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Advances in prebiotics for poultry: role of the caeca and oligosaccharides

Natalie K. Morgan[A,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Natalie K. Morgan
Curtin University, School of Molecular and Life Sciences, Kent Street, Bentley, WA 6102, Australia
Email: natalie.morgan@curtin.edu.au

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David Masters

ABSTRACT

Prebiotics are non-digestible carbohydrates that selectively stimulate the growth of beneficial bacteria. Prebiotic supplementation into poultry diets results in a decreased rate of pathogenic bacteria colonisation in the gastrointestinal tract. It also enhances production of volatile fatty acids and lactic acid, which provide the bird with energy. This results in improved host gastrointestinal health and productive performance. Oligosaccharides are the most notable prebiotics in poultry nutrition. Examples of prebiotic oligosaccharides include xylo-oligosaccharides, fructo-oligosaccharides, and galacto-oligosaccharides. Oligosaccharides are derived from hydrolysis of non-starch polysaccharides (NSP). They are manufactured from plant sources, synthesised by physiochemical methods or enzymatic processes. The effects of oligosaccharides occur primarily in the caeca; oligosaccharides bypass the small intestine and reach the caeca, where they are readily fermented by beneficial bacteria, such as those in family Lactobacillaceae and Bifidobacteriaceae. Caeca function is generally poorly understood, despite extensive reviews and studies in this field. A deeper understanding of the factors that influence ability of the caeca to effectively utilise oligosaccharides is warranted. This would allow new prebiotic products and NSP- degrading enzymes to be developed, targeted to specific diets and scenarios. This is required, given the lack of consistency observed in the outputs derived from different studies assessing oligosaccharide efficacy in poultry diets. A key hinderance to progression in this field is that authors rarely analyse the oligosaccharide content and composition in the test diets and products, or in the bird's gastrointestinal tract. This review examines the mechanisms behind how oligosaccharides induce prebiotic effects in poultry, by identifying the role of the caeca in NSP digestion and identifying the impact of oligosaccharides on caeca microbiota and short-chain fatty acid composition.

Keywords: caeca, enzymes, fibre, microbiota, non-starch polysaccharides, oligosaccharides, poultry, prebiotic, short-chain fatty acids.

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Introduction

The global demand for quality protein from poultry continues to increase. Meeting these demands requires development of feed technologies that beneficially affect animal performance and bird gastrointestinal health. This is particularly pertinent, given the current worldwide push to remove antibiotics from poultry feed. Application of prebiotics to poultry diets presents a promising approach. Prebiotics are non-digestible carbohydrates demonstrated to favourably manipulate the composition and fermentation patterns of gastrointestinal microbiota, selecting for beneficial species that profit the host (Ricke *et al.* 2020). The criterion for a qualifying prebiotic includes (a) not hydrolysed or absorbed in the upper gastrointestinal tract, (b) serve as a selective nutrient source for beneficial microbial communities in the gastrointestinal tract, and (c) induce physiological responses that benefit the host. Fermentation of prebiotics results in generation of short-chain fatty acids (SCFA), defined as the sum of volatile acids and lactic acid, which provide an important energy source for the bird and reduce gastrointestinal pH, thus inhibiting proliferation of acid-sensitive pathogenic bacteria species (Liu *et al.* 2021*a*, 2021*b*). The consequence is improved gastrointestinal and host health and resulting

enhanced bird productive performance. Oligosaccharides are the main source of prebiotics used in poultry diets. They are carbohydrates containing 2–10 monosaccharide units, derived from hydrolysis of non-starch polysaccharides (NSP). In recent years, there has been increased interest and research into the benefits of supplementing poultry diets with different types of oligosaccharides. The caeca are the primary site of oligosaccharide fermentation. To optimise the beneficial effects of oligosaccharides and develop improved and more consistent oligosaccharide products, it is necessary to better understand how the caeca utilise oligosaccharides.

Role of the poultry caeca in carbohydrate utilisation

It is well established that the caeca play a key role in carbohydrate digestion (McNab 1973; Svihus *et al.* 2013; Ramírez *et al.* 2022). This was identified almost a century ago by Radeff (1928), who observed that digestion of crude fibre was notably greater in birds with caeca than in those that had been caecectomised; 4% and 17% more crude fibre was digested from wheat and corn respectively, in the birds with intact caeca. Extensive research in this field has been conducted since, yet uncertainty still prevails about the role of the caeca in NSP utilisation. In contrast to readily digestible starches, NSP can by-pass the small intestine and reach the caeca undigested. NSP-degrading enzymes are frequently supplemented into commercial poultry diets to facilitate degradation of NSP into carbon sources that the microbiota can utilise. The form and circumstances under which these resulting NSP fractions can enter the caeca to be converted into SCFA are poorly understood. To achieve this, it is essential to first understand how the caeca operates and recognise factors that influence its efficacy as a site for carbohydrate fermentation.

Caeca anatomy

The caeca are paired blind pouches located at the junction of the ileum and colon. They are anoxic microbial habitats, hosting the highest microbial load and diversity of species in the gastrointestinal tract (Ramírez *et al.* 2022). Evolutionary selection for large caeca is observed in birds fed primarily plant-based diets (namely Galliformes and Anseriformes), whereas carnivorous birds have very small or no caeca (Hunt *et al.* 2019). This highlights that the caeca play a key role in fibre hydrolysis. The caeca in modern commercial broilers range from approximately 13 to 22 cm in length (N.K. Morgan and A. Wallace 2022, unpubl. data; Metzler-Zebeli *et al.* 2018). Caeca in laying hens are slightly smaller, at approximately 9–18 cm in length (Abdallah and Beshara 2015), possibly associated with their comparatively lower feed intake. The proximal region of the caeca contains villi, lymphoid cells, and goblet cells, supporting that nutrient absorption occurs here. In contrast, the medial and distal sections of the caeca contain poorly developed villi (Ferrer *et al.* 1991), suggesting that nutrient absorption does not readily occur in these regions. Compared with the small intestine, the caeca contain a higher abundance of lymphoid tissue (Casteleyn *et al.* 2010) and lower height and density of villi (Majeed *et al.* 2009; Elling-Staats *et al.* 2022). The presence of lymphoid nodules throughout the mucus membrane of the caeca provide evidence that the caeca play a role in maintaining gut immune homeostasis (Wickramasuriya *et al.* 2022).

