Review Article

Use of antibiotics in broiler production: Global impacts and alternatives

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ABSTRACT

Antibiotics are used to fight bacterial infections. However, a selective pressure gave rise to bacteria resistant to antibiotics. This leaves scientists worried about the danger to human and animal health. Some strategies can be borrowed to reduce the use of antibiotics in chicken farms. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health. Among these, the most popular are probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocins, bacteriophages, phytogenic feed additives, phytoncides, nanoparticles and essential oils.

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1. Introduction

The discovery of antibiotics was a success in controlling infectious pathologies and increasing feed efficiencies (Engberg et al., 2000). Antibiotics, either of natural or synthetic origin are used to both prevent proliferation and destroy bacteria. Antibiotics are produced by lower fungi or certain bacteria. They are routinely used to treat and prevent infections in humans and animals. However, scientific evidence suggests that the massive use of these compounds has led to increased problem of antibiotic resistance (Diarra et al., 2007; Forgetta et al., 2012; Furtula et al., 2010), and presence of antibiotics residues in feed and environment (Carvalho and Santos, 2016; Gonzalez Ronquillo and Angeles Hernandez, 2017), compromises human and animal health (Diarra et al., 2010). Hence, there is a growing need to find effective alternatives to control infectious diseases and limit the spread of resistant bacteria, but more importantly, keep antibiotics a useful tool for the future. This literature review synthesizes the current state of antibiotics use, as well as alternative strategies available in broiler chicken production.

2. Use of antibiotics in broiler chicken production

Over the past 50 years, the use of antibiotics combined with strict biosecurity and hygiene measures has helped the poultry industry to grow by preventing the negative impacts of many avian diseases (Bermudez, 2003). Even as biosecurity may be sufficient, vaccination can also be used as an additional measure. A vaccine

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provides assistance to the immune system by preparing it against certain pathogens such as viruses or bacteria to which it may be exposed in the future. Vaccination protocols and the type of vaccine used vary from country to country and from farm to farm. Many factors can influence the choice of vaccination method such as species, place, number of manpower, type of production, and production cycle. The choice of vaccination method also depends on general health status of poultry, maternal immunity, and vaccine costs (Rauw et al., 2009). Livestock vaccination against specific diseases is compulsory (e.g., Newcastle disease) in many countries (Belgium, Netherlands, Germany), while in other such as France only long-lived poultry (laying and breeding) are vaccinated (Rauw et al., 2009).

Antibiotics are not effective against fungal and viral pathogens. They only treat infectious diseases whose causative agents are bacteria. In general, antibiotics are used in phytosanitary treatments, fish farming, animal feed, and human or veterinary medicine where they can be used as a preventive or curative treatment. Antibiotics are classified according to their chemical family, mode of action and the species of bacteria on which they act. Bactericidal antibiotics kill bacteria and bacteriostatics weaken them by inhibiting their proliferation and facilitating their phagocytosis by the immune system. Thus, mortality rate decreases because animals become more resistant.

In intensive poultry farming, especially in North America, antibiotics such as tetracycline, bacitracin, tylosin, salinomycin, virginiamycin and bambermycin are often used (Diarra and Malouin, 2014). In the United States, tetracyclines represent more than two-thirds of antimicrobials administered to animals (Gonzalez Ronquillo and Angeles Hernandez, 2017), while in European Union (EU) they represent only 37% (Carvalho and Santos, 2016). In 2015, the overall sales of veterinary antimicrobial agent were 8,361 t in EU (ESVAC, 2017). This figure is calculated without counting growth promoters in animal production (Kummerer, 2009). The use of antibiotics as growth factors is not allowed in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) participating countries (ESVAC, 2017). In 2014, 1.5 million kg of active antimicrobial ingredients were distributed for use in animals in Canada, up 5% from 2013. For antimicrobials distributed, 99% were for farm animals and less than 1% were for pets. In 2014, 81% of the antimicrobials used in Canada on broiler farms were for prevention purposes. In the feed, 84% of these antimicrobials were used (Fig. 1). They were primarily intended to prevent necrotic enteritis caused by *Clostridium perfringens* and coccidiosis (CSCRA, 2016).
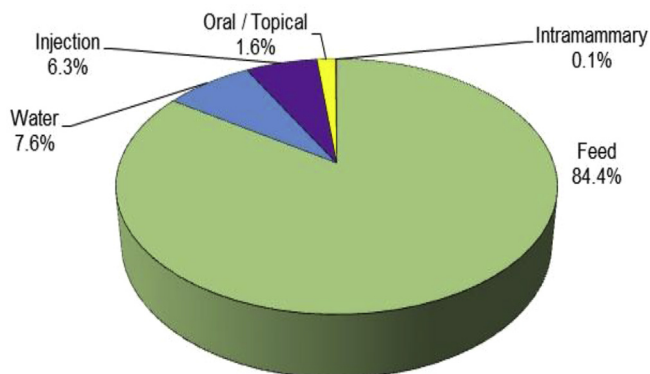

Fig. 1. Quantity of antimicrobials (% of total weight in kg) distributed for veterinary use by route of administration in Canada (CSCRA, 2016).

