Polyphenolic phytochemicals as natural feed additives to control bacterial pathogens in the chicken gut

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Introduction

Improvements in white meat productivity have increased significantly in the recent years, to meet the continuous increase in local and global demand for white meat and feed the growing population (Delgado 2005). As a result, huge efforts have been put to achieve higher level of effectiveness in poultry production such as improving diet and husbandry practices (Thornton 2010) which collectively led to improving feed conversion ratio (FCR) to 1.4 (Science 1999). High-quality diet costs around 60% of the production costs, but it increases the standards of poultry production due to its ability to maintain healthy intestines of these birds and avoid developing pathogenic diseases (Porter 1998). These transformations had positive impact on modern poultry industry such as decreasing the time required to get to the commercial weight (Zuidhof et al. 2014), improving food economy and ensuring its sustainability (Flock and Preisinger 2002), and reducing environmental pollution (Gerber et al. 2007).

Antibiotics used as a prophylactic agent have shown to have positive effects on the growth performance of chicken as a presumed result of reduced pathogen load, reduction in competition for nutrients in the small intestine, reduction of inflammation, and improvement of digestion (Thomke and Elwinger 1998). Also, they were used to fight bacterial infections as a therapeutic drug and at sub-therapeutic levels as feed ingredients, because they were shown to enhance growth, but unfortunately this led to the rise of the first incident of resistant Salmonella enterica ser. Typhimurium in 1963 (Dewey et al. 1997). The growth promoting effect of antibiotics was discovered in the 1940s (Hughes and Datta 1983), and later in the 1950s and 1960s, they were authorised with set guidelines by the EU to be used in animal feeds (Castanon 2007), which also made a contribution to an improved poultry productivity (Bunyan et al. 1977), but this resulted in negative consequences of selecting highly resistant bacteria leading to the emergence of global antibiotic resistant bacteria (ARB).
carrying antibiotic resistant genes (ARG) which was marked in the 1980s (Aarestrup 2003). This raised worries as these ARG would be transferred through the food chain from animals to man (Greko 2001), and this was proved by a European surveillance study conducted in 2005 that demonstrated the presence of ARB of animal origin among patients admitted to intensive care units (ICU) (Hanberger et al. 2009) which indicates that humans are direct recipient of these ARB and ARG. ARB were the reason behind high numbers of medical illnesses and even death among human (Cosgrove 2006).

As a result of the antimicrobial resistance (AMR) issue, the World Health Organization (WHO) set guidelines and recommendations to stop the use of antibiotics as growth promoters in 1997 (Caron et al. 2009). A year later in 1998, the EU imposed an initial ban on the use of antibiotics as feed additives in poultry feed and water (Dibner and Richards 2005), and this was followed by a complete ban on the use of prophylactic antibiotics in animal feed in 2006 (Millet and Maertens 2011). Later in 2013, the United States (US) Food and Drug Administration (FDA) ordered major medical manufacturers to stop labelling antibiotics as growth promoters. This came as a result of AMR and consumers demand for healthier antibiotic-free poultry products (Food and Administration 2013). Also recently in 2017, the FDA imposed new rules restricting the use of clinical antibiotics for the purpose of growth promotion in animal husbandry (Brüssow 2017). These precautionary measurements are being taken into consideration as they are important for the poultry welfare and its sustainability, and moreover for human (Casewell et al. 2003) as they are at the top of the food chain hierarchy. The emergence of poultry diseases has a negative economic impact and an increase in financial costs due to loss in poultry productivity and emergence of public health problems (Bryan and Doyle 1995). The previously mentioned rising issues in the poultry production has prompted a search for alternatives to control diseases (Si et al. 2006), and therefore looking for antibiotics alternative to serve the purpose of growth promotion and enhancement in the gut microbiota since diet has a direct impact on productivity and animal health (Borda-Molina et al. 2018), but this needs to be achieved without the current issue of AMR. There are many antibiotic alternatives used currently at commercial level such as probiotics, prebiotics, synbiotics, and bacteriophages (Gadde et al. 2017), but the focus of this literature review paper will be on phytochemicals.