A direct relationship between caeca anatomy and dietary carbohydrate content has been observed. For example, Longstaff *et al.* (1988) showed that caeca were heavier and longer in birds fed a diet with 200 g/kg pentoses and uronic acid than in those fed 200 g/kg glucose. Additionally, Józefiak *et al.* (2006) observed a significant increase in caeca weight and contents when a barley diet, with a higher soluble-NSP content, was fed than with an oat-based diet. Also, Rehman *et al.* (2008) found that feeding 1% inulin increased caeca weight. This highlights that caeca functionality may be dictated by the NSP content of the diet.

Caecal movements

Digesta from the ileum enters the colon, and then a portion of this digesta is pushed into the caeca through retrograde anti-peristaltic movements. Only soluble, low molecular-weight molecules can enter the caeca. This was illustrated by Björnhag and Sperber (1977), who found that nearly half of the particles present in ileal digesta were larger than 0.2 mm in size, but only 3% of the particles in caeca digesta were this large. The materials entering the caeca are filtered by a narrow opening and muscular ring of tissue anterior to the caecal opening, coupled with a meshwork of villi and ridges (Svihus *et al.* 2013). These materials are pushed towards the distal end and mixed by peristaltic movements by both circular and longitudinal contractions (Sacranie *et al.* 2007; Janssen *et al.* 2009). When the caeca are full, the amplitude of circular movements increases, heightening caecal digesta mixing through moving contents from the distal and proximal ends towards each other. Viscosity of the digesta influences its ability to enter the caeca. For example, Choct *et al.* (1996) observed that feeding as much as 6.6% viscous arabinoxylans into the diet did not increase caecal SCFA production, indicating that these materials were not able to enter the caeca. Similarly, Langhout and Schutte (1996) found that feeding viscous pectin had no impact on fermentation, on the basis of caecal SCFA concentration; however, feeding similar quantities of less viscous pectin did significantly increase fermentation.

Digesta retention in the caeca is relatively long, compared to retention in other gastrointestinal regions, due to infrequent emptying, allowing for increased fermentation, and thus heightened SCFA manufacture. This is illustrated by Warriss *et al.* (2004) who observed that digesta was still

present in the caeca of broilers that had been fasted for 24 h. Synchronised high-amplitude peristaltic movement of both the caeca and colon causes the caecal contents to be expelled. Antiperistaltic movements occur continuously in the colon, moving material from the anal opening into the caeca. This has been demonstrated by Sacranie *et al.* (2012), who injected Cr-EDTA soluble marker into the colon and observed that it was refluxed into the small intestine. This suggests that conditions in the caeca and lower digestive tract influence small-intestine microbiota composition. However, Glendinning *et al.* (2019) observed significant differences in microbiota composition among the duodenum, jejunum, ileum, and caeca, signifying that other factors may play a greater role in establishing ileal microbiota composition. Further research is warranted into the role that these antiperistaltic actions play in digestion of NSP.

Caeca microbiota composition

The gastrointestinal microbiome is recognised as a functional system of the bird, directly influencing animal health, productivity, and food safety. The largest concentration of microbial cells in the gastrointestinal tract are found in the caeca (Bindari and Gerber 2022). Bacteria are the primary component of the caeca ecosystem; Glendinning *et al.* (2020) concluded that 98.4% of the caeca microbiome originates from bacteria, 0.12% originates from Eukaryota (originated from viruses), and 0.31% originates form Archaea (single-celled prokaryotes). The most abundant phyla found within the caeca of adult birds are Firmicutes (Clavijo and Flórez 2018), with *Lactobacillus*, *Ruminococcus*, *Faecalibacterium*, and *Clostridium* being the most dominant genera within this phylum (Gong *et al.* 2002; Paraskeuas and Mountzouris 2019). The conformation of the caeca microbiota changes throughout the bird's life, dictated by diet and environmental conditions.

Impact of bird age on caeca microbiota composition

It is well established that microbiota composition changes with bird age, becoming more stable as the bird gets older. However, the exact age when it stabilises has yet to be determined. The identified bird age that caeca phylogenetic diversity stabilises has varied among studies, ranging from approximately 20 to 21 days (Torok *et al.* 2009; Ijaz *et al.* 2018; Kers *et al.* 2020), to 28 days (Lu *et al.* 2003) to over 42 days (Richards *et al.* 2019). Nonetheless, it is well established that initial inhabitation of microbes in the gastrointestinal tract occurs within the first 72 h post-hatch (Fathima *et al.* 2022; Pottenger *et al.* 2023). In modern commercial birds, the first bacterial inoculum is derived from the environment during incubation, hatching and delivery. Variability in these early environments explains why initial colonisation differs among birds from different hatcheries. For example, Kers *et al.* (2020) and Pedroso *et al.* (2016) presented that species of Clostridiaceae were the dominant species in the caeca of newly hatched birds, whereas Ballou *et al.* (2016) found species of Enterobacteriaceae to be the most abundant bacteria. Birds are then exposed to more diverse microbial environments on the farm, and ingest bacteria from the feed, water, and litter.

As the bird ages, the diversity of the caeca microbiota changes (Feye *et al.* 2020), transitioning from species that ferment and digest simple carbohydrates towards more complex structures, easing fibre fermentation (Stanley *et al.* 2013; Sergeant *et al.* 2014). This is because bacteria populate the upper intestine and remove the readily digested fermentable oligosaccharides and soluble polysaccharides, leaving the large intestine with NSP that are more difficult to digest, such as arabinoxylans and beta-glucans. Ballou *et al.* (2016) and Oakley *et al.* (2014) found that the caecal microbiota switched from being dominated by Enterobacteriaceae to being dominated by Firmicutes, such as Clostridiales, within the first 7 days of age. Also, Gong *et al.* (2008) found that caecal *Lactobacillus* content was 100 times higher at 3 days of age than at 42 days of age. Moreover, Lu *et al.* (2003) found that the dominant species in the caeca were *Clostridium saccharolyticum*, *C. oroticum*, and *C. orbiscindens* at 7 days of age, *Ruminococcus schinkii* and *Clostridium indolis* at 14–28 days of age and *Eubacterium* at 49 days of age. These outputs highlight a great deal of transition in caecal microbiota composition over the lifespan of the bird, addressing the importance of successful early colonisation of the caeca; founding of an efficient microbiota in the young bird may enhance its ability to face dietary and environmental challenges when older. For example, establishing a microbiota in young birds that is efficacious at NSP hydrolysis, by increasing the abundance of NSP-degrading bacterial species, could enhance dietary NSP utilisation when the bird is older. This was illustrated by Bautil *et al.* (2020), who found that supplementing wheat-based diets with 0.5% arabinoxylo-oligosaccharides sped up development of a fibre-fermenting microbiome, resulting in increased arabinoxylan solubilisation and fermentation in older birds. This approach of priming the microbiota in young birds to get targeted results in adult birds could potentially increase use of more economic and environmentally friendly sources of feed ingredients that are otherwise excluded from poultry diets due to their high NSP content. For example, Duke *et al.* (1984) adapted turkeys to either a low- or high-NSP diet and found that cellulose degradation in the adult bird was higher in those fed the high-NSP diet. However, it is not possible to provide a target optimum microbiota composition to aim for in young birds, because this is very much dependent on the conditions the birds are to be reared in. Notably, Stanley *et al.* (2013) ran three successive trials in which birds were obtained from the same breeder flock, were fed the same diet, and were reared in the same facility, and saw completely different caecal microbiota compositions. This suggests that measuring absolute values of different species may be meaningless. The