3. Antibiotic impacts

3.1. Impact on chicken growth, digestive tract and immune systems

The poultry industry uses antibiotics to improve meat production through increased feed conversion, growth rate promotion and disease prevention. Antibiotics can be used successfully at subtherapeutic doses in poultry production to promote growth (Barceló, 2007; Chattopadhyay, 2014; Engberg et al., 2000; Harms et al., 1986; Khodambashi Emami et al., 2012; Rosen, 1996) and protect the health of birds by modifying the immune status of broiler chickens (Lee et al., 2012). This is mainly due to the control of gastrointestinal infections and microbiota modification in the intestine (Dibner and Richards, 2005; Singh et al., 2013; Torok et al., 2011). The mechanism remains unclear, but antibiotics are likely to act by remodeling microbial diversity and relative abundance in the intestine to provide an optimal microbiota for growth (Dibner and Richards, 2005). For example, meta-genome sequencing approaches have demonstrated that diets with salinomycin (60 ppm) has an impact on microbiome dynamics in chicken ceca (Fung et al., 2013). Similarly, the use of virginiamycin (100 ppm) as a growth promoter has been associated with an increased abundance of *Lactobacillus* species in broiler duodenal loop at proximal ileum. This indicates that virginiamycin alters the composition of chicken gut microbiota (Dumonceaux et al., 2006). In addition, populations of *Lactobacillus* spp. in the ileum of chickens receiving feed containing tylosin, a bacteriostatic, are significantly lower than those in chickens receiving no tylosin (Lin et al., 2013). This decrease in *Lactobacilli* species following the use of antibiotics has been demonstrated in other studies (Danzeisen et al., 2011; Lee et al., 2012; Zhou et al., 2007). For reminder, *Lactobacillus* are the primary commensal bacteria for the production of bile hydrolase salt. The decrease in the *Lactobacillus* population in antibiotic-treated animals probably reduces the intestinal activity of the bile hydrolase salts, which would increase the relative abundance of conjugated bile salts, thus promotes lipid metabolism and energy harvesting and increases animal weight gain (Lin et al., 2013).

A change in the intestinal microbiota of chickens can influence their immunity and their health. However, changes in the intestinal microbiota of chickens can be influenced by several factors. These factors include housing conditions, exposure to pathogens, diet composition and the presence of antibiotics in feed (Lee et al., 2012).

3.2. Impact on meat quality and eggs

Campylobacter is a major cause of food-borne diarrheal diseases in humans. *Campylobacter* infections can be severe or fatal in immunocompromised or elderly people and very young children. *Escherichia coli* bacteria are very common and can also cause diseases. The most common type of *E. coli* infection that causes illness in people is called *E. coli* O157:H7. Salmonellosis is one of the most common and widespread food-borne illnesses in the world. *Salmonella* infections usually cause mild gastroenteritis. These 3 bacteria and others are monitored by specialized agencies around the world, for example, Public Health Agency of Canada in Canada, Food and Drug Administration (FDA) in USA, European Food Safety Authority (EFSA) in EU. Tens of millions of cases of these bacterial infections occur in humans every year worldwide. Each year in the United States there are approximately 76 million food-borne illnesses (Zhao et al., 2001). Approximately 325,000 cases result in hospitalization, and 5,000 cases are fatal, and 1.4 million cases are caused by nontyphoidal *Salmonella* serovars, *Campylobacter* spp. Nearly 2.4 million cases and 270,000 cases are caused by

pathogenic *E. coli*, including *E. coli* O157:H7 (Mead et al., 1999; Zhao et al., 2001).

According to CSCRA (2016) report, chicken contamination rates for *E. coli*, *Campylobacter* and *Salmonella* spp. are respectively 96%, 25% and 34% in Canada. In addition, antibiogram test revealed multi-pharmacological resistance in *Enterobacteriaceae* isolates from eggs and broiler meat (Diarra et al., 2010; Singh et al., 2010; Yulistiani et al., 2017). Eggs are frequently implicated in *Salmonella* transmission (Singh et al., 2010). This contamination is due mainly to the proliferation of pathogens in the intestines. There are secondary contaminations along the production line by resistant bacteria in foods of animal origin. Schwaiger et al. (2012) reported that the prevalence of multi-resistant of *Salmonella* was higher in retail samples compared to slaughterhouse samples.