### Bacterial infections in poultry

Bacterial infections in poultry are inevitable and are the cause of high mortality rate among birds (Porter 1998). These infections can be caused by a variety of Gram-negative and Gram-positive bacteria.

#### Escherichia coli (E. coli)

*E. coli* is a facultative anaerobe (Finegold et al. 1983), Gram-negative bacterium (Scheutz and Stockbaine 2015) belonging to the phylum Proteobacteria (Marchesi et al. 2016) and the family *Enterobacteriaceae* (Ewing 1986). *E. coli* is normally part of the human and animals’ intestinal natural microbiota (Ørskov and Ørskov 1992), being the most dominant aerobic bacterium (Savageau 1983) with $10^6$–$10^9$ colony forming unit (CFU) per cm of the poultry (chicken and turkey) intestine (Leitner and Heller 1992), and it is one of the first species to colonise the gut of human (Mitsuoka 1973) and animal (Hudault et al. 2001). Also, it is one of the best studied bacterial species and often used as a model microorganism because of their different commensal and pathogenic types, and with *E. coli* K12 being the most common reference strain (Hobman et al. 2007). The pathogenic type such as avian pathogenic *E. coli* (APEC) strains are the causative agent of colibacillosis in poultry (Gross 1994), which studies have shown to be of multi-resistant nature to 10 or more antibiotics (Lima Barbieri et al. 2017). Moreover, *E. coli* can be easily grown in the laboratory, as it needs simple growth requirements, grows at a fast rate, and extensive information is already provided in literature (Donachie and Begg 1970).

In terms of antibiotic resistance, *E. coli* is the most common carrier of extended spectrum β-lactamase (ESBL) genes which are located on plasmids that facilitate their transfer (Donachie and Begg 1970), and these genes are widespread among chickens (Machado et al. 2008). Ingestion of food of animals origin containing ARB becomes a source of ARB and their ARG in the human gut, and this might affect antibiotics use or might cause opportunistic diseases in the future (Smith et al. 2002). In human hosts, it is the main causative agent and is responsible for most cases of urinary tract infection (UTI) (Stamm and Hooton 1993). Therefore, the need for alternative control measurement and this can be through using natural phytochemicals. There are many studies that suggest the efficacy of using phytochemicals to control *E. coli* growth as summarised in Table 1.

#### Salmonella

*Salmonella* is a facultative anaerobe, Gram-negative bacilli bacterium belonging to the phylum Proteobacteria and the family *Enterobacteriaceae* (MacConkey 1905). It usually resides in the intestinal tract of animals and humans and it is the causative agent of Salmonellosis disease, where it infects food such as poultry meat and egg (Hikasa et al. 1982). Salmonellosis as a food-borne disease is known
as a public health issue causing concerns in industrial countries (D’Aoust et al. 1992) and responsible for high morbidity and mortality cases among human as indicated by the US Center for Disease Control and Prevention (CDC) (Mead et al. 1999). Among food-borne pathogens in the USA, Salmonella was found to be responsible for the highest percentage of these diseases (Gast and Porter 2020). Ingestion of foods of animal origin contaminated with AMR-resistant Salmonella is likely to be responsible for most of Salmonellosis diseases (Angulo et al. 1998). These AMR-resistant Salmonella were first arising from the misuse of antibiotics in poultry industry, and due to its zoonotic nature, it found its way to human (Angulo et al. 2000). In Denmark, it was found out that by decreasing the use of sub-therapeutic in poultry industry, it led to a significant decrease in the prevalence of antibiotic-resistant Salmonella in broilers (Evans and Wegener 2003).

