Fructooligosaccharide as a prebiotic: A substitute for antibiotic growth promoters in poultry production

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Abstract
Researchers are investigating the potential of new natural antibiotics and alternative compounds to deal with rising drug resistance among microbial populations. These alternatives are intended to preserve a healthy gut microbiota, hindering pathogenic organisms from attaching in early stages of life. Prebiotics are non-digestible feed components that can act as natural supplements that targeted intestinal microorganisms can use. They provide health benefits for hosts, cutting mortality rates, increasing growth, and improving feed efficiency. Prebiotics may also exert effects on host metabolism and immunity by producing favourable shifts to the gut microbiome. This review examines the impacts of fructooligosaccharides as prebiotics on the gut microbiome and physical composition, focusing particularly on their effect on poultry growth performance. The review will also emphasize knowledge gaps in this field as well as possible directions for future research.

Keywords: Broiler, gut microbiota, growth performance, immune response, prebiotic

Introduction
Antibiotic usage in animal feed has been widely used as a growth promoter with successful results, but its use has led to resistant bacteria and antibiotic residues in product, which can be a risk to public health [1]. To reduce this risk, many countries have restricted the use of antibiotics and encouraged the search for alternatives such as prebiotics, probiotics, symbiotic, enzymes, herbs, and essential oils to maintain efficient poultry production while ensuring safety [2]. Prebiotics are indigestible carbohydrates that can be utilized by beneficial gut microorganisms and have been fed to broilers, including fructan, oligofructose, inulin, fructooligosaccharides, galactan, galactooligosaccharides, XOS, pectin, fiber components, and milk oligosaccharides. Refined functional carbohydrates such as MOSs, β-glucan, and D-mannose from Saccharomyces cerevisiae can also be prebiotics [3]. Selecting the appropriate prebiotic for animals requires considering factors such as its resistance to gastric acidity and ability to be broken down by enzymes and absorbed across the intestinal wall [4]. Prebiotics have been found to selectively enrich beneficial microorganisms associated with health, as most of its benefits are mediated by altering the intestines’ microbiota [5]. Studies have shown that prebiotic supplementation can improve growth performance and immunity in broilers, as well as significantly alter the intestine's microbial population, increase villi length and optimize nutrient absorption [6, 7]. Thus, prebiotics are an important tool for maintaining a healthy gastrointestinal system.

Prebiotics-General Concepts
Prebiotics have been evolving since their introduction [8], but currently, experts from ISAPP define prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [9]. When orally administered, they are referred to as dietary prebiotics [10]. The most recent definition of prebiotics is non-digestible carbohydrates demonstrated to manipulate the composition and fermentation patterns of the gastrointestinal microbiota, facilitating the growth of beneficial species that promote host health [11].
To qualify as a prebiotic, the carbohydrate must meet three criteria: it must not be hydrolyzed or absorbed in the upper gastrointestinal tract; it must serve as a selective nutrient source for beneficial microbial communities in the gut; and it must spark physiological responses which benefit the host. Fermentation of prebiotics leads to the production of short-chain fatty acids (SCFAs), made up of lactic acid and volatile acids. These SCFAs provide an energy source for birds while also lowering gastrointestinal pH levels, counteracting the proliferation of pathogenic bacteria species [12, 13]. Prebiotic products typically include oligosaccharides like fructooligosaccharides, mannoooligosaccharides (MOS), glucooligosaccharides (GOS), trans glucooligosaccharides (TOS), xyooligosaccharides, soybean glucooligosaccharides and lactulose [14, 15].

