with about 50% of ingested lactose in a poultry diet reaching the caeca (Atkinson *et al.*, 1957). Here it is fermented to lactate, as higher concentration of caecal lactate have been found in lactose-fed broilers (van der Wielen *et al.*, 2002) compared to low levels found in commercially raised broiler chickens fed a lactose-free diet (Rehman *et al.*, 2007; 2008a). Lactose is believed to promote the growth of lactose-fermenting bacteria that either compete with *Salmonella spp.* colonisation or produce substances toxic to *Salmonella spp.* (DeLoach *et al.*, 1990). Chicks receiving dietary lactose showed larger numbers of *Enterobacteriaceae* and *Enterococcus spp*. on day 7 (van der Wielen *et al.*, 2002) or day 5 (Morishita *et al.*, 1982), while populations of *Lactobacillus spp*. remained unaffected in 2 week-old broilers (van der Wielen *et al.*, 2002). On the other hand, supplementation of lactose (2.5% or 4.5%) in the diets of broilers failed to affect the intestinal microbial population of *Enterococcus spp.*, *Lactobacillus spp*., *Clostridium spp*. or *Escherichia coli* (McReynolds *et al.*, 2007).

LACTOSE AND COLONISATION RESISTANCE

Dietary lactose, supplied either in drinking water or in feed, has been found to reduce the caecal colonisation of *Salmonella spp.* in broilers (Corrier *et al.*, 1990a and b; Hinton *et al.*, 1990 and 1991; Nisbet *et al.*, 1993) (*Table 4*). However, the protection against *Salmonella spp.* colonisation was greater in broilers when lactose was fed either in combination with an anaerobic caecal bacterial culture from adult broilers (Hinton *et al.*, 1990 and 1991; Nisbet *et al.*, 1993 and 1994) or with *Lactobacillus acidophilus* (Qin *et al.*, 1995). Nevertheless, lactose supplementation was unable to eliminate *Salmonella spp.* completely from birds. The anti-salmonella effect of dietary lactose may be attributed, in part, to an increased acidity of the digesta resulting from lactose fermentation. In salmonella-infected broilers, dietary lactose changed the intestinal bacterial fermentation profile characterised by a higher concentration of lactate and propionate and lower concentration of butyrate (Corrier *et al.*, 1990a and b; Hinton *et al.*, 1990 and 1991). Waldroup *et al.* (1992) suggested that supplementing poultry diets with lactose (at 7.5%) was not a viable means of reducing or eliminating the incidence of *Salmonella spp.* or levels on processed broiler carcasses.

Clostridium spp. were found less frequently in the caecal digesta of lactose-fed broilers (Morishita *et al.*, 1982). It has been demonstrated that dietary lactose either reduced the number and caecal colonisation of *Clostridium perfringens* (Takeda *et al.*, 1995) or the intestinal lesions associated with necrotic enteritis (McReynolds *et al.*, 2007) in broilers experimentally infected with *Clostridium perfringens*.

Apart from other prebiotics tested in broilers, a few reports are also available for lactosucrose and lactulose. Lactosucrose, an oligosaccharide composed of galactose, glucose and fructose, is produced from lactose and sucrose by ß-fructofuranosidase from *Arthrobacter spp*. (Fujita *et al.*, 1990). *In vitro* studies showed that lactosucrose was fermented by all species of *Bifidobacterium spp*. tested except *Bifidobacterium bifidum*, but not by many strains of putrefactive bacteria like *Clostridium perfringens* and *Enterobacteriaceae* (Fujita *et al.*, 1991; Hara *et al.*, 1994). Terada *et al.* (1994) studied the effect of supplementation of lactosucrose (0.15%) on the caecal microbial populations in broilers. Caecal concentrations of ammonia, phenol and cresol were decreased on day 62, while acetate and butyrate were higher on day 62 of lactosucrose consumption. Dietary lactosucrose elevated the caecal count of *Bifidobacterium spp*. on day 62 (*Table 2*). On day 20, the caecal population and the

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frequency of occurrence of lecithinase-negative *Clostridium spp*. was decreased without affecting lecithinase-positive *Clostridium spp*. However, lactosucrose did not affect the caecal population of *Enterobacteriaeceae* and *Lactobacillus spp*. (*Table 2*).