The genus Salmonella includes six sub-species of Salmonella enterica which is found to be responsible for diseases among warm-blooded animals (Gast and Porter 2020). There are more than 2600 serotypes of Salmonella enterica (Achtman et al. 2012), but Salmonella enterica serovars Heidelberg (SH) and Typhimurium (ST) are widespread among human and animal hosts (Zhao et al. 2008; Glenn et al. 2013). These bacterial pathogens are usually found to be responsible for food-borne outbreaks in human due to consumption of food products of animal origin (Authority et al. 2017). The misuse of antibiotics in poultry industry has led to the dissemination of antibiotic resistant Salmonella to ampicillin, chloramphenicol, quinolones and sulphonamide (Su et al. 2004). There are many factors that control the epidemiology of Salmonella infections such as (1) human demography, (2) human lifestyle, (3) human behavior, (4) industrial and technological revolutions, (5) changes in aviation industry, (6) bacterial adaptation, (7) status of public health infrastructure (Oaks et al. 1992), (8) human knowledge in food safety and health practices (Bruhn and Schutz 1999).

Table 1 A summary of the mechanisms of actions of carvacrol, thymol and oregano (at sub-MIC level) against some of the bacteria responsible for poultry infections

| Phytochemical | Bacteria         | Target site               | Mode of action                                                                                      | References              |
|---------------|-----------------|---------------------------|-----------------------------------------------------------------------------------------------------|-------------------------|
| Thymol/carvacrol | E. coli        | Heat and oxidative stress responses and iron transportation | Increased expression of membrane genes (pspD and pspG), heat responses genes (ibpB), oxidative stress responses genes (grxA and soxS) and iron transport gene (feoA) | Yuan et al. (2018)      |
| Carvacrol/oregano | E. coli    | Survival mechanism and multi-drug efflux system | Missense mutation in cadC and marR | Al-Mnaser and Woodward (2020) |
| Carvacrol      | E. coli        | Redox sensor system and multi-drug efflux system | Missense mutation in soxR and frameshift in marR | Chueca et al. (2018)    |
| Carvacrol/oregano | Salmonella  | Stress response            | Influence on the rpoS gene | Cariri et al. (2019)    |
| Carvacrol      | Salmonella     | Oxidative stress response  | Single nucleotide modification in the transcriptional regulators (yfhP and soxR) | Berdejo et al. (2020)   |
| Thymol         | E. coli        | Multi-drug efflux system  | Non-sense mutation in acrR gene encoding for the AcrAB repressor | Al-Kandari et al. (2019) |
| Thymol         | Salmonella     | Thermal stress response    | Upregulation in the expression of the chaperones (GroEl and DnaK) | Di Pasqua et al. (2010) |
| Thymol/carvacrol | Salmonella    | Virulence genes            | Downregulation in the expression of the main virulence genes (hilA, prgH, invA, sipA, sipC, sipD, sopB, sopE2) | Giovagnoni et al. (2020) |
| Carvacrol      | Campylobacter  | Motility systems           | Downregulation in the expression of genes encoding for motility systems (flaA, flaB and flgA) | Wagle et al. (2019)     |
| Carvacrol      | Campylobacter  | Thermal stress response    | Upregulation in the expression of the stress response genes (dnaK, grpE and groEL) | Windiasti et al. (2019) |
**Campylobacter**

Campylobacter is a microaerophilic, Gram-negative spiral-curved bacilli bacterium (Skirrow 2006) belonging to the phylum Proteobacteria and the family Campylobacteriaceae (Huang et al. 2020). It can be found in the intestinal tract of animals and human oral cavity with the ability to cause diseases in both hosts (Lee et al. 2016). Campylobacter species which inhabit poultry intestine and are associated with poultry diseases are Campylobacter jejuni and Campylobacter coli (Pezzotti et al. 2003) with the former specie being responsible for most of the infection cases. These bacteria species are the causative agents of enteritis and linked to chronic gastritis, gastric ulceration, and gastric cancer in human (Lee and Newell 2006). Campylobacter infection in human results from handling or ingestion of undercooked poultry meat contaminated with this pathogen, which 80% of the raw meat in the UK was found to be contaminated with it (Corry and Atabay 2001). Moreover, practicing low hygienic levels and food safety skills in the kitchen can lead to the spread of Campylobacter contamination with other undercooked food (Lee and Newell 2006).

