Paraprobiotics and postbiotics: Contemporary and promising natural antibiotics alternatives and their applications in the poultry field

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Abstract

With the high rise of drug resistance in microbial populations, there has been a surge in researches to find new natural antibiotics alternative compounds that can be used safely in both humans and animals. The main goals of using this category of alternatives are maintaining the gut microbiome in healthy conditions and preventing the attachment of pathogenic organisms at the early life stages. Probiotics, prebiotics, and synbiotics have been widely used for several years as growth promoters and as preventive measures against several enteric pathogens with successful results. Recently, paraprobiotics and postbiotics are derivatives of probiotic cultures and have been used in humans, animals, and poultry. They are regarded as immunostimulators, anti-inflammatory, antioxidants, and anti-microbial, as well as growth promoters. Till now, there is scanty information about the use of paraprobiotics and postbiotics in animals or in the poultry sector. Accordingly, this review article has focused on defining these new categories of natural alternatives with descriptions of their types, functions, and uses, especially in the poultry field.

Keywords: Immunomodulation, Paraprobiotics, Performance, Postbiotics, Poultry.

Introduction

Due to the rapid increase in human population worldwide, there is a continuous demand for animal protein. Poultry meat is considered as one of the most important, available, and valuable sources of animal protein (Zuidhof *et al*., 2014). A lot of diseases can affect the poultry industry, causing severe economic losses. Antibiotics are used to prevent such diseases and are also used as growth promoters and feed additives to improve the growth performance parameters (Durso and Cook, 2014). Unfortunately, prolonged, indiscriminate, and extensive administration of antibiotics in the poultry field have raised concerns relating to residues in meat and egg products (Shareef *et al*., 2009), development of antibiotic-resistant bacterial populations (Shazali *et al*., 2014), and consequently, there is an adverse effect on the health of poultry and humans (Ajuwon, 2015). Therefore, many countries have banned the administration of antibiotics in the feed. In 2017, the Federal Government imposed the Food and Drug Administration Veterinary Feed Directive to reduce the use of antibiotics as growth promoters. Nowadays, the biggest challenge facing producers of poultry is how to increase the production parallel with ensuring the safety and health of the product. In view of the prohibiting antibiotics use as growth promoters, several natural alternatives have recently gained attention, they were also encouraged and experimented for production of safe and foodborne disease-free products. Paraprobiotics and postbiotics are natural antibiotics alternatives.

There is still a dearth of information regarding the use of paraprobiotics and postbiotics for improving the health and maintaining the immune status of the host, as well as preventing diseases conditions. Hence, the objectives of this review article were to give more attention on paraprobiotics and postbiotics regarding their definitions, types, functions, and their uses, especially in the poultry field.

Types of natural antibiotic alternatives

Microbiota inhibiting the avian gut have an important role in the nutritional, immunological, and physiological status of the host as their compositions and activities are affected by feed (Rehman *et al*., 2007). These microbiota are adversely affected by the prolonged use of antibiotics. "Biotic" feed additives, probiotics, prebiotics, synbiotics, paraprobiotics, and postbiotics, are some of the promising antibiotics alternatives. They have been used to alleviate the problems of using antibiotics, improve both the production and the intestinal health, as well as control enteric pathogens (Jahromi *et al*., 2016).

Probiotics are sufficient numbers of defined and viable live bacterial or fungal cultures that have beneficial health functions in the host (Fuller, 1989). Probiotics can induce many obvious functions through alteration of gut microflora, colonization in specific intestinal niches (Howarth and Wang, 2013), and modulation of both cell-mediated and humoral immune responses (Shang *et al*., 2008). Although prebiotics are indigestible feed ingredients by animals, they can enhance the growth of specific beneficial microbial population numbers and

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inhibit the pathogenic ones (Alloui *et al*., 2013). Some researchers have described prebiotics as nutraceuticals or functional foods (Roberfroid, 2007). It was found that prebiotics could control the growth of some pa thogens, like *Salmonellae* and *Escherichia coli* (*E. coli*), stimulate the growth of beneficial *Lactobacilli* and *Bifidobacteria*, and accordingly improve the health and performance of animals (Bogusławska-Tryk *et al*., 2012). Synbiotics are combinations of probiotics and prebiotics in which prebiotic selectively favors the probiotic (Schrezenmeir and De Vrese, 2001).