focus instead should be on the metabolites being produced and functionality of the microbiota. This should include measurements of oligosaccharides, focussing on both quantity and size/structure, in the diet and caeca. It may also be advantageous to measure endogenous enzyme activity, to confirm whether the oligosaccharides are stimulating NSP-degrading microbiota species.

Impact of dietary NSP on caeca microbiota composition

NSP are considered anti-nutrients because the soluble component increases intestinal viscosity, and the insoluble component acts as a nutrient diluent and physical barrier to the gastrointestinal lining, thus reducing nutrient digestion and absorption (Bedford 2018; Morgan *et al.* 2021). NSP composition of the diet has a notable impact on the conformation of the caeca microbiota. For example, Crisol-Martínez *et al.* (2017) compared wheat- and sorghum-based diets and found that *Clostridium leptum* predominated in the caeca of birds fed wheat, whereas strains of *Lactobacillus crispatus* and Lachnospiraceae were the most abundant in birds fed sorghum. Moreover, Borda-Molina *et al.* (2021) and Kim *et al.* (2022) observed that feeding broilers wheat resulted in a higher presence of Lactobacillaceae and Bifidobacteriaceae in the caeca than did feeding corn. Similarly, Rodríguez *et al.* (2012) observed increased caeca lactobacilli when feeding wheat and barley, compared with feeding corn. This is because grains such as wheat and barley are richer in NSP, primarily xylan and β-glucan, and products derived from hydrolysis of these NSP provide substrates for beneficial Gram-positive bacteria species. Lactobacillaceae and Bifidobacteriaceae have particularly high glycosidase activity, meaning that they can readily utilise selective oligosaccharide substrates from complex plant cell-wall substrates (Modrackova *et al.* 2020; Shini and Bryden 2022), converting them into SCFA. This explains why numerous studies have presented high levels of caecal Bifidobacteriaceae and Lactobacillaceae, often correlated with improved bird performance, when feeding NSP-rich diets to poultry (Apajalahti *et al.* 2001; Engberg *et al.* 2004; Józefiak *et al.* 2010; González-Ortiz *et al.* 2020). Borda-Molina *et al.* (2021) also observed increased levels of *Bacteroides xylanisolvens* in the caeca of wheat-fed birds. *B. xylanisolvens* is known to exert xylanolytic activity (Mirande *et al.* 2010), highlighting that feeding xylan results in increased proliferation of caecal bacteria that are adept at xylan degradation and endogenous xylanase manufacture. Moreover, Sergeant *et al.* (2014), using metagenomic analysis, identified a considerable array of genes in the caeca that encode for polysaccharide- and oligosaccharide-degradation enzymes. This highlights the capacity of the microbiota to instigate NSP hydrolysis. The resulting SCFA generated because of oligosaccharide fermentation can also directly influence microbiota composition. For example, van der Wielen *et al.* (2000) observed a negative correlation between caecal *Enterobacteriaceae* concentration and actetate, propionate and butyrate. Additionally, Liao *et al.* (2020) found that *Escherichia–Shigella* concentration in the caeca was positively correlated with isobutyrate, and caeca *Salmonella* was negatively correlated with isovalerate, butyrate and acetate content. These outputs reconfirmed that the microbiota can indeed be manipulated to be more efficacious at utilising dietary NSP.

Impact of NSP-degrading enzymes on caeca microbiota composition

Undigested nutrients that escape the upper digestive tract, due to the presence of NSP, become substrates for the caeca. These substrates can fuel pathogenic bacteria species (Apajalahti and Vienola 2016). NSP-degrading enzyme supplementation combats the anti-nutritional effects of NSP, facilitating removal of digestible nutrients from the diet early in the digestive tract. The consequence is restricted flow of nutrients into the caeca. This forces the caeca microbiota to adapt to use NSP as its primary substrate, instead of starch and readily fermentable carbohydrates, because NSP is the only carbohydrate source available. This highlights the importance of developing a microbiota that is efficacious at fibre-degradation as soon as possible. *Clostridiales* are particularly effective at degrading plant polysaccharides (Wang *et al.* 2021; Bedford and Apajalahti 2022), suggesting that promoting proliferation of this species should be a priority.

It is common practice to switch diets as a bird grows, so as to meet evolving energy and protein requirements. This means that birds receive different ingredient combinations as they get older, usually increased cereals and reduced protein meals. The microbiota is subsequently forced to quickly adapt to changes in nutrient availability directly following diet changes, as highlighted by Ijaz *et al.* (2018). The changes in NSP content and composition provided during these diet transitions are largely ignored, which is concerning given that NSP is the primary source of fuel for the caeca, as highlighted above. Pathogenic bacteria can proliferate in this unstable environment. One approach to minimise the likelihood of this is to provide an ongoing supply of fermentable substrates throughout the bird's lifetime, so that the microbiota receives a consistent source of fuel, irrespective of diet composition (Bedford and Apajalahti 2022). Supplementing diets with NSP-degrading enzymes increases the abundance of fermentable substrates available, through hydrolysis of NSP into oligosaccharides. For example, Rodríguez *et al.* (2012) found that addition of xylanase and β-glucanase to wheat- and barley-based diets increased the number of bifidobacteria in the caeca. Another approach is to supplement the diets with a source of fermentable fibre, such as wheat bran or oat bran, ideally in combination with enzymes, or to directly supply oligosaccharides. For example, Morgan *et al.* (2022*a*) observed that supplementing sorghum-based diets with 2000 mg/kg xylo-oligosaccharides (XOS), xylanase and fermentable fibre (wheat bran) increased caecal SCFA concentration and xylanase and cellulase activity in 35-day-old broilers. The process of developing a microbiota that is

efficacious at NSP-degradation takes time, which is why it must be established early, and why effects of dietary oligosaccharide and NSP-degrading enzyme application may only be seen in older birds.