3.3. Impact on consumer health and the environment

In addition to bio-resistance, antibiotics abuse has resulted in drug residues in animal products (Gonzalez Ronquillo and Angeles Hernandez, 2017). Several antibiotics such as penicillin, tetracycline, macrolide, aminoglycoside and amphenicol have been detected in foods (Diarra and Malouin, 2014). Residues in livestock production can actually have adverse impact on human health; this is the case for tetracyclines, which interfere with teeth development in young children (Kummerer, 2009). This is also the case with beta-agonists, such as clenbuterol, leading sometimes to food poisoning and muscle tremors, palpitations and tachycardia (Chan, 1999). Clenbuterol is prohibited in EU. Some breeders use it to produce meat containing less fat and more protein. Further, chloramphenicol illustrates both potential problems (Gassner and Wuethrich, 1994). Gassner and Wuethrich (1994) have demonstrated the presence of chloramphenicol metabolites in meat products. These authors concluded a possibility link between the presence of these antibiotic residues in meat and the occurrence of aplastic anemia in humans.

The administration and restriction of the use of antibiotics in the EU are regulated by Directive 96/23/EC (European-Commission, 1996). This directive focuses on measures to monitor residues in animal products. Certain limits of antibiotic residues are imposed in food and animal products (meat and eggs). Limits on the quantities of antibiotic residues in eggs and chicken meat are reported in Table 1.

The global consumption of antibiotics in human and animal production is estimated between 1×10^5 and 2×10^5 t (Manzetti and Ghisi, 2014). Releasing thereby large quantities of antibiotics into the environment entertains the cycle of biotransformation and bioaccumulation of antibiotics in the environment. According to Manzetti and Ghisi (2014), the most vulnerable ecosystems to antibiotic contamination are confined aquatic ecosystems such as ponds and lakes and soils close to urban sites. Aquatic compartments, such as water and sediments, can thus play an important role in the transfer, evolution and ecology of antibiotic resistance genes (Marti et al., 2014). Large amounts of antibiotics administered to animals are excreted into the environment via urine and faeces (Carvalho and Santos, 2016). After metabolic changes in animals, 30% to up 90% of the dose consumed is found in the urine and feces as parent compounds and/or metabolite compounds (Carvalho and Santos, 2016). This makes sewage disposal systems one of the most important routes by which antibiotics can enter in the environment (Gonzalez Ronquillo and Angeles Hernandez, 2017) and contaminate even coastal waters (Chen et al., 2015).

Antibiotics' risks in the aquatic environment and sediments are important because they can influence aquatic life behavior (Kummerer, 2009). Antimicrobials have qualitative and quantitative effects on the microbial community residing in sediments,

Table 1
Maximum residue limits (MRL) for antimicrobials in poultry products applied in EU (adapted from Gonzalez Ronquillo and Angeles Hernandez, 2017).

Substance	Chemical group	Matrix	MRL, mg/kg
Tetracycline	Tetracyclines	Muscle	100
		Liver	300
		Kidney	600
		Eggs	200
Streptomycin	Aminoglycosides	Muscle	600
		fat	600
		Liver	600
		Kidney	1,000
Tilmicosin	Macrolides	Muscle	150
		Liver	2,400
		Kidney	600
		Skin and fat	250
Florfenicol	Amphenicols	Muscle	100
		Skin and fat	20
		Liver	2,500
		Kidney	750
Tiamulin	Pleuromutilins	Muscle	100
		Liver	1,000
		Skin and fat	100
		Eggs	1,000

which in turn can affect the degradation of organic matter (Kummerer, 2009). Residues of antibiotics present in the water contribute strongly to the maintenance, emergence and dissemination of bacterial populations with a low level of resistance and are ready to evolve towards resistance (Corvaglia, 2006). According to Chen et al. (2015), the spatial distribution of antibiotics in the marine environment is significantly correlated with environmental variables such as chemical oxygen demand (COD) and nitrates (NO_3—N).

The aquatic environment is considered as an important point for acquisition and spread of antibiotic resistance genes by bacteria (Devarajan et al., 2017). Studies (Caplin et al., 2008; Devarajan et al., 2017) demonstrated a widespread presence of antibiotic resistance determinants in aquatic sediment ecosystems. Devarajan et al. (2017) reported multi-resistance profiles in *Pseudomonas* spp. in aquatic sediment samples, which is potentially transferable to humans. Laroche et al. (2009) reported resistance genes in estuary samples, mainly carried by *Enterococcus* spp. and *E. coli*. In addition, a study Furtula et al. (2013) carried out on samples collected from 2 poultry farms showed that 58% of *Enterococcus* spp. isolates in surface waters and 100% of isolates in groundwater were resistant to more than an antibiotic. According to Carvalho and Santos (2016), the toxic effects of antibiotics in the aquatic environment increased when combined with other antibiotics.