**Mechanisms of action of prebiotics**

Prebiotics are metabolized by commensal microorganisms, leading to positive health benefits for the host [9]. These benefits are mainly found in lower regions of the gastrointestinal tract, such as in the ceca of birds, though some microbial hydrolysis can occur in upper sections like the crop [4]. In addition, prebiotics can help control and stabilize multiplication of pathogenic microflora through competitive exclusion mechanism [10]. This mechanism reduces colonization of intestinal epithelium by bacteria toxins and improves local immune system activity and nutrition of where epithelial cells are located [17]. In addition to providing energy and carbon sources for microorganisms which reside primarily in the colon, prebiotics also affect proliferation of *bifidobacteria* species. This leads to a reduction in growth rate of detrimental microorganisms and removal of hazardous toxins or enzymes. It can further enhance performance in animals and birds as well as reduce blood pressure or cholesterol levels [18]. Additionally, prebiotics are known to affect vitamin synthesis, specifically folic acid, nicotinic acid, B1, B2, B6 and B12 [19, 20]. Prebiotics act as substrates that improve bacterial activity and may thus improve animals’ performances [15, 21]. It is believed that different bacteria produce different enzymes because they have various preferences for prebiotics (Wilson and Whelan, 2017). Previously, the impact of dietary prebiotic supplementation was typically gauged by assessing the rise in the populations of *Bifidobacterium* spp. and *Lactobacillus* spp. [8]. However, recent advancements in sequencing techniques have shown that prebiotics can influence a wider range of microorganisms via cross-feeding processes [9]. Prebiotics are metabolized by bacteria into organic molecules that the host can use, whereas antibiotics cannot. It is important to restrict prebiotics to compounds that influence the metabolism of existing microorganisms. Furthermore, any medicinal component or feed ingredient which enhances intestinal micro-ecosystems can be classified as a prebiotic [10]. Ideally, these prebiotics should be resistant to gastrointestinal absorption, enzymatic hydrolysis and gastric acidity, as well as being selectively metabolized by beneficial commensal bacteria; this should result in systemic or local benefits for the host [8]. The underlying mechanisms of improving poultry performance are mainly related to prebiotic-mediated modifications in the gut microbiota [23, 24]. Prebiotics mainly alter the GIT microbiota, increasing the abundance of microbial species while providing energy for fermentation processes. Through fermentation, prebiotics produce SCFAs, supplying energy for epithelial cells and decreasing luminal pH. Moreover, balanced bacterial populations confer trophic, protective, and metabolic benefits to the host via products that can influence physiological processes [25].

**Applications of prebiotic supplementation in broiler chickens**

In recent years, numerous research studies have been conducted to explore the effects of prebiotic supplementation on growth performance, carcass characteristics, gut morphology, gut microbiota and immune response in broiler chickens.

**Effects of prebiotics on growth performance**

Undoubtedly, one of the main objectives of using food additives in the poultry industry is to improve productive performance, which is a major indicator of poultry welfare and is closely linked to the efficient utilization of nutrients and, consequently, to production profitability. The major impetus for conducting related research is to replace antibiotics as growth promoters with prebiotics in order to observe improvements in poultry performance [26]. Prebiotics can potentially promote growth through increased production of SCFAs in poultry—largely acetate, propionate, and butyrate—which can be directly absorbed by the intestine and used as an energy source for tissues; they also elevate metabolic activity within the intestine [27]. SCFAs may act as powerful regulators of insulin homeostasis in chickens and carbohydrate metabolism thus stimulating metabolic activity in striated muscle cells and possibly having an effect on muscle protein synthesis and consequently growth performance [28].