Lactulose, an isomeric form of lactose composed of galactose and glucose (Zentek *et al.*, 2002), has also been tested for alleviating the undesirable egg taints in layers (Zentek and Kamphues, 2002). *In vitro* trials demonstrated that lactulose was fermented by *Bifidobacterium spp*. (Rycroft *et al.*, 2001). Dietary lactulose and lactosucrose in broiler feed proved to be ineffective in eliminating *Salmonella spp*. (Chambers *et al.*, 1997).

Conclusions

There is a lack of reported clinical trials demonstrating the role of prebiotics on the intestinal bacteria and their potential impact on the colonisation resistance. For a better understanding and development of prebiotics, sound knowledge about the composition and activity of the intestinal bacteria and interaction between bacteria and host is a prerequisite. Use of molecular methods certainly may help in defining the microbial diversity and its response following the use of prebiotics. The dietary intervention with prebiotics tested in broilers and the change in the numbers of *Lactobacillus spp*. and *Bifidobacterium spp*. is still doubtful, as not all the prebiotics tested in broilers selectively enriched these two desirable bacterial populations. Likewise, the desirable ability of prebiotics to reduce the numbers of putrefactive and potentially pathogenic organisms seems to be questionable depending upon the data presented for broilers. The observed variation may be due to the compositions of feed, structure of the feed, the age of birds and, most importantly, the doses and types of prebiotics studied. As prebiotics vary in their molecular structure and chain length, it is unlikely that they are fermented at equal rates or in the same part of the intestine resulting in a variable intestinal bacterial fermentation spectrum. Apart from classical prebiotics, research for some other potential candidates is also needed to evaluate how they may affect the microbial ecology of the anterior GIT, especially the crop and gizzard that acts as gateway for the entrance of different enteropathogens. Therefore, it might be interesting to develop a prebiotic or a mixture of differentially fermentable carbohydrates that contains both rapidly fermentable carbohydrates (acting in the upper digestive tract) and more slowly fermentable carbohydrates that may influence the microbial community of the lower intestinal tract.

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Table 1 Name and composition of prebiotics tested in broilers.

Prebiotics	Chemical composition
Fructo-oligosaccharide	-D-Glc-(1←2)-(D-Fru-(1←2)-^{n} n = 2-4
Inulin	-D-Glc-(1←2)-(D-Fru-(1←2)-^{n}
Isomalto-oligosaccharides	Mixture of isomaltose, panose, isomaltotriose plus branched oligo-saccharides composed of 4 or 5 glucose moieties
Mannan-oligosaccharides	Multiple units of mannose
Kestoses	
Kestoses oligosaccharides	Mixture of 1-kestose, 6-kestose and neo kestose and their alpha fructofuranosyl anomers
Sucrose thermal oligosaccharide caramel	Mixture of fructose rich oligosacharides and difructose di-anhydrides
Galactooligosaccharides (transgalactosylated oligosaccharides)	Generally consists of oligosaccharide from tri-to pentasaccharide
Lactose	Galactose-Glucose
Lactose-derivatives	
Lactulose	4-*O*-ß-galactpyranosyl-D-fructose
Lactosucrose	4^{G}-ß-D-galactosylsucrose or 0-ß-D-galactopyranosyl-(1-4)-0-α-D-glucopyranosyl-(1-2)-ß-D-fructofuranoside

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Table 2 Influence of supplementation of different prebiotics on the caecal microbial population in broilers.