In the soil, antibiotic's behavior differs according to their physicochemical properties, soil characteristics, as well as climate conditions. Acid rain accelerates the antibiotics accumulation in animal manure and soil surface while long-lasting rains foster antibiotics' migration in deeper parts of the soil (Pan and Chu, 2017). According to Pan and Chu (2017), antibiotics leaching is higher in sandy soils than in clay and silty soils. Norfloxacin and tetracycline tend to persist in the soil surface while sulfamethazine and erythromycin pose a higher risk for deeper soil layers and groundwater. The soil can be also contaminated by antibiotics in litter. Animal bedding contains residues of antimicrobial compounds. Residues of bacitracin, salinomycin, penicillin and virginiamycin were detected in chicken litter at concentrations ranging from 0.07 to 66 mg/L (Furtula et al., 2010). When this bedding material is used as nitrogen amendment, the resistant bacteria can live in the soil for several months (Merchant et al., 2012). According to De Liguoro et al. (2003), biotransformation and biodegradation of antibiotics on agricultural sites can take up to 150 days. In addition, antibiotic

by-products in the environment remain bioactive and can be potentially more toxic, stable and mobile than their parent compounds (Carvalho and Santos, 2016). Bio-resistant bacteria (*Staphylococcus xylosus*) have also been reported in air in broiler farms (Vela et al., 2012). Liu et al. (2012a) have shown that airborne transmission causes the spread of epidemic diseases and also poses impend over public health.

3.4. Antibiotic and bacterial resistance

Scientific evidence suggests that the use of antimicrobials in livestock production can promote bacterial resistance in treated animals (O'Brien, 2002). Antibiotic resistance is defined as the ability of microorganisms to proliferate in presence of an antibiotic that generally inhibits or kills microorganisms of the same species (RUMA, 2016). Resistance is by mutation or acquisition of genes carried by mobile genetic elements such as transposons, integrons, plasmids or phages (Kempf and Zeitouni, 2012). Chicken harbors large proportion of *Enterobacteriaceae* resistant to aminosides in its digestive tract and tetracycline in its meat (Guillot et al., 1977; Yulistiani et al., 2017). Bacterial resistance to antibiotics has been the subject of several studies in the recent years (Diarra et al., 2007; Forgetta et al., 2012; Furtula et al., 2010, 2013; Johnson et al., 2012). In one study on *Salmonella enterica* isolates collected from poultry farms in British Columbia (Canada), Diarra et al. (2014) showed that more than 43% of the isolates were simultaneously resistant to ampicillin, amoxicillin-clavulanic acid, ceftiofur, cefoxitim and ceftriaxone. Another Canadian study (Diarra and Malouin, 2014) highlights the existence of different stereotypes of *Salmonella*, isolated from broiler farms, resistant and multi-resistant to antibiotics. In addition, antibiotic resistance in *Enterococci* (Silbergeld et al., 2008), *Mycoplasma gallisepticum* (Pakpinyo and Sasipreeyajan, 2007) and *Salmonella* spp. (Manning et al., 2015) isolated in broilers have been reported. A study in Germany (Schwaiger et al., 2012) showed that resistant and multi-resistant isolates are very common in chicken meat. Another study in Italy (Bacci et al., 2012) reported that 86% of *S. enterica* isolated from chicken carcasses were resistant to tetracycline, while 30% of isolates showed multipharmacological phenotypic resistance to ampicillin, sulfamethoxazole and tetracycline. In Ecuador, a study by Braykov et al. (2016) showed that tetracycline resistance was detected in 78% of production bird (broilers and laying hens). More than half of the isolates were resistant to sulfisoxazole and trimethoprim-sulfamethoxazole (69% and 63%, respectively).

Bacterial resistance to animal antibiotics is a public health issue. In Canada, for example, poultry meat may play a role in human infections (Diarra et al., 2010; Manges et al., 2007). In addition, Hur et al. (2011) founded that isolates of *S. enterica* from egg and chicken carcasses were resistant to penicillins, sulfisoxazole, treptomycin, tetracycline and quinolones. *S. enterica* isolates were resistant to at least 21 antibiotics used by the authors. Most isolates harbored genes associated with SPI-1 and SPI-2 and the spv operon, which are known to be associated with human infections. This represents a threat to human health. This situation is mainly due to the misuse of certain antibiotics such as penicillins, tetracyclines, macrolides and aminoglycosides (Diarra and Malouin, 2014). The abusive use of antibiotics and the associated selection pressure have led to decreased therapeutic efficacy and created populations of antibiotic-resistant microorganisms. Antibiotic resistance may spread over time despite the suspension of antibiotic use. Indeed, strains of *E. coli* resistant to trimethoprim and streptomycin have been shown to persist for several weeks in a chicken farm without using the antibiotics mentioned above (Chaslus-Dancla et al., 1987). On the other hand, antibiotic resistance is lower in organic farms (Hegde et al., 2016). Thus, it is imperative to determine the exact sources and ecology of resistant bacteria in order to develop strategies to stop their proliferation (Diarra and Malouin, 2014).