Role of oligosaccharides as prebiotics

Oligosaccharides contain 2–10 monosaccharide units. These monosaccharides can be either linear or branched and are connected by α- or β-glycosidic linkages (Patel *et al.* 2014). Oligosaccharides are manufactured from plant sources, such as legumes, wholegrains and some cruciferous vegetables and fruits (Mussatto and Mancilha 2007). The NSP in these sources is hydrolysed into oligosaccharides, usually in the presence of enzymes. A review by Jahan *et al.* (2022) presents the sources and production techniques for common oligosaccharides used in poultry diets. Recently there has been increased interest into the benefits of supplementing poultry diets directly with oligosaccharides produced *in vitro*, as opposed to relying on the bird to manufacture them *in situ* in the digestive tract in the presence of supplemental enzymes (Morgan *et al.* 2019). As highlighted by Jahan *et al.* (2022) and in Tables 1 and 2, supplemental oligosaccharides have shown to improve bird gastrointestinal health and performance, but there is a lack of consistency among studies regarding the magnitude of their impact. This is due to variation in the size and structure of the oligosaccharides, which directly influences how and which microbiota species respond to them. The key issue with research in this field is that the majority of researchers do not measure the oligosaccharide concentration or composition (e.g. degree of polymerisation) in the diet, or in the gastrointestinal digesta. This makes it difficult to compare and interpret outputs from bird trials testing oligosaccharide efficacy. This needs to be rectified. Historically, measuring oligosaccharides has been time-consuming and expensive, requiring specialised equipment. However, there has been promising advances in this field in the past 5–10 years, particularly in analysis of XOS (Samanta *et al.* 2015; Ribeiro *et al.* 2018; Morgan *et al.* 2020). It is hoped this will improve the quality of oligosaccharide research in animal nutrition. Moreover, the lack of studies published in this field, particularly in laying hens, means that trends cannot yet be identified. A wider range of studies, featuring different diets, oligosaccharides derived from different substrates, and birds under varying degrees of challenge, are required. Availability of new methods for measuring oligosaccharides and increased publication of robust and repeatable studies in this field will shape development of improved and more consistent oligosaccharide products.

Properties of oligosaccharides

Diet type and enzyme treatment dictate the size and physiochemical properties of oligosaccharides. Endo-acting NSP-degrading enzymes randomly cleave the backbone of the target NSP, generating polymeric fragments. Successive hydrolytic events result in production of progressively smaller fractions (De Maesschalck *et al.* 2015). The aim is to manufacture oligosaccharides, as opposed to complete hydrolysis into monomers; unabsorbed pentose sugars stimulate microbial activity in the intestinal tract, causing inflow of water into the intestinal lumen via osmosis, resulting in increased moisture content in the excreta and potential litter-quality issues (Zyla *et al.* 1999; Mateos *et al.* 2012). Variability observed in response to supplemental oligosaccharides may be due to the presence of de-branching and exo-activities in the intestinal tract causing oligosaccharides to be broken down into constituent sugars, removing their prebiotic effects (Bedford and Apajalahti 2022).

Oligosaccharides generated from hydrolysis of NSP vary in degree of polymerisation (DP), monomeric units and types of linkages. An example are XOS, which are formed by xylose residues linked through β-(1→4)-linkages. The number of xylose residues in XOS vary from 2 to 10, forming xylobiose (X_2; $C_{10}H_{18}O_9$), xylotriose (X_3; $C_{15}H_{26}O_{13}$), xylotetraose (X_4; $C_{20}H_{34}O_{17}$) etc. (Morgan *et al.* 2020). Bacteria species vary significantly in their ability to use XOS of differing chain lengths (Aachary and Prapulla 2011); for example, bifidobacteria show a preference for shorter, un-substituted XOS, and struggle to use branched arabino-XOS (Okazaki *et al.* 1990). This was illustrated by Courtin *et al.* (2008), who found that adding 0.2% XOS to broiler diets had no effect on caecal enterobacteria and lactobacilli but did increase bifidobacteria concentration, after 1 week on the supplement. Dale *et al.* (2020) also observed that caeca bacteria prefer longer oligosaccharides, of DP 3 or larger. This knowledge can be used to develop oligosaccharide products tailored to specific scenarios, such as stimulating beneficial bacteria species that may otherwise be lacking.

The concentrations and structural features of NSP differ widely among different ingredients and batches of the same ingredient (Nguyen *et al.* 2021); for example, the distribution of branches along the main backbone may be more regular for some ingredients than others (Morgan *et al.* 2020). NSP structures also vary within different sections of the grain, such as the endosperm compared with the aleurone and bran (Burton and Fincher 2014). For example, Olukosi and Bedford (2019) found that response of wheat to xylanase varied greatly depending on the endosperm structure of the wheat. This means that it is not possible to predict *in situ* oligosaccharide manufacture in the gastrointestinal tract on the basis of the quantity of NSP or fibre measured in the ingredient being fed, as several factors influence response to enzyme application. Nonetheless, efficacy of NSP-degrading enzymes could be assessed by measuring the size and structure of oligosaccharides manufactured. This also reiterates that direct oligosaccharide application maybe more reliable than enzyme application alone.

Table 1. Effect of dietary supplementation of oligosaccharides derived from non-starch polysaccharides on poultry caeca microbiota composition.