Despite numerous probiotics' notable beneficial effects, inconsistent results demonstrate that probiotics need to be more specialized for animal species and their overall efficacy is debated. Bacterial organisms of some probiotics show and acquire antibiotic-resistance genes encoded by plasmids that transfer between microorganisms (Gueimonde *et al*., 2013). Moreover, it was detected that probiotics can have a negative impact on the host by inducing local inflammation in healthy hosts and increasing the severity of tissue inflammation in the hosts with inflammatory bowel disease (IBD) (Tsilingiri *et al*., 2012). Subsequently, in the near future, probiotics might be completely substituted with paraprobiotics and postbiotics.

Definition of paraprobiotics
Probiotics contain both living and dead cells with a low shelf life (Lahtinen, 2012). It was found that the dead cells of probiotics could be fractionated and their metabolites showed a wide range of bioactivities (Adams, 2010). Inactivation of probiotics occurs by heat, chemicals such as formalin, radiation with gamma or ultraviolet rays, and sonication (Zorzela *et al*., 2017). The inactivation process may affect the structural composition of the cells and may influence their immunomodulatory activities (Taverniti and Guglielmetti, 2011). Accordingly; Taverniti and Guglielmetti (2011) proposed a term paraprobiotic (ghost probiotics) to define the non-viable, intact, or broken microbial cells or crude cell extracts (complex chemical composition), which, when taken in adequate amounts either through oral or topical routes, provide benefit to the human or animal consumer.

Paraprobiotics cultures
Different species of bacterial cultures were used as paraprobiotics. The most common paraprobiotic species are *Lactobacilli* and *Bifidobacterium* as they proved their efficacy after inactivation, especially with heat.

Function of paraprobiotics
Immunomodulation and anti-inflammatory
Paraprobiotics were found to maintain their immunomodulatory capacity beyond their viability (Taverniti and Guglielmetti, 2011). It was suggested that the killed probiotics may be as effective as the live ones in modulating inflammatory stimulation, after experiments in young rodent (Lopez *et al*., 2008). Dead probiotic bacteria showed different biological

responses in the stimulation of gut immune system, anti-inflammatory effects, reduction of interleukin (IL)-8 production, and stimulation of IL-6 production (Adams, 2010). Heat inactivation of *Lactobacillus casei*) and *Lactobacillus fermentum* revealed a modulation of inflammatory responses through regulating IL-10, B-defensin, and other pro-inflammatory cytokines, while inactivation of *Bifidobacterium breve* and *Bifidobacterium bifidum* showed an increase in the secretion of IL-10, which is an anti-inflammatory cytokine (Habil *et al*., 2014). Guglielmetti *et al*. (2008) proved that dead probiotics have an adhesive cell surface lipoprotein, which was detected in strains of *B. bifidum* and anti-inflammatory substances in *Lactobacillus acidophilus* and *Lactobacillus plantarum*. Inactivated preparations of cultures containing *Lactobacillus rhamnosus*, *L. plantarum*, *L. acidophilus*, *Lactobacillus helveticus*, *L. casei*, *B. breve*, *B. bifidum*, and *E. coli* induced effective immune responses (Taverniti and Guglielmetti, 2011). Heat-killed *L. rhamnosus* decreased lipopolysaccharides, induced pro-inflammatory mediators, as well as increased anti-inflammatory mediators in gastrostomy-fed young rats (Li *et al*., 2009). Several inactivated and non-viable probiotic cultures were found to be immune potentiators through different mechanisms, including increasing salivary gene expression (Kotani *et al*., 2010), modulation of T-cell responses (Hirose *et al*., 2006), and stimulation of IgA production (Konstantinov *et al*., 2008). The immunomodulatory effects of heat-inactivated *L. plantarum* strains were tested in humans and pigs and the results revealed that his type of paraprobiotic increased the innate and acquired immune responses due to the production of interferons type 1 and IL-12 (Hirose *et al*., 2006). In a gnotobiotic mouse model, heat inactivation of *B. breve* helped in immunemodulation and regulation which suppressed the production of pro-inflammatory cytokine production in spleen cells and affected the gene expression, as well as intestinal metabolism (Sugahara *et al*., 2017).