**Body weight**

Previous studies have looked into the influence of dietary FOS supplementation on the growth performance of broiler chickens. Yusrizal and Chen, (2003) [29] observed that FOS improved body weight gain (BWG). This was also seen by Xu et al., (2003) [30] who supplemented a basal diet with 2.0, 4.0, and 8.0 g/kg of FOS; Yang et al. (2009) [31] similarly reported an improvement in BWG in antibiotic-free groups which had been supplemented with FOS at 21 d of age. Shang and Kim, (2016) [32] noted a positive effect on BWG after giving birds FOS as well. Moreover, Al-Surrayai and Al-Khalaifah, (2022) [33] found that body weight and weight gain were significantly higher when birds were fed prebiotics containing 0.3%, 0.5%, or 0.7% fructooligosaccharides (FOS). The results echo those of Froebel et al. (2019) [6]; Ibrahim et al. (2021) [7]; Kridtayopas et al. (2019) [34]; Mookiah et al. (2014) [35]; Rehman et al. (2020) [36]; Reznichenko et al. (2021) [37]; Salianeh et al. (2011) [38]; Tayeri et al (2018) [39]; and Wang et al. (2015) [40], which suggest that prebiotic supplementation leads to noteworthy improvements in both body weight and weight gain for broilers. However, some previous studies have yielded contrast results with the broiler performance of the FOS supplementation. Williams et al. (2008) [41] observed that daily live weight gain in the 0.6 g/kg FOS-supplemented group was lower than that in the control group. Kim et al. (2011) [42] however, found no difference between the 0.5% FOS-supplemented and control groups in terms of weight gain. Furthermore, Biggs et al. (2007) [43] and Telg and Caldwell, (2009) [44] reported no remarkable growth changes when broiler chickens were fed diets containing 0.4%, 0.8%, or 1% FOS respectively. Findings from recent studies have revealed that adding prebiotics to broiler diets does not have a significant impact on growth performance. Al-Khalifa et al. (2019) [45] demonstrated this when 5 g/kg of fructooligosaccharides...
(FOS) in the diet of broilers had no effect on body weight gain. Furthermore, Askri et al. (2022) [46] found no statistically significant difference in body weight or weight gain after administering three levels of prebiotics (1 g, 1.5 g, and 2 g) to a control group. Similarly, Salehimanesh et al. (2016) [47] showed that prebiotics did not account for any difference in body weight or growth between treatments (p>0.05). In addition, research of Maiorano et al. (2017) [48] established that there were no major distinctions in final body weight or weight gain between experimental groups that consumed two different types of prebiotics. As well, Waqas et al. (2018) [49] affirmed that the dietary prebiotic supplementation did not exert (p>0.05) body weight and body gain. Many factors such as age, sex, and health status of the birds, environmental hygiene, experiment protocols, and inclusion level of prebiotics all can affect growth performance Yang et al. (2009) [51].

Feed intake
The studies conducted by Abdel-Raheem and Abd-Allah, (2011) [50] and Froebel et al. (2019) [6] both observed that prebiotic supplementation improved feed intake compared to a control group (p<0.05). Riad et al. (2010) [51] reported that supplementing prebiotic at 1 g/kg diet doses significantly increased feed intake in comparison to the other groups. Supplementation with prebiotics has been shown to decrease gastric emptying time, which then resulted in an increased amount of feed intake by broilers, as indicated by Altaf-ur-Rahman et al. (2009) [52] and Abdel-Raheem and Abd-Allah, (2011) [50]. In contrast to these findings, some researchers found that supplementing prebiotics in the broiler diet decreases feed intake [53]. Kim et al. (2011) [42] reported that overall feed intake did not differ between the 0.5% FOS supplemented group and control group. Subsequent research by Al-Surryai and Al-Khalifaif, (2022) [53] also showed that different levels of FOS prebiotic treatments did not have any impact on feed intake. Continuing in this line, Al-Khalifaifa et al. (2019) [45] concluded that including 5 g/kg fructo-oligosaccharides (FOS) in a broiler chicken diet had no effect on feed intake either. Additionally, Maiorano et al. (2017) [48] demonstrated through their study that two different prebiotics failed to produce significant differences in feed intake among the experimental groups. Salehimanesh et al. (2016) [47] found that a prebiotic dose of 0.5 g/kg caused a decrease in feed intake for broiler chicks, echoing the findings of Salianeh et al. (2011) [58]. Similarly, Sarangi et al. (2016) [54] concluded that FOS prebiotics had no significant impact on cumulative feed consumption during their experiment period (p>0.05). Collectively, these studies illustrate the limited influence of prebiotic ingredients on the feed intake of broiler chickens.