Oligosaccharide	Oligosaccharide supplementation (mg/kg)	Species	Age	Diet	Effect	Reference
Arabinoxylo-oligosaccharides (AXOS)	0, 2000	Broiler	10–21 days	Wheat–soybean meal	• No effect of AXOS on microbiota.	Keerqin *et al.* (2017)
Arabinoxylo-oligosaccharides (AXOS)	0, 2000	Broiler	10–21 days	Wheat–soybean meal	• No effect of AXOS on microbiota.	Morgan *et al.* (2019)
Fructo-oligosaccharides (FOS)	0, 5000, 10 000	Layer	60–65 to 63–68 weeks	Corn–soybean meal	• Reduced viable number of *Salmonella enteritidis* with 10 000 mg/kg FOS.	Adhikari *et al.* (2018)
Fructo-oligosaccharides (FOS)	0, 4000	Broiler	1–35 days	Wheat–soybean meal	• Increased *Lactobacillus* and reduced coliform with FOS.	Akbaryan *et al.* (2019)
Fructo-oligosaccharides (FOS)	0, 2000, 4000, 8000	Broiler	1–49 days	Corn–soybean meal	• Increased total anaerobes and *Bifidobacterium* with 4000 mg/kg compared to 0 mg/kg FOS. • Increased *Lactobacillus* with 2000 and 4000 mg/g, compared with 0 mg/kg FOS. • Reduced *Escherichia coli* with 2000 and 4000 mg/g, compared with 0 mg/kg FOS.	Xu *et al.* (2003)
β- galacto-oligosaccharides (β-GOS)	0, 1000, 2000, 5000	Broiler	0–35 days	Corn–soybean meal	• Increased *Lactobacillus* with 2000 and 5000 mg/g β-GOS. • No effect of β-GOS on coliforms and *Clostridia*.	Yousaf *et al.* (2017)
Isomalto-oligosaccharides (IMO)	0, 5000, 10 000	Broiler	1–42 days	Corn–soybean meal	• Increased *Lactobaccilli* and *Bifidobacteria* at Day 21 with 5000 and 10 000 mg/kg IMO. • Increased *Lactobaccilli* at Day 42 with 10 000 mg/kg IMO. • Increased *Bifidobacteria* at Day 42 with 5000 and 10 000 mg/kg IMO. • Reduced *Escherichia coli* and total aerobes at Day 21 with 5000 and 10 000 mg/kg IMO. • Reduced *Escherichia coli* at Day 42 with 10 000 mg/kg IMO.	Mookiah *et al.* (2014)
Isomalto-oligosaccharides (IMO)	0, 3000, 6000, 9000, 12 000	Broiler	0–49 days	Corn–soybean meal	• No effect of IMO on microbiota.	Zhang *et al.* (2003)
Isomalto-oligosaccharides (IMO), raffinose oligosaccharides (RFO) and chitooligosaccharides (COS)	0, 3000 mg/kg (IMO and RFO) and 30 mg/kg COS	Broiler	1–56 days	Corn–soybean meal	• Increased *Bacteroidetes*, *Tenericutes*, *Euryarchaeota*, and *Spirochaetae* with IMO in the starter phase. • Increased *Lactobacillus* with RFO and COS. • Increased *Ruminocaceae* and *Lachnoclostridium* with IMO and RFO.	Chang *et al.* (2022)
Inulin, oligofructoses (OF), short-chain fructo-oligosaccharides (SCFOS), transgalacto-oligosaccharides (TOS)	0, 4000	Broiler	0–21 days	Dextrose–isolated soy protein	• SCFOS reduced *Clostridium perfringens*. • No effect of the oligosaccharides on *Bifidobacteria*, *Lactobacilli* or *Escherichia coli*.	Biggs *et al.* (2007)
Xylo-oligosaccharides (XOS)	0, 5000	Broiler	0–39 days	Wheat–rye–soybean meal	• Increased abundance of *Clostridium* cluster XIVa with XOS, including *Anaerostipes butyraticus*. • Increased *Lactobacillaceae* with XOS.	De Maesschalck *et al.* (2015)

(*Continued on next page*)

Table 1. (*Continued*).

Oligosaccharide	Oligosaccharide supplementation (mg/kg)	Species	Age	Diet	Effect	Reference
Xylo-oligosaccharides (XOS)	0, 100, 200, 300, 400, 500	Layer	28–36 weeks	Corn–soybean meal	• *Bifidobacterium* spp. increased linearly with increasing XOS. • *Escherichia coli* reduced linearly with increasing XOS. • No impact on total bacteria counts or *Lactobacillus*.	Ding *et al.* (2018)
Xylo-oligosaccharides (XOS)	0, 50, 2000	Broiler	0–35 days	Sorghum–soybean meal	• No effect of XOS on microbiota.	Morgan *et al.* (2022*a*)
Xylo-oligosaccharides (XOS)	0, 50, 2000	Layer	39–47 weeks	Wheat–corn–soybean meal	• *Bifidobacterium* concentration reduced when feeding 2000 mg/kg XOS.	Morgan *et al.* (2022*b*)
Xylo-oligosaccharides (XOS)	0, 1000, 2000	Broiler	0–35 days	Corn–soybean meal	• Increased *Lactobacillus* with 2000 mg/kg XOS.	Pourabedin *et al.* (2015)
Xylo-oligosaccharides (XOS)	0, 2000	Broiler	0–10 days	Corn–soybean meal	• Reduced caecal colonisation of *Salmonella enteritidis* with XOS. • *Lactobacillus*, *Roseburia* and *Clostridium* greater with XOS, in *Salmonella enteritidis*-infected birds.	Pourabedin *et al.* (2017)
Xylo-oligosaccharides (XOS)	0, 5000	Broiler	0–21 days	Corn–soybean meal	• No effect of XOS on microbiota.	Samanta *et al.* (2017)
Xylo-oligosaccharides (XOS)	0, 25, 50, 75, 100	Broiler	0–42 days	Corn–soybean meal	• No effect of XOS on microbiota.	Suo *et al.* (2015)
Xylo-oligosaccharides (XOS)	0, 200, 400	Layer	50–62 weeks	Corn–soybean meal	• Higher Firmicutes and lower Bacteroidetes with XOS. • Higher *Bacilli*, *Lactobacillales*, *Lactobacillaceae*, *Lactobacillus*, *Erysipelotrichia*, *Erysipelotrichales*, *Erysipelotrichaceae* with 200 mg/kg XOS. • Higher Bifidobacteriales (and derivatives Bifidobacteriaceae and *Aeriscardovia*) with XOS. • Lower Epsilonproteobacteria (and derivatives Campylobacterales, Campylobacteraceae, *Campylobacter*) with XOS.	Zhou *et al.* (2021)
Galacto-oligosaccharides (GOS) and xylo-oligosaccharides (XOS)	0, 1000	Broiler	1–70 days	Corn–soybean meal	• Reduced diversity of microbiota with XOS and GOS; increased *Ruminococcaceae, Barnesiellaceae* and *Acidaminococcaceae* and decreased *Bacteroidaceae* and *Lactobacillaceae*. • *Alistipes* dominant with GOS. • *Faecalibacterium* and *Bacteroides* dominant with XOS.	Yang *et al.* (2022)

Prebiotic effects of oligosaccharides

Oligosaccharides are not degraded by gastric acid or digestive enzymes and are not absorbed by intestinal mucosa (Abd El-Hack *et al.* 2020). This ensures that they reach the distal intestinal tract intact, where they can be fermented by probiotic bacteria in the caeca. The beneficial technological features of oligosaccharides include stability at acidic pH, heat resistance, ability to achieve significant biological effects at low daily doses, low calorie content and no toxicity (Carvalho *et al.* 2013).