Campylobacter and Salmonella share similar infection outcome and zoonotic nature, but Campylobacter are fastidious and differ in their metabolic and stress responses as demonstrated by genomic analysis (Lee and Newell 2006). Among zoonotic diseases, Campylobacteriosis has been reported to be the most frequently spread among humans in the EU (Hugas et al. 2009; Westrell et al. 2009), and the major source of infection was fresh broiler chicken meat infected with Campylobacter jejuni (Authority 2005; Wingstrand et al. 2006). Therefore, reducing the occurrence of Campylobacter in poultry meat would decrease its infection cases among human and that would be through various preventative measures: (1) vaccination (Wyszyńska et al. 2004), (2) bacteriophages (Wagenaar et al. 2005), (3) bacteriocins (Line et al. 2008), (4) organic acids (Chaveerach et al. 2004), (5) probiotics (Ghareeb et al. 2012), (6) antibiotics. However, administration of fluoroquinolone antibiotic in poultry industry has led to the emergence of resistant Campylobacter and was found to be responsible for 10% of Campylobacter diseases in human (Randall et al. 2003). Hence, the need to look for alternative ways.

**Clostridium perfringens**

Clostridium is an anaerobic, Gram-positive spore-forming bacterium (Songer and Meer 1996) belonging to the phylum Firmicutes and the family Clostridiaceae (Wiegel 2015). Clostridium perfringens is a bacterium of ubiquitous nature and it is part of animal and human gut microbiota (Miller et al. 2010). At the same time, it is one of the frequent zoonotic bacterial pathogens that causes foodborne diseases outbreak in humans after Campylobacter and Salmonella (Buzby and Roberts 1997). It costs poultry industry economically as it is the main causative agent of necrotic enteritis (Van der Sluis 2000). Necrotic enteritis infections can be in the form of acute or subclinical, with the former being responsible for higher mortality rates among broiler chickens (Kaldhusdal and Lovland 2000). The acute form of the infection leads to the formation of severe necrosis in the mucosal layer of the small intestine, thereby increasing the rate of death (Gazdinski and Julian 1992). While, the subclinical form of the infection causes reduced absorption and digestion, decrease in the weight gain and an increase in the FCR due to the damage in the intestinal mucosa (Kaldhusdal and Hofshagen 1992). At the level of bacterial gut microbiota, this infection leads to its disturbance which can be reversed by feeding a monoculture of Lactobacillus acidophilus or Streptococcus faecalis (Fukata et al. 1991). The classification of Clostridium perfringens puts them into five toxinotypes (A, B, C, D and E) according to the ability to produce four major toxins (α, β, ε and i). The subclinical form of the infection is caused mainly by Clostridium perfringens type A producing alpha toxin and to a less degree by Clostridium perfringens type C producing alpha and beta toxin (Songer and Meer 1996). The European ban on the use of antibiotics as a growth promoter has been associated with the wide spread of necrotic enteritis (Immerseel et al. 2004). Hence, the need to find alternatives to control this pathogen.

**Phytochemicals**

Phytochemicals are natural plant products produced as secondary metabolites of which some possess antimicrobial effects (Metabolites 2004), and natural sources of feed additives and have been proven to be generally recognised as safe (GRAS) (Hashemi et al. 2008). These secondary metabolites can be non-digestible carbohydrates and compounds such as lignin, resistant protein, polyphenols and carotenoids, some of which are considered anti-oxidants (Saura-Calixto et al. 2004) and display anti-microbial activities (Wink 2004). They differ in chemical structure, biological activity, plant source, and method of production. In other words, they are natural sources of growth promoters coming from plants, herbs, or spices (Hashemi and Davoodi 2010). They come in different materialistic form: solid in the form of dried leaves or ground powder, or liquid in the form of an essential oil (Gadde et al. 2017). They differ in their composition, extraction method, storage conditions, growth stage, the source of plant and its geographical location (Dhami and Mishra 2015). Generally, phytochemicals are categorised into five main groups; terpenoids, polyphenols, organosulfur compounds, phytosterols, and alkaloids (Somani et al. 2015). However, polyphenols represent the major and main
compounds of these phytochemicals (Gadde et al. 2017), hence the focus of this current paper.