Feed conversion ratio
In the study by Al-Khalifai et al. (2019) [45], it was found that supplementation of fructo-oligosaccharides (FOS) at 5 g/kg improved growth performance in broilers, with a greater feed gain ratio than those not given prebiotics (p<0.05). A positive effect on feed efficiency was also demonstrated by Shang and Kim, (2016) [52] when FOS were supplemented to the diet, as well as improved feed conversion ratios by Xu et al. (2003) [30] when FOS levels of 2.0, 4.0, and 8.0 g/kg were included. Similarly, Rehman et al. (2020) [56] observed enhanced FCR after additions of 1g and 1.5 g/kg prebiotics to the basal diet. Multiple scientific studies have demonstrated that supplementing with prebiotics, either in large or small quantities; can result in improved feed efficiency. This was confirmed by Abdel-Hafeez et al. (2017) [55]; Mookiah et al. (2014) [58]; Nipkiran et al. (2013) [56]; Riad et al. (2010) [51]; Hussein et al. (2020) [57]; and Žikić et al. (2011) [58], who observed a significant effect on the feed conversion ratio as a result. Generally, this improvement is due to an increase of beneficial microorganisms in the gastrointestinal tract following prebiotic supplementation, as explained by Çınar et al. 2009. On the other hand, Al-Surryai and Al-Khalifaif, (2022) [43] found that prebiotics fructooligosaccharides (FOS) at the rates of 0.3, 0.5, and 0.7% had no effect on feed conversion ratio (FCR). Williams et al. (2008) [41] reported no difference in FCR between the 0.6 g/kg FOS-supplemented group and the control group. Kim et al. (2011) [42] similarly showed that there was no difference in the feed conversion rate of the 0.5% FOS supplemented group compared to the control group. Maiorano et al. (2017) [48] observed a lack of differences in FCR among groups provided with two different prebiotic supplements, which was also corroborated by Sarangi et al. (2016) [54], where no variation in feed conversion ratio was found as a result of prebiotic supplementation. These outcomes agreed with several researchers who observed that adding prebiotics had no significant effect on feed conversion ratio [6, 38-40, 47, 59].

Livability
Kim et al. (2011) [42] and Froebel et al. (2019) [6] both reported that mortality did not differ between the 0.5% FOS-supplemented group or treatment groups and the respective control groups. However, Riad et al. (2010) [51] found a different result; supplementation prebiotic at 1 g/kg diet doses significantly decreased the mortality rate in comparison to other groups involved in their study. These results indicate that there is potential for prebiotic supplementation to increase performance and decrease mortality rates in broilers, although further research is needed to validate these findings. The differences in results of previous experiments regarding the growth performance of broiler chickens can largely be attributed to a variety of factors, such as genotype, environmental conditions, hygiene, management methodologies, nutritional system and diet composition. Furthermore, this variance may also be due to the lack of consistent research protocols and procedures employed in different studies. Significant discrepancies have been noted when comparing data from similar tests that had differing experimental designs or were collected with varying degrees of accuracy. As such, it is essential that researchers account for any potential variability among research materials and methodologies in order to ensure more consistent and accurate findings.

Effects of prebiotic on carcass traits
Supplementation of prebiotics to animal diets has been found to enhance performance as well as the quality of carcasses [60, 61]. Yusirizal and Chen, (2003) [29] determined that FOS supplementation increased the carcass weight in broiler chickens. Equally, Xu et al. (2003) [30] reported improved carcass traits when FOS was added to diet at concentrations of 2.0, 4.0, and 8.0 g/kg feed. Saiyed et al. (2015) [62] concluded that the addition of 500 g/tonne prebiotic to a basal feed significantly improved dressing percentage, abdominal fat weight, and abdominal fat percentage (p<0.05). Moreover, Zhao et al. (2016) [63] found that inclusion of a prebiotic in diets resulted in an elevated yield in carcasses among treatments. Wang et al. (2015) [40] conducted a study which yielded results that indicating a notable increase in carcass
traits with supplementation of prebiotics at 0.07%, 0.10%, and 0.13% in broiler chicken diet. Furthermore, Abdel-Hafeez et al. (2017) [55] additionally found, through significant statistical analysis (p<0.05), marked relative weight increase in the gizzard and proventriculus, spleen, bursa of Fabricius, and both caeca upon administering prebiotics to broilers. In contrast, several studies have investigated the impact of prebiotics as feed additives on carcass characteristics and meat quality, without finding any considerable difference as a consequence of supplementation. Sarangi et al. (2016) [54] evaluated the effect of prebiotics on the carcass traits of broiler chickens and found no significant change in dressing percentage, carcass percentage, heart weight, liver weight, gizzard weight, wing percentage, breast percentage, back percentage, thigh percentage or drumstick percentage (p>0.05). Additionally, Rehman et al. (2020) [36] looked at variables such as liver, pancreas, gizzard and heart weights and small intestine and cecum lengths without observing meaningful alterations due to dietary supplementation with prebiotics. Askri et al. (2022) [46] noted that there were no significant differences in carcass yields, breast muscle, and thigh weights (p>0.05), which was consistent with the previous study of Chumpawadee et al. (2009) [64]. Abu Shulukh et al. (2017) [65] also found that prebiotics had no significant influence on carcass parameters. Wang et al. (2015) [49] and other authors including Konca et al. (2009) [66], Mahmud et al. (2005) [67], Midilli et al. (2008) [68], and Salehimanes et al. (2016) [47] similarly showed no noteworthy changes in carcass yield when prebiotics were added to the diet in their respective reports. The existing literature does not yet provide a comprehensive understanding of how prebiotics as feed additives influence carcass characteristics and meat quality.