Impact of oligosaccharides on caeca microbiota composition

Numerous studies have presented that feeding oligosaccharides directly modifies the caeca microbiota, as highlighted in Table 1. Similar changes to caecal microbiota composition have also been observed in response to dietary NSP-degrading enzyme application (Józefiak *et al.* 2010; Munyaka *et al.* 2016; González-Ortiz *et al.* 2020), verifying that the positive effects of application of these enzymes can

Table 2. Effect of dietary supplementation of oligosaccharides derived from non-starch polysaccharides on poultry caeca short-chain fatty acid (SCFA) concentration.

Oligosaccharide	Oligosaccharide supplementation (mg/kg)	Species	Age	Diet	Effect	Reference
Arabinoxylo-oligosaccharides (AXOS)	0, 2000	Broiler	16–21 days	Wheat–soybean meal	• Increased total SCFA and acetic, isovaleric, valeric, succinic and lactic acid with AXOS, compared with feeding 2000 mg/kg arabinoxylan.	Keerqin *et al.* (2017)
Arabinoxylo-oligosaccharides (AXOS)	0, 2000	Broiler	0–21 days	Wheat–soybean meal	• Increased total SCFA and acetic, butyric, isovaleric and lactic acid, compared with feeding 2000 mg/kg arabinoxylan.	Morgan *et al.* (2019)
Fructo-oligosaccharides (FOS)	0, 4000	Broiler	1–35 days	Wheat–soybean meal	• Increased acetic and butyric acid with FOS.	Akbaryan *et al.* (2019)
Fructo-oligosaccharides (FOS)	0, 4100	Broiler	28–42 days	Corn starch isolated soybean protein	• Increased valeric acid with FOS.	Cao *et al.* (2005)
Fructo-oligosaccharides (FOS)	0, 3750, 7500	Layer	100–105 to 101–106 weeks	Corn–soybean meal	• Increased total volatile fatty acids, propionate, butyrate, and lactic acid with FOS.	Donalson *et al.* (2008)
Isomalto-oligosaccharides (IMO)	0, 5000, 10 000	Broiler	1–42 days	Corn–soybean meal	• Increased total volatile fatty acids (VFA), total non-VFA, and acetic, butyric, lactic and succinic acid at Day 21 with 5000 and 10 000 mg/kg IMO. • Increased propionic acid at Day 21 with 10 000 mg/kg IMO. • Increased total VFA, total non-VFA, and acetic, lactic and propionic acid at Day 42 with 5000 and 10 000 mg/kg IMO. • Increased butyric acid at Day 42 with 10 000 mg/kg IMO.	Mookiah *et al.* (2014)
Isomalto-oligosaccharides (IMO)	0, 3000, 6000, 9000, 12 000	Broiler	0–49 days	Corn–soybean meal	• No effect of IMO on SCFA concentration.	Zhang *et al.* (2003)
Isomalto-oligosaccharides (IMO), raffinose oligosaccharides (RFO) and chito-oligosaccharides (COS)	0, 3000 mg/kg (IMO and RFO) and 30 mg/kg COS	Broiler	1–56 days	Corn–soybean meal	• Increased butyric acid and valeric acid with IMO.	Chang *et al.* (2022)
Soybean oligosaccharides (SBO)	0, 500, 2000, 3500, 5000	Broiler	0–42 days	Corn–soybean meal	• Increased formate with 5000 mg/kg SBO. • Increased acetate with 3500 mg/kg SBO.	Liu *et al.* (2021*a*)
Soybean oligosaccharides (SBO)	0, 6000	Broiler	0–49 days	Corn–soybean meal	• Increased acetic and propionic acid with SBO. • Reduced butyric and lactic acid with SBO.	Zhu *et al.* (2020)
Xylo-oligosaccharides (XOS)	0, 250, 1000	Broiler	0–29 days	Wheat, wheat bran–soybean meal	• Increased total SCFA and acetic, propionic and n-valeric acid concentration with XOS, compared with feeding xylanase (16 000 XU/kg).	Craig *et al.* (2020*a*)
Xylo-oligosaccharides (XOS)	0, 250	Broiler	0–21 days	Wheat, wheat bran, corn, wheat germ, barley–soybean meal	• Increased propionic acid with XOS.	Craig *et al.* (2020*b*)
Xylo-oligosaccharides (XOS)	0, 100, 200, 300, 400, 500	Layer	28–36 weeks	Corn–soybean meal	• Linear increase in butyrate and acetic acid concentration.	Ding *et al.* (2018)

(*Continued on next page*)

Table 2. (*Continued*).

Oligosaccharide	Oligosaccharide supplementation (mg/kg)	Species	Age	Diet	Effect	Reference
Xylo-oligosaccharides (XOS)	0, 500, 1000	Broiler	0–21 days	Corn–soybean meal	• No significant effect of XOS but tended ($P < 0.01$) to decrease isobutyrate concentration.	Lin *et al.* (2022)
Xylo-oligosaccharides (XOS)	0, 50, 2000	Broiler	0–35 days	Sorghum–soybean meal	• Total SCFA and acetic acid higher with 2000 mg/kg XOS, compared with 0 and 50 mg/kg, only when wheat bran present. • Butyric acid higher with 2000 mg/kg XOS, compared with 0 mg/kg, only when wheat bran present. • Propionic and succinic acid higher with 2000 mg/kg XOS, compared with control. • Valeric acid higher with 2000 mg/kg XOS, compared with 0 and 50 mg/kg. • No impact of XOS on formic, isobutyric, isovaleric or lactic acid.	Morgan *et al.* (2022*a*)
Xylo-oligosaccharides (XOS)	0, 50, 2000	Layer	39–47 weeks	Wheat–corn–soybean meal	• No effect of XOS on SCFA.	Morgan *et al.* (2022*b*)
Xylo-oligosaccharides (XOS)	0, 1000, 2000	Broiler	0–25 days	Corn–soybean meal	• Increased acetic acid concentration with 2000 mg/kg XOS.	Pourabedin *et al.* (2015)
Xylo-oligosaccharides (XOS)	0, 50, 100	Broiler	0–42 days	Corn–soybean meal	• Increased total SCFA and acetic acid with 100 mg/kg XOS.	Singh *et al.* (2021)
Xylo-oligosaccharides (XOS)	0, 150	Layer	74–82 weeks	Corn–soybean meal	• Increased acetic acid concentration with XOS.	Xiao *et al.* (2020)
Xylo-oligosaccharides (XOS)	0, 2	Broiler	0–42 days	Corn–soybean meal	• Increased acetate and butyrate with XOS.	Yuan *et al.* (2018)

