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Saccharomyces cerevisiae based postbiotics: Assessment of their effects on the health and productive performance of poultry

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Abstract
As drug resistance in microbial population increases, researchers are investigating novel natural antibiotics and alternative substances that can be employed safely in both humans and animals. These alternatives strive to sustain a healthy gut microbiome and obstruct the adhesion of harmful organisms at premature life milestones. The use of probiotics, prebiotics, synbiotics, and their derivatives such as para probiotics and postbiotics have proven to be effective in promoting growth and preventing enteric pathogens. Postbiotics have been observed to possess a range of beneficial properties, including immunostimulation, anti-inflammation, antioxidant abilities, antimicrobial effects and promotion of growth. Nevertheless, there is not much information about how postbiotics can be used in animals or the poultry industry; therefore this review article seeks to bring greater understanding to those possibilities.

Keywords: broiler, growth performance, gut health, immune response, postbiotic

Introduction
The global impact of antibiotics being utilized for the promotion of growth in poultry production has been immense. It has led to a shift in the intestinal flora of chickens, better disease control and immunization capacities (Lee *et al.*, 2012) [2], but also resulted in unchecked indiscriminate usage of antibiotics resulting in bacterial resistance and antibiotic residue being present in animal products, causing a health hazard for animals and humans alike (Gonzalez Ronquillo *et al*., 2022) [3]. Due to the necessity of it, European nations and many others have imposed a ban on using antibiotics in animal feed. As a response, alternatives that can still provide high levels of animal production are being looked into and explored (Diarra and Malouin, 2014) [4]. Recently, due to the need for alternatives to traditional antibiotics, there has been extensive research into utilizing animal antibiotic replacements in livestock production (Rafiq *et al.*, 2022) [5]. These include prebiotics, probiotics, synbiotics, and postbiotics, which have been subject to extensive research to tailor more specific and secure biological control products for the industry. Such substitutes can help mitigate antibiotic resistance issues while providing safe and efficient food for animals and humans. Recently, postbiotics have gained vast attention as preferred substitutes for probiotics due to their ability to reproduce the same effects as probiotics without having to use living cells (Choe *et al.,* 2012) [6]. Postbiotics have been found to possess numerous beneficial effects such as improved gut health, which is essential for animal nutrition; prevention of the expansion of pathogenic bacteria allows for optimal nutrient uptake and enhanced growth in livestock (Kareem *et al.,* 2014) [7]; and potentially even gastro-protective effects mediated by immunomodulation (Kareem *et al.,* 2014) [7]. Thus, postbiotics present a promising avenue in the research of novel biotechnological solutions for animal nutrition.

Concept of postbiotics
In 2019, the International Scientific Association for Probiotics and Prebiotics (ISAPP) gathered a team of specialists to assess the meaning and scope of postbiotics. The panel outlined a definition of postbiotics as “preparations of inanimate microorganisms and/or their components that confers a health benefit on the host” (Salminen *et al.,* 2021) [8]. In order for postbiotic preparations to be successful, they must comprise either inactivated microbial cells or their components, with or without accompanying metabolites that contribute to beneficial outcomes (Yelin *et al.,* 2019) [9]. This consensus from leading experts provides an important framework for understanding these complex preparations and their potential applications.

Different strains of microbes produce a number of soluble compounds, including enzymes, short-chain fatty acids, peptides, lactic and acetic acids, plasmalogens, endo- and exo-polysaccharides, ethanol, polyphosphates, teichoic acids, diacetyl, lactocepins, B vitamins, cell-surface proteins, muropeptides, hydrogen peroxide and teichoic acids (Rad *et al*., 2020) (Rad *et al.*, 2020) [10]. Various bacterial cultures were employed as paraprobiotics, and *Lactobacilli* and *Bifidobacterium* being the most popular. After inactivation, mainly with heat, they proved to be efficient. Cell wall components and cytoplasmic extracts taken from different species of *Lactobacilli* such as *L. acidophilus*, *L. casei*, *L. fermentum*, *L. rhamnosus*, *Lactobacillus reuteri* and *Lactobacillus johnsonii* were all found to be very effective postbiotics (Cicenia *et al.*, 2016; Johnson *et al.*, 2019) [11, 12]. In addition, *Faecalibacterium prausnitzii* (Iweala *et al.*, 2019) [13], and *Bacillus coagulans (*Abbas *et al.*, 2018) [14]; *Bifid bacterium* species (Timmer *et al.,* 2014) [15] are known for their postbiotic properties. Strains of *L. plantarum*, either alone or in combination, have been identified as the most frequent producers of postbiotics (Reuben *et al.,* 2021) [16]. Furthermore, *Saccharomyces cerevisiae* is used to generate postbiotics via anaerobic fermentation in a particular medium and subsequent liquid drying (Chan *et al.,* 2022) [17].