4. Alternatives to the use of antibiotics

Consumers' pressure and worries towards harmful effects of antibiotic use and the ban of antibiotics in EU have prompted researchers to think about alternatives to antibiotics (Diarra and Malouin, 2014). The aim of these alternatives is to maintain a low mortality rate, a good level of animal yield while preserving environment and consumer health. Much research has been carried out to look for natural agents with similar beneficial effects of growth promoters. There are indeed a number of non-therapeutic alternatives that can substitute antibiotics use. Among these, the most popular are probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocins, bacteriophages, phytogenic feed additives, phytocides, nanoparticles and essential oils.

4.1. Phytogenic feed additives

Phytogenic feed additives (PFA) derived from plants, herbs and spices are used to improve animal performance. They have been very successful because of their positive effects on growth, improved immune system and reduced stress response. Recent results showed that PFA were good alternatives to antibiotics (Frankic et al., 2009; Ghasemi et al., 2014; Toghyani et al., 2011; Windisch et al., 2008) and promoted broiler chicken growth (Ghasemi et al., 2014; Li et al., 2015; Toghyani et al., 2011). For example, inclusion of cinnamon 2 g/kg of the diet had a positive effect on growth performance at 28 days of age (974 vs. 850 g) and at 42 days of age (2,111 vs. 1,931 g) (Toghyani et al., 2011). Also, inclusion of *Lippia javanica* at 5 g/kg in broiler feed had beneficial effects on average daily gain (ADG) in the grower period (67 vs. 30 g), slaughter weight (2,213 vs. 1,967 g) and fatty acid profiles of broiler chicken meat (Mpofu et al., 2016). According to Mpofu et al. (2016), phytogenic extracts in *L. javanica* leaf meal can stimulate glycolysis and increase utilization of energy production and ultimately growth. In addition, a mixture of garlic (5 g/kg) and black pepper (1 g/kg) powder had positive effects on weight gain and broiler chicken consumption index (Kirubakaran et al., 2016).

The study by Jarriyawattanachaikul et al. (2016) showed that certain herbs, especially *Cratoxylum formosum*, can have an antimicrobial activity against *E. coli, C. jejuni* and *S. aureus* isolated from chicken caecum. At a dose of 150 mg/kg for 39 days, PFA contained extracts from fennel (*Foeniculum vulgarae* var. *dulcemil*), Melissa balm (*Melissa officinalis* L.), peppermint (*Mentha arvensis* L.), anise (*Pimpinella anisum* L.), oak (*Quercus cortex*), clove (*Syzygium aromaticum* L.), and thyme (*Thymusvulgaris* L.) was as effective as bacitracin methylene disalicylate in controlling inoculated *Clostridium*, *Salmonella* and *E. coli* in broiler chicken (day 28) (Wati et al., 2015). Some medicinal plants have anti-microbial and antioxidant properties (El-Ghorab, 2006; Mahboubi and Haghi, 2008). The use of pennyroyal (*Mentha pulegium* L.) in broiler chicken diets has shown positive effects. Goodarzi and Nanekarani (2014) showed that the use of 2% of pennyroyal in broiler feed has positive effects on their ADG (49 g compared with the negative control [43 g] and *Virginimacin* control [49 g]) and carcass traits.

A positive influence of PFA has been reported on the immune system. Indeed, supplementation with 7 g/kg of neem (*Azadirachta indica*) induced favorable influences on the immune responses of broiler chickens and ADG compared with chicken supplemented with flavophospholipol (Landy et al., 2011). Similarly, supplementation with 10 to 20 g/kg of black seed (*Nigella sativa* L.) improved plasma lipid profile (triglyceride and low density lipoprotein) and antibody-mediated immunity (Ghasemi et al., 2014). In a

comparative study, between the use of lincomycin and licorice extract (*Glycyrrhiza glabra*) (0.1%, 0.2% or 0.3% in drinking water) in broiler chickens, Khamisabadi et al. (2015) concluded that licorice extract can reduce abdominal fat and serum levels of low density lipoprotein cholesterol and total cholesterol without any adverse effects on broilers performance and immune status.