Presently, plant-based natural therapies are of increased popularity, because consumers are becoming aware of concerns regarding synthetic additives (Hammer et al. 1999) as well as the dangers of antibiotic use as discussed earlier. Scientific research changed the perception of food including phytochemicals from being an energy source to that of health promoting supplements because of their bioactive roles (Berner and O’Donnell 1998). Therefore, it is crucial to understand the scientific background behind the beneficial roles of phytochemicals as anti-microbial agents (Mitscher et al. 1987), and the process of using them as an alternative to antibiotics. The scientific interests in phytochemicals are due to the rising problem of ARB in poultry industry, consumers demand, and the EU ban on the usage of antibiotics for growth promotion. The biological mechanism of action of these phytochemicals is not very well-understood, but it depends on their chemical structure (Hashemi and Davoodi 2010).

Phytochemicals used as poultry feed additives can improve animal’s health and performance because of their anti-microbial, anti-stress (Wang et al. 1998) and anti-oxidant properties (Valenzuela 1995), and their ability to modulate gut microbiota (Hashemi et al. 2009) and enhance immune responses (Chowdhury et al. 2018). The improvements in the animal health performance can be observed in the form of an increased body weight, feed intake and FCR. Physically, the positive effects included an improved carcass quality, meat quality and nutritional values (Valenzuela-Grijalva et al. 2017). The efficiency of these phytochemicals is determined by intrinsic and extrinsic factors such as animal’s nutrition and health, type of diet and environment (Giannenas et al. 2003). They can act as prebiotics by enhancing the growth of beneficial bacteria and suppressing the growth of pathogenic bacteria (Cencic and Chingwaru 2010) which leads to the enhancement in the gut microbiota (Hashemi and Davoodi 2010). Thus, they reward the host by shaping gut microbiota in a beneficial way (Laparra and Sanz 2010). On the bacterial level, previous research has demonstrated that using phytochemicals as feed additives results in a decrease in the population of E. coli and also an increase in the activity of specific digestive enzymes (Jang et al. 2007) such as amylase in the intestinal system of female broiler chickens (Lee et al. 2003) and maltase in the intestinal system of male broiler chickens (Xu et al. 2003).