Effect of postbiotic on growth performance

Studies have shown that postbiotics are a viable alternative to antibiotics for encouraging the growth of chickens, with research results suggesting they can be as effective as or even more successful than antibiotics. The antimicrobial properties of postbiotics are believed to play a part in their effectiveness by limiting pathogenic bacteria in the gastrointestinal tract while promoting growth, similarly to antibiotics. Zeinali and Mohammadi (2022) [18] reported that supplementing feeds with fermented *Saccharomyces cerevisiae* at 0.1%, 0.3%, or 0.5% had a significantly better daily weight gain and feed conversion ratio compared to control groups (p<0.05). Gao *et al*. (2009) [19] conducted a study on the effects of adding *Saccharomyces cerevisiae* fermentation product to poultry diets on average daily gain and concluded that birds given the fermented product at 0.25% and 0.50% had significantly improved average daily gain compared to those fed only a basal diet (P=0.04); similar results were observed by Chaney *et al.* (2023) [20]. Furthermore, Chuang *et al.* (2021) [21], Yasar and Yegen, (2017) [22], Kang *et al*. (2015) [23], Linh *et al.* (2021) [24], Liza *et al.* (2022) [25], Roto *et al.* (2017) [26], M'Sadeq *et al.* (2015) [27] and Ismael *et al.* (2022) [28] found that supplementing with *Saccharomyces cerevisiae* fermented product improved growth and feed utilization, however this had no effect on livability or feed intake in any of these studies. Despite the potential growth-promoting properties of *Saccharomyces cerevisiae* fermented product (SCFP), multiple studies have been unable to conclusively establish its efficacy in this regard. Lensing *et al.* (2012) [29] and Nelson *et al.* (2018) [30] found no noteworthy disparity in body weight or feed conversion ratio after SCFP supplementation, while Cortés-Coronado *et al.* (2017) [31] observed no changes in final body weight, average daily gain, feed intake, feed conversion ratio or liveability even when different amounts of SCFP were tested. This is also reaffirmed by subsequent studies, including varying doses of SCFP such as 0.625, 1.250, and 2.500 kg/ton (Oliveira *et al.*, 2022) [32], 250 and 500mg/kg (Lin *et al.*, 2023) [33], 0.1% (Chuang *et al.* 2021) [21], and 0.0625%, 0.125% and 0.25% (Firman *et al.* 2013) [34].

Effect of postbiotic on Carcass traits

Carcass characteristics are a critical factor in broiler production, with numerous studies conducted to assess the impact of postbiotics on carcass traits. While most researchers conclude that postbiotics have no effect on carcass traits, the exact mechanisms behind this remain unclear. In the study conducted by Oliveira *et al.* (2022) [32], no significant difference (p>0.05) in carcass yield or breast yields was observed when 0.625, 1.250 and 2.500 kg/ton of *Saccharomyces cerevisiae* fermentation products were added to broiler diets.

Similarly, Zeinali and Mohammadi, (2022) [18] found that supplementing fermented *Saccharomyces cerevisiae* at 0.1, 0.3 and 0.5 percent levels did not have any significant impact on carcass traits, weight of thigh, breast, or wing (p>0.05). This further appears to be true when varying doses of SCFP are incorporated into diet formulations, such as 250, 750, 1,500 g/t (Aristides *et al.*, 2018)[35]; 5.0 and 10 g/ kg (Yasar and Yegen, 2017) [22]; 2, 3, or 4% (Linh *et al.*, 2021) [24]; 3.5% and 7% (Liza *et al.*, 2022) [25]. This finding is consistent across all studies, suggesting that postbiotics may not be directly influencing carcass traits after all.