be attributed to oligosaccharide production, not just elimination of the anti-nutritional effects of NSP. Oligosaccharides have bifidogenic properties, promoting the growth of beneficial bacteria such as *Bifidobacterium adolescentis*, *B. longum*, *Lactobacillus brevis* and *L. fermentum* (Moura *et al.* 2007; Pourabedin *et al.* 2015). For example, Ding *et al.* (2018) observed a linear increase in bifidobacteria concentration in the caecum of White Lohmann laying hens with increasing dietary XOS supplementation, ranging from 0% to 0.05%. This confirmed that oligosaccharides cause beneficial bacteria species to be more dominant, meaning that they can competitively inhibit the growth of harmful bacteria, such as *Escherichia coli*. This was also illustrated by Xu *et al.* (2003) who observed that supplementing 4 g/kg fructo-oligosaccharides to corn–soybean-based diets increased bifidobacteria and lactobacilli and decreased *E. coli* concentration in the caeca of broilers. However, it should be noted that a dosage of 4 g/kg oligosaccharides is very high; as illustrated in Tables 1–3, most poultry studies test lower supplementation levels, rarely exceeding 2 g/kg. It may be disadvantageous to supplement high volumes of oligosaccharides into research diets, as these diets are likely to be unbalanced, and these high quantities would not be used in a commercial setting due to costs. Bird studies testing high supplementation levels of oligosaccharides are valuable for observing the mechanisms of their effect and how they respond in the gastrointestinal environment, but it is equally important to test levels that could be applied in commercial diets. Reduced caecal *E. coli* has also been observed when feeding broilers mannan-oligosaccharides (MOS; Baurhoo *et al.* 2007). However, care should be taken when interpreting data from research measuring bird responses to MOS, because the source of MOS in these studies is often yeast cell walls, as opposed to MOS derived from soybean meal. This is important because MOS derived from yeast have a different structure and are largely insoluble, so are polymeric, not oligomeric (Bedford and Apajalahti 2022). Researchers need to clearly define the substrate the oligosaccharides are derived from, measure the oligosaccharide concentration in the diet, and ensure test diets are balanced when designing and publishing studies in this field.

The preference of different bacteria species for different oligosaccharides was presented by Rycroft *et al.* (2001), in which *in vitro* fermentation of seven oligosaccharides

Table 3. Effect of dietary supplementation of oligosaccharides derived from non-starch polysaccharides on non-starch polysaccharide (NSP) utilisation.

Oligosaccharide	Oligosaccharide supplementation (mg/kg)	Species	Age	Diet	Effect	Reference
Arabinoxylo-oligosaccharides (AXOS)	0, 5000	Broiler	0–35 days	Wheat–soybean meal	• Increased water extractable arabinoxylan (AX) digestibility, determined in the ileum and caeca. • Total AX in ileum, and water extractable AX in the ileum and faeces, reduced by XOS.	Bautil *et al.* (2020)
Xylo-oligosaccharides (XOS)	0, 250, 1000	Broiler	0–29 days	Wheat–soybean meal	• Increased ileal NSP degradability with 1000 mg/kg XOS (determined from water-unextractable rhamnose, fructose, arabinose and galactose concentration). • Water-extractable fructose higher with 250 mg/kg compared to 1000 mg/kg XOS.	Craig *et al.* (2020*a*)
Xylo-oligosaccharides (XOS)	0, 50, 2000	Layer	39–47 weeks	Wheat–corn–soybean meal	• Increased ileal soluble NSP degradability with 2000 mg/kg and 10% wheat bran.	Morgan *et al.* (2022*b*)

(fructo-oligosaccharides (FOS), inulin, XOS, lactulose, isomalto-oligosaccharides, galacto-oligosaccharides (GOS) and soybean-oligosaccharides) by faecal bacteria was assessed, along with resulting SCFA production. All prebiotics were shown to increase bifidobacteria numbers, but total bacteria numbers were increased only by inulin and XOS. Additionally, it was observed that FOS produced the highest populations of lactobacilli, GOS resulted in the greatest decrease in Clostridia numbers, and caecal SCFA generation was highest with lactulose and GOS. Moreover, Yang *et al.* (2022) supplemented corn-based diets with either 1% GOS or 1% XOS and found that *Alistipes* were the dominant bacteria in the caeca of birds fed GOS, but *Faecalibacterium* and *Bacteroides* were dominant in birds fed XOS. These outputs suggest that there may be merit in using more than one oligosaccharide in a diet, to fuel a wider range of beneficial bacteria species. Further research is warranted into which oligosaccharide combinations are optimal, on the basis of the composition of the diet.

Dietary oligosaccharide application is expected to be most beneficial in broilers at an age of approximately day 10–20, given that this is when the caeca microbiota is rapidly changing, coupled with a likely change in diet from starter to grower. This is also the time when the microbiota is transitioning from dependence on starch and readily fermented carbohydrate sources to NSP, suggesting that nutrient availability may be limited, so an extra source of fuel would be beneficial. Alongside improved microbiota composition, oligosaccharides can also favourably manipulate the physical structure of the gut. For example, Ayman *et al.* (2022) and Li *et al.* (2019) found that supplementing chitosan-oligosaccharides into corn-based diets increased intestinal villus height and villus:crypt ratio. The mechanisms behind how oligosaccharides influence these parameters is not yet understood but could be associated with their ability to reduce bacteria proliferation in the lumen and produce SCFA, enhancing ability of epithelial cells to proliferate.