It has been also shown that the use of spices in the feeding of broiler chickens could have a lipotropic effect. This effect is achieved by affecting lipid metabolism by the transport of fatty acids (Cross et al., 2007). Some aspects of the effects of PFA remain unclear (Wati et al., 2015). However the reason for using these plants is the many chemical components with antibacterial, antioxidant and conservative activity they possess (Mabona et al., 2013). According to Platel and Srinivasan (2004), it is likely that essential oils contained in PFA promote intestinal functions by stimulating bile secretion, digestive enzymes and mucus. Biologically active constituents of the plants are mainly terpenoids, phenolics, glycosides and alkaloids. Phytogenic feed additives promote growth by reducing the incidence and severity of subclinical infections, improving nutrient uptake (Huyghebaert et al., 2011). The phytogenic efficacy of broiler chickens depends on inclusion level in feed and the physiological period of the chicken (Mountzouris et al., 2011). Most beneficial effects of additives are observed in the growth and finishing period.

The use of PFA contributes to the maintenance of regular digestive function and chicken microflora (Mountzouris et al., 2011). According to Wati et al. (2015), supplementation with PFA in broiler chicken diets can improve nitrogen (N) retention and raw fiber digestibility, and may induce decreased digestive transit time compared to negative (without antibiotics) or positive control (supplemented with bacitracin methylene disalicylate [BMD]). The use of PFA does not alter carcass or meat quality. For example, the use of garlic and cinnamon (2 to 4 g/kg for both) in broiler feed had no effect on olfactory meat parameters (Toghyani et al., 2011). Li et al. (2015) concluded that phytoncide (0.5 to 1 g/kg) extracted from korean pine could be considered as an alternative to tylosin. Inclusion of phytocides, molecules excreted by trees in the air, has been able to improve performance and to reduce excreted gas emissions in broiler chickens (Li et al., 2015). Excreta ammonia, total mercaptans, acetic acid, and hydrogen sulfide all linearly decreased (P = 0.02, 0.04, 0.05 and 0.02, respectively) with increasing levels of phytoncide (Li et al., 2015).

Some research on chicken feed additives has focused on nanoparticles. Certain nanoparticles such as silver (Ag) and copper (Cu) as well as certain oxidized metals (Al_2O_3, Fe_3O_4, CeO_2, ZrO_2) may also have antimicrobial action and can be beneficial when used in feeds (Gangadoo et al., 2016).

4.2. Essential oils

Essential oils are the hydrophobic liquid of odoriferous and volatile aromatic compounds of a plant. Essential oils can be natural (vegetable origin) or synthetic. Only a few essential oils have useful antibacterial properties. The most used are thymol, trans-cinnamaldhyde, carvacrol and eugenol. Their modes of action lie in their interference with the enzymatic system of the bacteria and the modulation of immune responses and inflammation. Some studies (Khattak et al., 2014; Peng et al., 2016; Pirgozliev et al., 2015) showed that essential oils are promising alternatives to growth promoter antibiotics (e.g., avilamycin) in improving chicken production. Essential oils can also play a preventive and curative role in necrotic enteritis in broilers (Jerzsele et al., 2012). The use of essential oils has a positive effect on growth, meat and carcass quality as well as chicken health. Peng et al. (2016) reported that adding oregano essential oil (*Origanum genus*) at 300 and 600 mg/

kg in broiler chicken feed increased ADG. According to the authors, this result may be related to increased villus height and decreased crypt depth in the jejunum of broiler chickens. In addition, the administration of 600 mg/kg of feed of oregano essential oil improved the percentage of thigh muscle and decreased abdominal fat percentage in broiler chickens. Nevertheless, peppermint *(Mentha piperita)* was a good alternative to virginiamycin in broiler chickens (Khodambashi Emami et al., 2012).

4.3. Probiotics

Probiotics are defined as "live micro-organisms, when administered in adequate amounts, confer a health benefit to the host" (WHO, 2001). Probiotic feed supplementation improves growth, feed efficiency and intestinal health (Ghasemi et al., 2014; Giannenas et al., 2012; Samli et al., 2007). This improvement is achieved by reducing intestinal pH, intestinal bacteria composition and digestive activity. Mechanisms of action of probiotics include stimulation of endogenous enzymes, reduction of metabolic reactions that produce toxic substances, and production of vitamins or antimicrobial substances (Hassanein and Soliman, 2010). Probiotic bacteria produce molecules with antimicrobial activities such as bacteriocins which inhibits toxins' production and pathogens' adhesion (Pan and Yu, 2014). On the other hand, probiotics stimulate the immune response and increase resistance to colonization of bacteria (Hassanein and Soliman, 2010).