Antimicrobial and maintaining of gut health
Heat-inactivated *Lactobacilli* were found in the intestinal lumen (Maldonado Galdeano and Perdigon, 2004), which enhanced the gut epithelial barrier (Montalto *et al*., 2004) and inhibited the pathogen adhesion to the gut mucosa through competitive exclusion (Ouwehand *et al*., 2000). In newly hatched chicks, killed strains of *Enterococcus faecalis* prevented vancomycin-resistant *enterococcus* intestinal colonization (Sakai *et al*., 2006). In patients with viral hepatitis C, paraprobiotic of *E. faecalis* culture reduced alanine aminotransferase levels due to the change in microbiota (Oo *et al*., 2016). Inactive *Saccharomyces boulardii* shortened the duration of diarrhea in acute rotavirus gastroenteritis children after oral administration (Grandy *et al*., 2010). *Bifidobacterium lactis* treatment shortened the diarrheal period in acute rotavirus gastroenteritis in 5 months to

5-year-old children after oral rehydration (Erdogan *et al*., 2012).

Definition of postbiotics

Postbiotics are soluble non-viable metabolites produced by a bacterial or probiotic metabolic process (Tsilingiri *et al*., 2012; Klemashevich *et al*., 2014). So, postbiotics shared probiotics with the same mode of action but differed being non-living organisms (Thanh *et al*., 2009). Moreover, postbiotics are fed products such as organic acids and bacteriocins that are produced by beneficial intestinal microbiota and are able to reduce the gut pH, prohibit opportunistic pathogens proliferation, and consequently have a positive influence on host health (Aguilar-Toalá *et al*., 2018). Bacteriocins, short-chain fatty acids, peptides, and proteins are considered as postbiotics inhibitory metabolites (Cicenia *et al*., 2014). Postbiotics have been classified as promising alternatives to antimicrobials due to their multiple health benefits.

Postbiotics producers

The cell wall components and cytoplasmic extracts of various *Lactobacilli* species, including *L. acidophilus*, *L. casei, L. fermentum*, *L. rhamnosus, Lactobacillus paracasei, Lactobacillus delbrueckil subsp. Bulgaricus, Lactobacillus gasseri, L. helveticus*, *Lactobacillus reuteri*, and *Lactobacillus johnsonni*, were found to be highly effective postbiotics (Vidal *et al*., 2002; Matsuguchi *et al*., 2003; Choi *et al*., 2006; Kim *et al*., 2011; Cicenia *et al*., 2016; Tiptiri-Kourpeti *et al*., 2016; Johnson *et al*., 2019). In addition, *Bifidobacterium* species (Tejada-Simon and Pestka, 1999), *Faecalibacterium prausnitzii* (Sokol *et al*., 2008), and *Bacillus coagulans* (Jensen *et al*., 2010) showed postbiotics properties. Strains of *L. plantarum*, either alone or in combination, are the most common postbiotics producers (Thanh *et al*., 2009; Thu *et al*., 2011). The strains of *L. plantarum* demonstrated efficiency in rats (Loh *et al*., 2009), pigs (Loh *et al*., 2013), broiler chickens (Thanh *et al*., 2009; Loh *et al*., 2010; Rosyidah *et al*., 2011; Jahromi *et al*., 2016; Kareem *et al*., 2016, 2017), layer chickens (Loh *et al*., 2014), and ruminants (Izuddin *et al*., 2019), as well as humans (Kim *et al*., 2011). *Saccharomyces cerevisiae* is also a yeast species that has been used for the production of metabolites after anaerobic fermentation in a proprietary medium and drying of the liquid (Jensen *et al*., 2007).