**Effects of prebiotic on gut microbiota**

The gastrointestinal microbiome is recognised as a functional system of the bird, directly influencing animal health, productivity, and food safety. The gastrointestinal microbiome is identified as a functional system of birds, directly impacting animal health, productivity, and food safety. The caeca of adult birds have been found to contain the highest concentration of microbial cells in the gastrointestinal tract [109]. Bacteria make up the bulk of this ecosystem; Glendinning et al. (2020) [70] determined that bacteria account for 98.4%, eukaryota (originating from viruses) account for 0.12%, and archaea (single-celled prokaryotes) contribute to 0.31%. The most abundant phyla include Firmicutes [71], with Lactobacillus, Ruminococcus, Faecalibacterium, and Clostridium being dominant genera in this phylum [72, 73]. However, the composition of the caeca microbiota is not static; it can be modified throughout a bird's life cycle due to diet and environmental factors. Studies have found that the use of prebiotics as an effective strategy to modify and regulate the gut microbiome is beneficial, especially when dietary FOS supplements are included. Research conducted by Choi et al. (1993) [74] indicated that broiler chickens supplemented with 0.22% fructooligosaccharides had raised levels of bifidobacteria and lactobacilli, as well as a decreased occurrence of Clostridia perfringens and Escherichia coli in their ileal content. Further biochemical and culture-based research demonstrated that FOS supplementation boosts gut fermentation, increases SCFA production, stimulates growth of beneficial bacteria like bifidobacteria and lactobacilli, while at the same time inhibiting development of pathogenic bacteria such as Salmonella spp., Clostridia perfringens, and Escherichia coli [5, 10, 42, 75]. Geier et al. (2009) [76] reported that providing FOS at a rate of 5 g/kg to Cobb 500 birds was associated with an elevated ileal Lactobacillus profile. Paraskeuas and Mountzouris, (2019) [77] observed a reduction in Salmonella in caeca of broilers; however, no significant alterations occurred for lactobacilli, clostridia, enterococci or anaerobic bacteria populations [77]. Discovered that supplementing 3% fructooligosaccharides to broilers caused a rise in lactobacilli numbers and reduced C. perfringens amounts found within caecal content. Conversely, Biggs et al. (2007) [43] did not detect any variation in Bifidobacterium, Lactobacillus, Clostridium perfringens or Escherichia coli populations after feeding 0.1-0.4% FOS to 21-day old chickens.

**Effects of prebiotic on gut morphology**

Assessing the gut morphology of birds is a key factor in determining their digestive tract health and performance. Stressors that affect the intestine can cause changes to the intestinal mucosa, like a decrease in villus length and an increase in crypt depth [78]. It is widely believed that increasing villus height and decreasing crypt depth could lead to improved digestive and absorption capacities due to increased absorption area and lower tissue turnover rate within the gastrointestinal tract [47, 69]. According to Xue et al. (2003) [30], supplementing with 0.4% FOS significantly elevated (p<0.05) ileal villus height, jejunum and ileal microvillus height, as well as VH: CD ratio while also reducing crypt depth in the jejunum and ileum. Shang et al. (2018) [79] observed that the supplementation of 0.4% FOS in broiler chickens' diets had a positive effect on their intestinal morphology, as indicated by enhanced villus height and microvilli in both the jejunum and ileum, whilst crypt height declined. Shang et al. (2015) [50] also found that villus height, crypt height, and total mucosal thickness improved in birds offered 0.5% FOS. Akbaryan et al. (2019) [77] further revealed an increase (p<0.05) of villus height and CD for the group supplemented with 0.4% FOS compared to the control group. Obianwuna et al. (2022) [81] similarly reported a significant increase (p<0.05), shown by greater villi heights, villi widths, ratio of villi height to crypt depth, and reduced crypt depth when birds were provided with 0.3% or 0.6% FOS respectively. These changes in the structures of intestinal mucosa are likely credited to FOS strengthening a favorable intestinal microbial environment [78]. However, Adhikari et al. (2018) [19] found that villus height, crypt depth, and their ratio were not significantly different between treatments when FOS was added to the broiler diet at a rate of 0.5% or 1%. Khodambashi Emami et al. (2012) [82] also observed that the groups supplemented with antibiotics had shorter crypt depths and higher VH: CD ratios compared to those supplemented with FOS.