Administration of *Enterococcus faecium* in chicken feed had an antibacterial effect on bacterial microflora in the small intestine (Levkut et al., 2012). Similar results were reported with *Streptomyces* sp. (Latha et al., 2016) and *Bacillus subtilis* (Zhang et al., 2013). In a study (Zhang et al., 2013), comparing *B. subtilis* with enramycin, widely used as a feed additive for chickens to prevent necrotic enteritis, administration of 105 cfu of *B. subtilis* UBT-MO2/kg in broiler feed increased body weight by 4.4% and relative weight of the thymus. In addition, the treatment reduced NH_3 and H_2S concentrations in chicken excretions leading to less odor emissions.

Probiotics have positive effects on poultry meat quality (Hassanein and Soliman, 2010; Popova, 2017). They improve pH, color, fatty acid profile, chemical composition, water retention capacity and oxidation stability (Popova, 2017). The probiotics affect the protein and fat contents of meat and thus the meat quality. Abdurrahman et al. (2016) reported that lipid oxidation is one of the main causes of deterioration in feed quality. This hypothesis can be confirmed by other studies that showing the inclusion of *Aspergillus awamori* and *Saccharomyces cerevisiae* in chicken feed reduced blood saturated fatty acids and increased the polyunsaturated (Saleh et al., 2012). Another similar study of Liu et al. (2012b) showed that treatment with *Bacillus licheniformis* significantly increased the protein content and the respective essential and aromatic amino acids (Liu et al., 2012b). Feed containing *B. licheniformis* improves meat color, juiceness and flavor of broiler chickens (Liu et al., 2012b). These factors are very important in terms of consumer appreciation especially the color.

Probiotics may also have anticoccidial role. Results of Giannenas et al. (2012) suggest that treatment with probiotics may mitigate the impact of parasitic infection on chickens in the absence of anticoccidial infections. The use of probiotics exerted coccidiostatic effect against *Eimeria tenella*. This can help to minimize the risk and spread of coccidiosis and maintain intestinal health.

4.4. Organic acids

Organic acids are conservation agents used to protect feed from microbial and fungal proliferation (Kum et al., 2010). These acids are mainly carboxylic acids carrying a hydroxyl group on alpha

carbon such as malic, lactic and tartaric acids. The organic acids can also be simple monocarboxylic acids such as acetic, formic, butyric and propionic acids. The antimicrobial action of organic acids is due to the fact that non-dissociated acids can diffuse through lipophilic bacteria membrane and disrupt enzymatic reactions and transport system (Cherrington et al., 1991). Some studies (Hassan et al., 2010; Nava et al., 2009) showed that organic acids addition to broiler feed promotes growth, feed conversion rate and feed utilization. Adding organic acids in drinking water gives young chicks a protective efficacy against *Campylobacter* infection (Chaveerach et al., 2004). These acids also have a protective action against *E. coli* (Izat et al., 1990). Thus, it has been shown (Mohammadagheri et al., 2016) that supplementation with citric acid (2%) can improve cell proliferation epithelial and villi height of gastrointestinal tract. Organic acid blend, formic and propionic acid supplementation (0.0525% in drinking water) generates more homogeneous and distinct populations in the intestinal microbiota and increases the colonization of *Lactobacillus* spp. in ileum of chicken (Nava et al., 2009). These changes in the intestinal microbiota and the increase in *Lactobacillus* populations show that organic acid can be used as an alternative to antibiotics (bacitracin in this study) to reduce pathogenic bacteria in the gastrointestinal tract (Nava et al., 2009).

Some organic acids would play a role in digestion. Indeed, a diet with low digestible protein in chicken leads to more protein reaching the gut, resulting in an increase in protein fermentation. Protein fermentation produces ammonia, branched-chain fatty acids, volatile fatty acids and intermediate products such as lactate and succinate as well as gases (hydrogen, carbon dioxide and methane). Some of these compounds may have adverse effects on growth performance (Bikker et al., 2007). Organic acids, such as butyric acid, added as a feed additive can be used to improve the digestibility of ileal proteins from poorly digestible protein sources (Adil et al., 2010).

Butyric acid is a saturated carboxylic acid produced in the cecum and colon of animals via the fermentation of carbohydrates such as dietary fiber and unabsorbed starch (Hu and Guo, 2007). Butyric acid is a readily available source of energy for intestinal epithelial cells and stimulates their multiplication and differentiation, as a result improves the feed efficiency in chickens (Adil et al., 2010; Józefiak et al., 2004). Indeed, Hu and Guo (2007) showed that body weight gain in chickens increased linearly during the period from 0 to 21 days as the dietary supplementation of butyrate increased. Further, according to Hu and Guo (2007) dietary supplementation of butyrate influenced feed conversion ratio in a positive quadratic fashion during the period from 0 to 42 days. Qaisrani et al. (2015) reported that diet supplemented with butyric acid improved the growth performance of chickens fed proteins of low digestible sources.