Functions of postbiotics

Immunomodulation and anti-inflammatory

Postbiotics have exhibited immunostimulation properties. Both cell-mediated and humoral immune responses have been activated. The cell wall components and cytoplasmic extracts of *Bifidobacterium* sp. and *Lactobacilli*, including *L. acidophilus*, *L. casei*, *L. delbrueckil* subsp. *Bulgaricus*, *L. gasseri*, and *L. helveticus*, showed ability to stimulate immune cells and produced cytokines and nitric oxide *in vitro* (Tejada-Simon and Pestka, 1999). Moreover,

Lee *et al*. (2002) found that the purified cell wall supernatants and cell-free extracts of *Bifidobacterium* provoked the rapid activation of macrophages through the formation of actin filament and development of vacuole and cytokines production. The modulation of pro-inflammatory responses of the host by lipoteichoic acids of probiotics bacteria as *L. casei* and *L. fermentum* was proved (Matsuguchi *et al*., 2003). It was detected that *F. prausnitzii* exhibited anti-inflammatory effects on cellular and trinitrobenzenesulfonic acid colitis models as a result of secreted metabolites that block NF-kappa B activation and IL-8 production (Sokol *et al*., 2008). Jensen *et al*. (2010) suggested that the cell wall components and metabolites of *B. coagulans* can possess strong immune modulating effects *in vitro*; in addition, the anti-inflammatory effects and production of IL-10 are important in the treatment of IBD, as well as in enhancing healthy immune system. In this regard, Tsilingiri *et al*. (2012) showed that the cell-free supernatants of *L. paracasei* could protect against the inflammatory activities of invasive *Salmonella* in healthy tissues, as well as in lowering ongoing inflammatory processes in IBD tissues. Also, Kim *et al*. (2011) demonstrated that the lipoteichoic acids of *L. plantarum* attenuated the pro-inflammatory signaling induced by *Shigella flexneri* in human monocytic THP-1 cells, which showed by a significant reduction in tumor necrotizing factor (TNF)-α and IL-1β production. In the same context, feeding of lambs with metabolites of *L. plantarum* induced an increase in the level of IL-6 mRNA and decrease in IL-1β, IL-10, and TNF mRNA (Izuddin *et al*., 2019). The sonicated cell suspensions of *L. casei* proved inhibitory activity against colon carcinoma cells in mice and anti-proliferative and apoptotic cell death in cell lines (Tiptiri-Kourpeti *et al*., 2016). Cicenia *et al*. (2016) found that the postbiotic mediators of *L. rhamnosus* protected colonic human smooth muscle cells from lipopolysaccharides, which induced myogenic damage. Recently, other types of bacterial species were used for production of postbiotics. In broiler chickens, combinations of insulin and postbiotics revealed an upregulation of mRNA expression of IL-6 and reduction of expression of IFN and lipopolysaccharide-induced tumor necrosis factor-alpha factor gene (Kareem *et al*., 2017). Cultures of *Pediococcus acidilactici*, *L. reuteri*, *Enterococcus faecium*, and *L. acidophilus* were tested in broiler chickens and demonstrated great efficiency in improving the innate immunity if used alone; moreover, they inhibited the activation of immune response if used with pathogenic *Clostridium perfringens* (Johnson *et al*., 2019).

Antioxidant

The *in vitro* antioxidant effects of intracellular contents of *Streptococcus salivarius* subsp. *Thermophillus* and *L. delbrueckii* subsps. *Bulgaricus* (Ou *et al*., 2006), as well as *L. acidophilus*, *L. jonsonnii*, and *L. brevis* were studied (Ji *et al*., 2015). The results demonstrated

that these cultures inhibited lipid peroxidation and showed resistance to hydrogen peroxide and hydroxyl radical scavenging activity and glutathione peroxidase activity. Postbiotics exhibited antioxidant effects due to an increase in the content of uronic acid which has an antioxidant effect as well as the chelating capability of ferrous ion which is involved in the formation of free radicals. Moreover, the antioxidant action of postbiotics may be related to the production of antioxidant enzymes as glutathione peroxidase, superoxide dismutase, and nicotinamide adenine dinucleotide oxidase and peroxidase. Choi *et al*. (2006) detected that the soluble intracellular polysaccharide fraction contents of *L. acidophilus*, *L. brevis*, *L. casei*, and *L. rhamnosus* could be considered as anticancer substance due to selectivity for human cancer cells and antioxidative effect in the food industry. The antioxidant activity of *S. cerevisiae* metabolites was demonstrated in human leukocytes (Possemiers *et al*., 2013). In poultry, *L. plantarum* producing postbiotics showed antioxidative activity under heat stress (Humam *et al*., 2019).