**Carvacrol, thymol and oregano**

Thymol (2-isopropyl-5-methylphenol) (Fig. 1) and carvacrol (5-Isopropyl-2-methylphenol) (Fig. 2) are phenolic compounds (Kim et al. 2016) and they are the main constituents of the essential oils of oregano (Fig. 3). They are structural l isomers, sharing the same chemical structure in the form of a phenolic ring but differing in the location of hydroxyl groups (Ultee et al. 2002). Moreover, carvacrol is the key ingredient of oregano essential oil that is extracted from plants of the genus *Origanum* (Kintzios 2002), but its abundance in plants differs from one species to another (Gounaris et al. 2002). Thymol, carvacrol, and oregano share the same chief components which are monoterpenic phenols consisting of two main ingredients of γ-terpinene and p-cymene (Kokkini 1996). Carvacrol and oregano exhibit anti-microbial activities against pathogenic microorganisms whether from plant, animal or human sources, and these microorganisms include bacteria and fungi (Baricevic and Bartol 2002).
Carvacrol and thymol as feed additives showed enhanced growth promoting effects on anti-oxidant enzyme activities, immune responses, digestive enzyme activities among broiler chickens (Hashemipour et al. 2013). Oregano oil containing carvacrol and thymol is effective against E. coli in a dosage-dependent manner (Friedman et al. 2002; Al-Mnaser 2019; Alvarez et al. 2019). Generally, the phytochemicals (e.g. carvacrol) with a high percentage of other phenolic compounds display potent anti-bacterial properties (Guynot et al. 2019). As anti-bacterial agents, the main mechanism of action appears to be disruption of the integrity and functionality of the cell wall and cell membrane structures (Sikkema et al. 1995). At minimum inhibitory concentration (MIC) level, they disrupt the outer membrane structure of Gram-negative bacteria, increasing the permeability of cell membrane, leading to leakage of cellular energy sources in the form of adenosine tri-phosphate (ATP) (Gill and Holley 2006) and may also result in the bursting of the bacterial cell (Sikkema et al. 1995). These essential oils are highly hydrophobic and thus can readily integrate into and transition across the bacterial cell membrane (Sikkema et al. 1995). Interestingly, exposing bacteria to sub-lethal concentrations of these phytochemicals leads to changes in the ratio of unsaturated and saturated fatty acid component of the cell membrane (Di Pasqua et al. 2006) suggesting that bacteria develop an adaptive response upon exposure. Furthermore, oregano oil exhibits high biological activities resulting in growth promotion when used as feed additives in poultry (Giannenas et al. 2005). Another study showed that oregano extract (Origanum vulgare) contains a high phenolic content that exhibits anti-oxidant properties (Gómez-Estaca et al. 2009). More recent studies showed that broiler chickens fed diet supplemented with oregano resulted in the following: (1) significant increase in the digestive enzyme chymotrypsin and enhanced protein digestion (Basmacioğlu Malayoğlu et al. 2010), (2) significant increase in body weight, higher anti-oxidant activity of serum, significant decrease in cecal E. coli population resulting in an increased growth performance (Roofchaee et al. 2011), (3) significant increase in body weight and significant decrease in FCR among broilers chickens infected with Eimeria species (Pajić et al. 2019). Moreover, oregano and other herb extracts can suppress the growth of harmful coliform bacteria, but do not affect the growth of beneficial bacteria (Namkung et al. 2004).

In vitro study demonstrated the anti-bacterial activity of carvacrol and oregano by decreasing the number of Salmonella and Campylobacter jejuni in chicken cecal content (Johny et al. 2010), which was further supported by an in vivo study suggesting the efficacy of using these phytochemicals as feed additives in 10 days old broiler chickens due to the significant reduction in the number of Campylobacter in chicken ceca (Arsi et al. 2014). The mode of action of the carvacrol treatment against Campylobacter jejuni can be due to its ability to act as membrane destabilization agent and therefore increasing the susceptibility and cell membrane damage of this bacteria (Windiasti et al. 2019). A longer period study covered 35 days showed that the absence of Campylobacter sp. at day 21 was due to the increase in the number of Lactobacillus sp. with probiotic beneficial effects and thereby improving chicken health by preventing Campylobacter infection (Kelly et al. 2017). As for the spore-forming bacteria Clostridium perfringens, carvacrol, oregano and thymol have showed their ability in preventing their sporulation and controlling their numbers in meat (Juneja and Friedman 2007). Another study further supported the anti-bacterial efficacy of thymol and carvacrol when used as feed additives in broiler chickens challenged with Clostridium perfringens leading to an improve in the chicken gut health supported by the presence of Lactobacillus strains with probiotic beneficial properties (Du et al. 2015). Table 1 provides a summary of the proposed mode of actions of these phytochemicals in further details and at the genetic level of the bacterial cell.