Reference	Treatments	Age	Total		*Bifidobacterium spp.*	*Lactobacillus spp.*	*E. coli/ coliforms*	*Bacteroides*	*Clostridium spp.*	*Eubacterium spp.*
			Anaerobes	Aerobes						
Terada *et al.* (1994)	Control	62 d	10.7[a]	9.3	9.3[a]	9.4	8.9*	10.5	8.9	9.5
	LS (0.15%)		10.5[b]	9.4	9.7[b]	9.3	9.0	10.3	9.1	9.2
Orban *et al.* (1997)	Control	28 d	10.9	9.4	6.0[b]	9.2[a]	9.0	ND	6.2	ND
	STOC (3.7%)		10.9	9.0	7.5[a]	8.6[b]	8.9	ND	6.0	ND
	STOC (7.5%)		10.9	9.1	7.4[a]	8.9[b]	9.0	ND	6.1	ND
Patterson *et al.* (1997)	Control	28 d	10.6	ND	9.0[b]	9.6[b]	ND	ND	6.3	ND
	TPK (10%)		10.8	ND	10.4[a]	10.4[a]	ND	ND	6.0	ND
Fukata *et al.* (1999)	Control	21 d	10.6	ND	9.9	9.8	9.1	8.8	ND	ND
	FOS (0.1%)		10.7	ND	9.6	9.2	8.9	8.6	ND	ND
Spring *et al.* (2000)**	Control	10 d	9.3	.....Not determined.....		7.4	8.7	.....Not determined.....		
	MOS (0.4%)		9.1			7.6	8.5			
Fernandez *et al.* (2002)	Control	30 wk	10.0	ND	8.3	8.8	7.6	9.3	5.0	7.5
	MOS (2.5%)		10.0	ND	8.5	9.0	7.1	8.8	5.5	8.2
van der Wielen *et al.* (2002)	Control	14 d	ND	.....Not determined.....		9.1	7.8*	.....Not determined.....		
	Lactose (5%)		ND			9.3	8.2			
Kleessen *et al.* (2003)	Control	35 d	ND	9.1[a]	.....Not determined.....		8.3[a]*	ND	6.1[a]	ND
	TP (0.5%)		ND	8.2[b]			6.5[b]	ND	3.6[b]	ND
Xu *et al.* (2003)	Control	49 d	9.6[b]	ND	8.4[b]	8.4[b]	7.7[a]	.....Not determined.....		
	FOS (0.4%)		9.9[a]	ND	8.9[a]	9.1[a]	7.2[b]			
Yusrizal and Chen (2003)	Control	42 d	ND	8.9	ND	9.0	8.7[b]	.....Not determined.....		
	Inulin (1%)		ND	8.5	ND	9.1	7.6[a]			
	OF (1%)		ND	8.7	ND	9.0	7.7[a]			
Zhang *et al.* (2003)	Control	49 d	ND	8.5	ND	8.8	7.9	.....Not determined.....		
	IMO (0.6%)			8.4		8.8	7.9			
Cao *et al.* (2005)	Control	42 d	.Not determined		8.4[a]	10.1	6.9	11.2	4.3[a]	9.9[a]
	FOS (0.4%)				9.1[b]	10.4	6.6	11.0	3.0[b]	10.4[b]
Thitaram *et al.* (2005)**	Control	21 d	10.4	ND	9.1[a]	9.9[§]	ND	.....Not determined.....		
	IMO (1%)		10.7	ND	10.1[b]	10.3	ND			
Yang *et al.* (2007)	Control	14 d	8.9	ND	ND	8.4[§]	8.4	ND	5.1	ND
	MOS (0.2%)		9.1	ND	ND	8.8	8.7	ND	5.1	ND

LS = lactosucrose, STOC = sucrose thermal oligosaccharide carmel, TPK = thermally-produced kestoses, FOS=fructo-oligosaccharides, ND = not determined, MOS = mannan-oligosaccharides, TP = topinambur syrup, OF = oligofructose, IMO = isomalto-oligosaccharides, d = days, wk = weeks, * *Enterobacteriaceae.*, [ab]denote differences ($p<0.05$) for a reference. ** Birds were challenged with salmonella, [§]Total lactic acid bacteria

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Table 3 Influence of supplementation of different prebiotics on selected bacterial fermentation metabolites in jejunum and caecum of broilers.