**Effects of prebiotic on Immune response**

Many authors have cited that the use of prebiotics in poultry diets enhances bird immunity due to the preferential development of beneficial microbiota, resulting in greater production of substances such as bacteriocins and SCFA. Not only are these capable of hindering pathogen growth, they also act within the body's signalling pathway for the immune system [83, 84]. A study conducted by Rehman et al. (2020) [36] explored the effect of two levels of prebiotics (1g and 1.5 g/kg) in the basal feed on antibody titer for infectious bursal disease (IBD). Results indicated an improvement in IBD antibody titers compared to the control group (P = 0.026), which may be attributed to the interaction effect of prebiotics.
However, Saliarne et al. (2011) [38] determined that prebiotics did not influence antibody responses against IBD. Al-Khalifa et al. (2019) [45] conducted research to assess the effects of fructo-oligosaccharides (5 g/kg) and mannan-oligosaccharide derived from Saccharomyces cerevisiae (5 g/kg) on immune response in broilers, observing an increase in NDV antibody production with diets containing prebiotics (p<0.05). Salehimanesh et al. (2016) [47] obtained comparable results, determining that prebiotic supplementation at 0.9 g/kg in the base diet increased antibody titer against Newcastle virus disease in broiler chicken. Murarolli et al. (2014) [85] also noted a rise in antibody production against the Newcastle disease virus in the group given prebiotics. However, Rehman et al. (2020) [36] documented that two levels of prebiotic supplementation (1g and 1.5 g/kg) to a basal feed did not provoke any significant variation (p> 0.05) in antibody titer towards the Newcastle disease strain among various treatment groups of broiler chickens. Saliarne et al. (2011) [38] determined that prebiotics had no noticeable effect on antibody responses to the Newcastle disease pandemic; similar results were seen by Akbaryan et al. (2019) [77], with 4% FOS having no bearing on NDV titres.

## Effects of prebiotic on serum biochemical profile

Serum metabolites can be used to gauge the degree of organ or tissue damage. Tang et al. (2017) [86] discovered that there were no notable disparities (p> 0.05) between prebiotic-supplemented treatment groups concerning serum triglyceride, uric acid, total protein, and blood sugar levels. Similarly, Abdel-Hafeez et al. (2017) [55] noted that prebiotics did not affect globulin, albumin, total protein concentrations in serum or glucose measurements; yet it caused a decline in overall cholesterol compared to the control groups. Muhammad et al. (2020) [87] also established that prebiotic (0.5, 1 and 1.5 g/kg diet) did not significantly differ among the parameters of serum biochemistry and cholesterol levels. Alkhalf et al. (2010) [88] likewise determined that adding prebiotics to the broiler diet had no impact on serum albumin, globulin, and total protein or glucose values. In a study by Abdel-Wareth et al. (2018) [89], serum cholesterol and LDL-cholesterol levels were significantly reduced when chickens were fed a diet including prebiotics at 0.5 or 1 g/kg. Beski and Al-Sardary, (2014) [90] also reported that total cholesterol and LDL concentrations were lower in broilers who had a dietary prebiotic of 2.5 or 5 g/kg compared to those on the control diets. It is widely believed that prebiotics lower blood cholesterol levels by limiting intestinal lipid absorption due to bile acid binding. This, in turn, drives better cholesterol excretion from the body and increased hepatic synthesis of new bile acids [91, 92]. Scientists agree that liver synthesis of bile acids from cholesterol is one of the best ways for excreting cholesterol from the body [93].

## Conclusion

Fructooligosaccharide has the potential to serve as an alternative to feed-antibiotic in poultry, potentially improving their productive performance and health status. However, additional research is needed to be conducted under controlled conditions in order to fully understand its mechanism of action and determine the optimal dietary inclusion for optimum growth performance and healthy birds.

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