Tannins are polyphenolic compounds [2,3-dihydroxy-5-[(2R,3R,4S,5R,6S)-3,4,5,6-tetras[3,4-dihydroxy-5-(3,4,5-tri hydroxybenzoyl)oxybenzoyl]oxy]oxan-2-yl]methoxy[carboxyl]phenyl] 3,4,5-trihydroxybenzoate] (Fig. 4) (Kim et al. 2016) categorised into four groups: (1) condensed tannins or proanthocyanidins, (2) hydrolysable tannins, (3) phlorotannins from brown algae, and (4) complex tannins (Suvanto et al. 2017; Brus et al. 2018), and come in different chemical structures (Lillehoj et al. 2018). The presence of different hydroxyl groups at different positions in its structure believes to be behind its ability to bind with the carboxyl groups of the proteins (Wang et al. 2016).
Moreover, different chemical composition and structure of tannins makes it bacterial species-specific (Huang et al. 2018). In nature, tannins originate in several plant species, specifically in the inedible parts (i.e. bark or wood) (Brus et al. 2018). Some of these tannins are responsible for defending the plants and others give the plants their odor or color (Redondo et al. 2014). Tannins have been known for their ability to promote growth and their anti-microbial properties, making them a broiler feed additive of choice in South America (Lee et al. 2021). Though, the exact mechanism of action of tannins is still poorly understood (Smith and Mackie 2004), the proposed inhibitory activities involve its interaction with proteins, bacterial cell membrane (Hemingway and Laks 2012), and chelation of iron metals (Scalbert 1991). Other intracellular activities include inhibiting enzymatic and metabolic activities that result in bacterial cell morphological changes (Liu et al. 2013). A recent study showed the immunomodulation effect of tannins when used as a feed additive, and this was due to a significant increased expression of cytokines (IL-6 and IL-10) in the cecal cells. This resulted in an altered metabolism, enhanced gut health and bird growth, and feed efficiency (Lee et al. 2021).

On a larger scale, inclusion of tannins in the poultry diet had health promoting effects on growth and intestinal effects (Schiavone et al. 2008), and increased feed efficiency (Redondo et al. 2014). The health promoting effects on growth can include a decrease in lipid oxidation and cholesterol level, an increase in the content of beneficial fatty acids, and an increase in body weight (Starčević et al. 2015). On the bacterial level, it can lead to changes in the gut morphology and type of bacteria and thereby increasing their biodiversity in the gut of broiler chickens (Viveros et al. 2011). Also, it can act as an anti-oxidant and provides a source of vitamin E in animal nutrition (Brenes et al. 2008). On the physiological level of the broiler, inclusion of tannins leads to the following: (1) red blood cell growth and maturation of the small intestine (Iji et al. 2001), (2) elongation of the villi of the small intestine, and (3) increased number of cell mitosis of the duodenum (Khambualai et al. 2009). Collectively, these result in increased weight gain on a daily basis and in total, and enhanced feed utilization (Lee et al. 2021). On the broiler production level, tannins increase the quality and nutritional values of meat (Mannelli et al. 2019), and eggs (Minieri et al. 2016).

Tannins as an anti-bacterial agent can reduce the occurrence of avian diseases and transmission of zoonotic pathogens (Hassan et al. 2020). Examples on this can include the following: (1) inhibit the growth of E. coli by acting as
anti-biofilm and anti-motility agents (Dakheel et al. 2020), (2) inhibit the growth of Salmonella by acting as anti-quorum sensing and anti-virulence agent (Sivasankar et al. 2020), (3) inhibit the growth of Campylobacter sp. which may be resorted to their ability to bind to the proteins and enzymes within the bacterial cell (Nagayama et al. 2002), (4) inhibit the growth of Clostridium perfringens by acting as a membrane destabilization agent (Kaimudin and Manduapessy 2020) and as an anti-toxin agent (Elizondo et al. 2010), (5) chelates iron that is crucial for the growth of most pathogenic bacteria (Chung et al. 1998). Table 2 provides a summary of the proposed mode of actions of tannins in further details and at the genetic level of the bacterial cell.

### Conclusion

Antibiotic resistance issue made the scientific community shift their perspective and search for antibiotic alternatives. One of the antibiotic alternatives is the use of natural plant products, phytochemicals. This review has focused on four polyphenolic phytochemicals with promising results which make them good candidates to be used as feed additives in the poultry industry instead of antibiotics. However, more studies need to be done to increase our understanding in the long-term used of these phytochemicals and how will they affect us as humans considering that we are at the end of the food chain. This AMR issue will continue to increase in the coming years due to the current overuse of anti-bacterial compounds during this coronavirus pandemic. Therefore, the need to increase our understanding in this area and find effective alternatives is very crucial and of high importance.

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