Impact of oligosaccharides on short-chain fatty acid production

Measurement of SCFA concentration in the caeca can provide an indicator of NSP degradation. For example, Józefiak *et al.* (2004) observed that grain sources with varying NSP compositions responded differently to supplementation with combined xylanase, β-glucanase and protease, verified on the basis of resulting differing caecal SCFA content and concentration. Caecal fermentation of readily fermentable carbohydrates, such as starch and saccharides, typically results in production of lactate, but utilisation of more complex NSP, by families of bacteria species, such as *Ruminococcaceae* and *Lachnospiraceae*, promotes acetate and butyrate production (Mahmood and Guo 2020; Bedford and Apajalahti 2022). Lactate produced in the ileum can either be directly absorbed and used as an energy source or enters the caeca where lactate-utilising bacteria ferment it further into another SCFA, usually butyrate (Broekaert *et al.* 2011; De Maesschalck *et al.* 2015). This cross-feeding mechanism is beneficial to the bird because butyrate increases intestinal epithelial integrity by fuelling epithelial cells, displays anti-inflammatory properties, and increases the metabolic activity of the microbial ecosystem (Guilloteau *et al.* 2010; Canani *et al.* 2011). Luminal pH is also likely to be stabilised by the relationship between lactate-producing and lactate-utilising bacteria (Belenguer *et al.* 2006). A more acidic pH is favourable in the gastrointestinal tract because it prevents colonisation by pH-sensitive pathogenic bacteria, thus promoting growth of beneficial bacteria (Fathima *et al.* 2022). The high content of SCFA in the caeca means that it is usually slightly acidic, ranging from 6.3 to 6.9 in modern broilers (Asare *et al.* 2021).

Recent studies have presented beneficial effects of dietary supplementation of oligosaccharides on caecal SCFA concentration, as presented in Table 2. Zhu *et al.* (2022) fermented soybean-oligosaccharides using *in vitro* batch incubation inoculated with broiler caecal microbiota and observed that oligosaccharides increase SCFA production, namely propionic, butyrate and lactic acid, as well as gas production, suggesting bacterial growth-stimulating activities. This highlights the potential to use *in vitro* techniques, as opposed to just live-bird trials, to assess the efficacy of oligosaccharide products. Supplementing oligosaccharides into diets has been the most common route of administration, but a study by Singh *et al.* (2022) found that injecting 3 mg of xylotriose into broiler chicken eggs enhanced production of caecal acetate, butyrate, and total SCFA when measured in birds at 28 days of age, compared with the control. These studies highlighted the direct benefits of oligosaccharides on SCFA production and signified the need for research into new techniques and methods for assessing, developing, and administering oligosaccharides to poultry.

NSP-degrading enzymes stimulate caecal bacteria to produce endogenous NSP-degrading enzymes. This was shown by Bedford and Apajalahti (2018), Bautil *et al.* (2021) and Morgan *et al.* (2019) who observed that rearing birds on diets with xylanase resulted in a caecal microbiome with heightened xylanase activity, and generally increased xylan degradation. The same has also been observed with direct oligosaccharide application into diets; for example, Morgan *et al.* (2022*a*) observed increased caeca SCFA concentration and xylanase and cellulase activity from supplementing 2000 mg/kg XOS and wheat bran into sorghum-based diets fed to broilers. This suggests that the increased SCFA observed in the presence of oligosaccharides and NSP-degrading enzymes may be an indicator of the ability of these supplements to manipulate the microbiome to produce more endogenous enzymes, as opposed to just prebiotic effects. The ramifications of this on NSP degradability are still unclear, due to a lack of studies presenting this data, as highlighted in Table 3.

Gonzalez-Ortiz *et al.* (2019) recently proposed a new category of feed additives, stimbiotics, defined as products with the capacity to stimulate an NSP-degrading microbiome to increase NSP fermentability, at doses that are too low to contribute in a meaningful manner to an increased SCFA content. Supplemental oligosaccharides fall into this category. Bautil *et al.* (2019, 2020) and Morgan *et al.* (2022*a*, 2022*b*) presented the stimbiotic effects of XOS, with low dietary concentrations shown to stimulate production of endogenous xylanase in the caeca, resulting in enhanced degradation of xylan and increased SCFA manufacture. A stimbiotic can be the same molecule as a prebiotic, such as an oligosaccharide, but stimbiotics are fed at concentrations below that capable of directly supporting fermentation (Bedford and Apajalahti 2022). Moreover, response to stimbiotics can take multiple weeks (Morgan *et al.* 2021), whereas prebiotics are fermented straightaway, resulting in SCFA manufacture within hours of consumption (Pourabedin and Zhao 2015). Both stimbiotics and prebiotics will be used by similar bacteria species, but prebiotics are quantitatively fermented by the bacteria into SCFA, whereas stimbiotics stimulate growth and activity of the bacteria. Further research is warranted into the mode of action of stimbiotics, and factors that influence their efficacy, such as diet type, environmental challenges and bird age and physiological status.

Conclusions

Oligosaccharides stimulate proliferation of beneficial bacteria species in the caeca, resulting in increased production of valuable SCFA and heightened degradation of NSP. Application of enzymes to generate oligosaccharides *in situ* in the gastrointestinal tract is advantageous, but the effects are variable, as they are dictated by the NSP substrate in the diet. Direct application of oligosaccharides mitigates this issue. However, there is currently a deficit of data presenting the effects of oligosaccharide application in poultry. This could be attributable to how complex and time-consuming it can be to produce and measure oligosaccharides. This will improve, given the global interest in finding and testing alternative products to in-feed antibiotics, with oligosaccharides being a key contender. Presently, it is not possible to draw strong conclusions about why there is variability among studies testing the same oligosaccharide, or fully understand the mechanisms behind their effects. This is primarily because authors very rarely measure the quantity and composition of the oligosaccharides in the test diets. To rectify this, robust research trials that measure the oligosaccharides in the test diets, and preferably also in the digesta, need to be conducted. Different diets and scenarios need to be examined, as it may be that a more tailored approach to oligosaccharide application is required, primarily on the basis of the composition of the diet. This information will allow improved oligosaccharide products, with prebiotic and stimbiotic properties, to be developed for the poultry industry.

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Author affiliation
[A]Curtin University, School of Molecular and Life Sciences, Kent Street, Bentley, WA 6102, Australia.