Anti-proliferative
Postbiotics showed anti-proliferative activity against cancer cells via regulation of immune response. The sonicated cell suspensions of *L. casei* proved inhibitory activity against colon carcinoma cells in mice and anti-proliferative and apoptotic cell death in cell lines (Tiptiri-Kourpeti *et al*., 2016). These types of bacteria produce some inhibitory components like proteins, nucleic acids, or lipopolysaccharides, which reduce the activity of metalloproteinase-9 activity and consequently inhibit the cancer colon. The postbiotic mediators of *L. rhamnosus* protected colonic human smooth muscle cells from lipopolysaccharides which induced myogenic damage (Cicenia *et al*., 2016). Some postbiotics produce fatty acids such as butyrate, propionate, and acetate, which are very important for homeostasis of energy balance, cholesterol, and glucose, as well as regulation of lipid metabolism.

Antibacterial
It was demonstrated that lipoteichoic acids from *L. johnsonni* and *L. acidophilus* were able to maintain gut homeostasis and were used as a treatment of diseases mediated by Gram-negative bacteria or their components (Vidal *et al*., 2002). Postbiotics metabolites of *L. plantarum* with prebiotic (inulin) showed great inhibitory effect of different bacterial pathogens such as *E. coli*, *Salmonella typhymurium*, vancomycin-resistant *enterococci*, and *Listeria monocytogens* (Kareem *et al*., 2014). Supernatants of *L. paracasei* cells protected human healthy tissues from invasive *Salmonella* and downgrading the ongoing inflammatory processes in tissues with IBD (Tsilingiri *et al*., 2012). Postbiotic mediators of *L. rhamnosus* that were collected at different growth stages protected colonic mucosal cells from Gram-negative bacteria (Cicenia *et al*., 2016). The fermentation products of *S. cerevisiae* cultures clearly showed promising successful results regarding the increase in the animal's growth rate, as well as the decrease in the intestinal colonization of pathogens. It was proved that *S. cerevisiae* fermentation metabolites reduced the colonization of both *S. typhimurium* and *Campylobacter coli* in pigs and broiler chickens (Price *et al*., 2010; Hofacre *et al*., 2015; Feye *et al*., 2016; Rubinelli *et al*., 2016). Johnson *et al*. (2019) concluded that water treatment of broilers with postbiotics metabolites of *P. acidilactici*, *L. reuteri*, *E. faecium*, and *L. acidophilus* in the presence of pathogenic *C. perfringens* provoked the activation of immune responses to this pathogen.

Intestinal health and performance parameters
Postbiotics maintain the gut health, improve the intestinal mucosal barriers integrity, and enhance the inflammatory mediator secretion. It was found that the addition of postbiotics to broiler rations improved the intestinal villi, increased production of lactic acid bacteria, and reduced *Enterobacteriaceae* and fecal pH, resulting in increased growth performance parameters and enhanced immune response and gut health (Thanh *et al*., 2009, Loh *et al*., 2010; Rosyidah *et al*., 2011; Kareem *et al*., 2017). In lambs fed postbiotics produced from *L. plantarum*, there were improvements in the height and width of rumen papillae and improvement in the integrity of the intestinal barrier (Izuddin *et al*., 2019). In piglets, liquid metabolite combinations produced by *L. plantarum* helped in increasing the growth performance parameters, including birth weight, overall weight gain, and average weight gain per day, as well as reduction in diarrhea production (Loh *et al*., 2013). Similarly, in layers, these metabolites contributed to improving egg quality and reduction in plasma and yolk cholesterol (Loh *et al*., 2014). Kareem *et al*. (2016) demonstrated that the addition of a combination of postbiotics and prebiotic containing inulin in the ration of broiler chickens improved the total body weight and the feed efficiency, and maintained the intestinal mucosal structure, growth factor 1, and growth hormone receptor mRNA expressions. Feeding of broiler chickens with postbiotics produced by *L. plantarum* indicated improvement of growth performance, intestinal morphology, and maintained gut microbiota, even under heat stress conditions (Humam *et al*., 2019).

Conclusion
The overall numerous health benefits arising from the use of paraprobiotics and postbiotics in humans, animals, and poultry can be classified as favorable alternatives to antibiotics. As the types and exact mechanisms of paraprobiotics and postbiotics are not fully elucidated, further research work and additional information are necessary to discover other cultures' sources and understand the characteristics and mode of actions especially in the field of poultry.

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