Effect of postbiotic on Intestinal Morphology

Maintaining a healthy digestive system in broilers is imperative for optimum growth performance and good health, as it greatly facilitates nutrient absorption due to its increased surface area. Studies have shown that higher nutrient absorption rates in chickens are linked to a longer villus length, a shallower crypt depth, and an increased villus to crypt depth ratio. This ratio has been definitively proven to be beneficial in terms of nutrient uptake. Hence, improving this ratio can lead to improved nutrient absorption (Johnson *et al.*, 2019; Jha *et al.,* 2020) [12, 36]. Ismael *et al.* (2022) [28] and M'Sadeq *et al.* (2015) [27] both conducted studies with the integration of *Saccharomyces cerevisiae* fermented product (SCFP) at 0.625 kg/ton and *Saccharomyces cerevisiae* cell wall extract at 200, 400, and 800 mg/kg in broiler chicken diets, respectively. This resulted in significant differences seen in villi length, crypt depth, and villi length to crypt depth ratio when compared to the control group (*[*<0.05). Lin *et al.* (2023) [33], supplementation with 250mg and 500mg/kg of SCFP resulted in decreased crypt depth together with increased villus height and a higher villus crypt ratio compared to the control group (p<0.05). Additionally, Chuang *et al.* (2019) [37] found that administering 0.1% SCFP significantly raised villus height as well as the villus-crypt ratio (p<0.0001); however, there was a lessened crypt depth (p=0.0002). In contrast to the above observations, Chuang *et al.* (2021) [21] demonstrated that the postbiotics developed by the co-fermentation of *Saccharomyces cerevisiae* and phytase using wheat bran as a substrate at doses of 5% and 10% led to a significantly greater villus height (p<0.05); on the other hand, no significant difference was seen regarding crypt depth and the villus height and crypt depth ratio between all groups included in the study. Firman *et al.* (2013) [34] reported that *Saccharomyces cerevisiae* fermentation product (SCFP) at 0.0625, 0.125, and 0.25% had no statistical differences in villi height, or crypt depth.

Effect of postbiotic on Gut Microbiota

Several studies have found that postbiotics created by *Saccharomyces cerevisiae* offer multiple beneficial impacts on health and can inhibit a variety of gut pathogens such as *E.*

coli, Salmonella typhimurium, and *Vancomycin-resistant Enterococcus*, suggesting they might be used as an alternative to antibiotics. These postbiotics contain several antimicrobial components--including short-chain fatty acids, peptides, proteins, and organic acids--which reduce the pH level of the gut to prevent growth of pathogens and foster positive poultry health (Aguilar-Toalá *et al.*, 2018) [38]. In their study, Chuang *et al.* (2021) [21] reported increased concentrations of *Lactobacillus* spp. in the caecum in response to postbiotics formed by *Saccharomyces cerevisiae* at 10%, contrasting previous studies such as Chuang *et al.* (2019) [37] and Kang *et al.* (2015) [23], which found no significant enhancement or differences in *Lactobacillus*, *Escherichia coli*, and *Salmonella* levels with dietary supplementation of SCFP or fermented rice bran, respectively. In contrast, Roto *et al.* (2017) [26] and Gingerich *et al.* (2021) [39], found that adding SCFP at 1.25 g/kg and 1.5 kg/MT significantly reduced *Salmonella* concentrations compared to control groups, implying potential inhibitory effects of *Saccharomyces cerevisiae* on pathogenic bacteria when used at specific concentrations.

Effect of postbiotic on Immune Response

Feeding broilers with microbial fermented feed has been shown to increase lactic acid bacteria in their intestines, consequently boosting their immune function. Research conducted by Ismael *et al.* (2022) [28] indicates that augmenting the diet of broiler chickens with 0.625 kg/ton of *Saccharomyces cerevisiae* fermented product (SCFP) can significantly enhance the antibody titer in response to NDV vaccines in comparison to control birds, which is supported by similar findings reported by Cortés-Coronado *et al.* (2017) [31], Xiao *et al.* (2013) [40], Hand, (2020) [41]; Tukaram *et al.* (2022) [42] and Abd El-Ghany *et al.* (2022) [43]. Conversely, Danladi *et al.* (2022) [44] showed no notable differences between treatment groups when broiler chickens' postbiotic supplements were included in a basal diet, indicating the importance of accurate nutritional provision for a successful immune response.

Conclusion

Postbiotics have become increasingly popular as a potential feed supplement that could potentially enhance the health and productivity of birds. An in-depth analysis of the literature has displayed that postbiotic dietary supplements can significantly affect growth performance, gastrointestinal microbiome, gastrointestinal development, and immunity. This combination of biological activities makes postbiotics an appealing alternative to traditional chemical-based antimicrobial growth promoters in poultry diets.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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