4.5. Prebiotics

Prebiotics are non-digestible feed components that are potentially beneficial to host health because of their fermentable properties that stimulate bacteria growth and/or activity in the ileum and caecum (Gibson and Roberfroid, 1995). It generally consists of short chain polysaccharides and oligosaccharides. Several prebiotics are generated from yeast cell walls and fermentation products. Prebiotics are not digestible by the host but commensal intestinal bacteria can metabolize them to produce short chain fatty acids like propionate, acetate and butyrate (Józefiak et al., 2008). These prebiotic components have positive effects on poultry productivity and contribute to a healthy intestinal tract and can be a good alternative to antibiotics (Morales-Lopez et al., 2009; Zhang et al., 2005). When ingested, the prebiotics alter the caecal microbial composition resulting in changes in the proteobacteria

and changes in the genus and family of bacteria which causes change in growth (Park et al., 2016).

The addition of a product rich in mannose and mannoproteins in chicken feed significantly increased the number of intestinal villus cells (Baurhoo et al., 2007). Further, administration of mannanoligosaccharide (0.2%) in the chicken diet conferred intestinal health benefits over antibiotics. These advantages are expressed by a reduction of pathogenic bacteria, a morphological development (height of the villus and number of goblet cells) and an increased colonization by beneficial bacteria (Baurhoo et al., 2009). Another way of administration reported by Bednarczyk et al. (2016) is the ingestion of *in ovo* prebiotics in the chicken embryo. It is an effective practice that can replace antibiotic supplementation in water after hatching. The doses of prebiotics used *in ovo* are 10 times lower than after hatching.

4.6. Amino acids and enzymes

The feed additive enzymes are produced through fungi and bacteria fermentations. They are used to maximize feed conversion. Enzymes facilitate components degradation such as proteins, phytates and glucans. For example, endo-b-1-4-xylanases and b-1-3,1-4-glucanases have been used in wheat and barley diets of broilers to improve their digestion (Cowieson et al., 2006). Also, phytase enzyme can increase villus width and decrease crypt depth which can improve ADG (Mohammadagheri et al., 2016). Lysins are bacteriophage endolysins representing an innovative alternative therapeutic option of antibacterial. Lysins are phage-encoded peptidoglycan hydrolases which bring about the bacterial cell lysis when applied exogenously to Gram-positive bacteria (Fenton et al., 2010; Rios et al., 2016). According to Volozhantsev et al. (2011), administration of a combination of a group of lysins containing peptidases, amidases and lysozymes produces an antimicrobial effect against *C. perfringens* in poultry. For example, Ply3626 lysine is an enzyme which has been shown lytic activity against several strains of *C. perfringens*, which is an important cause of food poisoning and leads to economic losses in poultry production (Fenton et al., 2010; Zimmer et al., 2002).

4.7. Other studies

Other trials aiming at identifying compounds with positive effects on chicken health and productivity have focused on the use of natural products such as milk and propolis, resinous mixture produced by honey-bees. An immunodulatory effect was observed in broilers fed a feed containing propolis (Daneshmand et al., 2015). Broilers fed this had heavier lymphatic organs and a higher concentration of antibodies against Newcastle virus. Inclusion of milk kefir (2%) in chicken drinking water had a significant effect on body mass and chicken consumption index between 28 and 42 days of breeding (Toghyani et al., 2015).

5. Conclusion

Over the years, antibiotics have played an important role in fighting infectious diseases and stimulating poultry growth. Scientific evidence suggests that their large-scale use has led to antibiotic resistance and residues in the food and environment, particularly aquatic ecosystem, which can lead to public health problems. Many trials of potential alternatives to antibiotics have shown very relevant results. These alternatives give equal or better effects to antibiotics (namely good livestock performance), reduce mortality rates and protect environment and consumer health. Application of the results generated by these studies in feed industries, as well as livestock breeders and veterinary practice is

very appealing. Some studies show that antibiotic use can be dropped or reduced. It should also be noted that some trials have shown the efficacy of vaccination of broiler chickens as a prophylactic treatment against necrotic enteritis induced by *C. perfringens* (Caly et al., 2015). Another way of research in the future is to test the interactive effect of using combinations of these alternatives. The aim will be to maintain a high level of viability and optimum productivity in poultry antibiotic free farms.

Conflicts of interest

The authors declare no conflict of interest.

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