Reference	Treatment	Segment	Age	Lactate	Ammonia	Short chain fatty acids (µmol/g of contents)			
						Total	Acetate	Propionate	Butyrate
Corrier *et al.* (1990b)	Control	Caecum	10 day	BDL	ND	105.3	73.1[a]	23.6	8.7[b]
	Lactose (5%)			8.6	ND	75.1	45.7[b]	26.0	4.4[b]
	Lactose (10%)			23.1[a]	ND	65.9	39.4[b]	26.0	0.5[a]
Hinton *et al.* (1990)	Control	Caecum	10 day	9.7[a]	ND	111.3	69.3[a]	29.3	12.8[a]
	Lactose (2.5%)			29.4[b]	ND	70.9	44.8[b]	23.0	3.1[b]
Hinton *et al.* (1991)	Control	Caecum	10 day	2.2[a]	ND	67.4	55.3	6.8[a]	10.2[a]
	Lactose (2.5%)			13.8[b]	ND	72.4	47.0	29.7[b]	3.3[b]
Terada *et al.* (1994)	Control	Caecum	60 day	4.7	150.0[a]	52.7	35.4[a]	12.0	5.3[a]
	LS (0.15%)			6.2	75.3[b]	95.4	70.0[b]	14.8	10.6[b]
Zhang *et al.* (2003)	Control	Jejunum	7 week	….Not determined….		4.7	4.0	0.5	0.2[b]
	IMO (0.3%)					1.6	1.2	0.1	0.3[a]
Zhang *et al.* (2003)	Control	Caecum	7 week	….Not determined….		59.5	47.6	2.0	9.9
	IMO (0.3%)					39.2	35.8	2.0	1.4
Cao *et al.* (2005)	Control	Caecum	42 day	….Not determined….		155.9	100.0	13.6	37.2
	FOS (0.4%)					150.8	108.0	12.0	35.9
Rehman *et al.* (2008a)	Control	Jejunum	35 day	41.6[a]	5.8	……..Not determined……..			
	Inulin (1%)			70.2[b]	6.2				
Rehman *et al.* (2008a)	Control	Caecum	35 day	ND	22.3[a]	98.2	72.0	16.6	7.3[a]
	Inulin (1%)			ND	13.9[b]	101.3	68.7	15.5	15.2[b]
Rehman *et al.* (2008b)	Control	Caecum	42 day	….Not determined….		60.7	40.7	6.0	7.4
	Inulin (1%)					57.6	37.6	6.5	9.5
Yang *et al.* (2007)	Control	Caecum	35 day	….Not determined…..		84.2	48.5	4.4	12.6
	MOS (0.2%)					81.5	45.6	3.8	12.4

BDL = below detection limit, ND = not determined, MOS = mannan-oligosaccharides, LS = Lactosucrose, [ab] denote differences ($p < 0.05$) for a reference

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Table 4 Caecal count of *Salmonella typhimurium* in broilers supplemented with lactose with or without caecal anaerobic microflora culture .

Reference	Challenge dose	Day of challenge	Dose (%)	Source	Age (day)	*Salmonella* count (log_{10}cfu/g)			
						Control	Lactose	CA	CA+Lactose
Hinton *et al.* (1990)	10^8	3	2.5	Water	10	6.1^b	4.7^c	5.2^c	3.3^d
	10^6					5.7^b	4.0^c	4.1^c	0.4^a
Corrier *et al.* (1990a)	$10^6/10^8$	3/13	7	Feed	40	2.58^a	ND	2.63^a	1.79^b
Corrier *et al.* (1990b)	10^6	3	2.5	Water	10	5.36^b	1.42^a	….Not determined….	
			5	Feed			1.82^a		
			10	Feed			1.59^a		
Hinton *et al.* (1991)	10^6	3	2.5	Water	10	6.06^a	2.69^c	3.9^b	0.51^d
Nisbet *et al.* (1993)	10^4	3	5	Feed	10	6.27^a	4.57^b	4.13^b	2.21^c
Nisbet *et al.* (1994)	10^4	3	3	Feed	10	4.81^a	3.38^{ab}	4.25^a	1.93^{bc}
			4				3.52^a		0.84^c

CA = Caecal anaerobic culture, * Birds were challenged on day 3 (10^6) and on day 13 (10^8)
Means with different superscripts for a same reference differ ($p < 0.05$) from control birds

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