



Use of Antimicrobials from Plants in Feed as a Control Measure for Pathogenic Microorganisms

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Abstract

Animal Feed has become an increasing critical component of the integrated food chain, in 2010 about 1000 mt of animal feed was produced globally and 150 mt in the EU27. The animal feed has an important impact in the human health. The farm or feedlot is the origin of microorganisms introduced onto carcasses during slaughter and dressing. It appears that changes in diet and management practices could precipitate increased shedding of pathogens. Additionally, antibiotics are used in animals, not only for treatment or prevent diseases, but also to promote growth. As a result of the use of antibiotics, food can contain antibiotic-resistant bacteria and resistance genes with important public health consequences.

Although antibiotics are banned as growth promoters in the European Union and some other countries, this is not the case throughout the WHO European Region. Travel and the globalization of trade further increase the risk of spreading antibiotic-resistant bacteria.

Keywords: Animal feed; *Campylobacter jejuni*; Salmonella; *Escherichia coli*; Bacteria

Numerous studies carried about for evaluating the antimicrobial activity of plants, vegetables and agro-food by-products have demonstrated their efficiency against different pathogens. The use of these vegetables (plants and by-products) for obtaining natural additives with antimicrobial properties could be used in feed, after the adequate studies, to reduce antibiotics consumption with a natural feeding. Another approach with significant research is feed animals with probiotics ("good bacteria") to competitively exclude the pathogens.

According to EFSA meeting report (2012) related to foodborne pathogens from zoonotic origin, *Campylobacter jejuni*, *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* have been described with incidence in broiler flocks and raw animal origin products.

Campylobacter jejuni was responsible of the most human zoonotic confirmed cases in 2010. Moreover, it is of concern the human campylobacteriosis significant increase occurred in the last five-year at European Union (2006-2010). The most important reservoir continues to be broiler meat at European Union level, with 30 % of fresh broiler meat units positive for *Campylobacter*, varying from 3.1 % to 90.0 % (EFSA, 2012).

Regarding *Salmonella* and although the observed reduction in salmonellosis cases mainly due to successful *Salmonella* control programmes, salmonellosis still remains as an important disease with economic impact as it may affect animal performance and may result in foodborne disease in humans through the eggs and carcass contamination. The costs associated with non-typhoidal *Salmonella* infections are estimated at nearly \$2.4 billion dollars annually, which includes costs due to loss of productivity and medical treatment costs. A poultry producer suffers losses due to *Salmonella* infection of the flock including loss of birds and production time [1].

Salmonella is most often detected in fresh broiler at different levels of the production chain, with *Salmonella*-positive samples proportion varying between 0.2 % and 27.8 % but on average, at European Union level, 1.2 % of the samples were positive. The highest contamination

levels (27.8 %, single samples) were reported by Hungary in non-read-to-eat (RTE) meat preparations at retail [2]. It is remarkable the prevalence of the five serovars: *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella infantis*, *Salmonella virchow* and *Salmonella hadar*. Among them, *Salmonella enteritidis*, *Salmonella typhimurium* are serovars most frequently associated with human illness [3,4]. Human *Salmonella enteritidis* cases are most commonly associated with the consumption of contaminated eggs and poultry meat, while *Salmonella typhimurium* cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat [2].

Due to the risk for unborn child, infants, and the elderly by means of *Listeria monocytogenes* infection, it will be considered in the present study because of *Listeria* spp. is one of the most relevant zoonotic agents with food safety implications by consumption of ready-to-eat in the last products of animal origin (e.g., fowl meat) (4.9 % of RTE products of animal origin are non-meeting the criterion of absence of *Listeria monocytogenes* in 25 g of product at processing level) [5].

In Europe 2005, 380,000 European Union citizens were affected by infectious zoonotic diseases, 5311 food-borne outbreaks were reported involving 47,251 people and resulting in 5330 hospitalizations and 24 deaths. *Campylobacter* and *Salmonella* reported the highest number of cases, 197,363 and 176,395, mainly related to fresh poultry meat and eggs, poultry and pig meat, respectively [6]. *Yersinia enterocolitica* reported 9630 cases and *Escherichia coli* VTEC caused 3314 cases, which were mainly associated to fresh bovine meat. *Listeria monocytogenes*

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reported 1439 cases, mainly related to RTE products, and *Brucella mellitensis* accounted for 1218 cases [6].

One of the greatest challenges in the meat industry is to keep the product safe and free from pathogen contamination. Several pre-slaughter interventions are used to reduce intestinal carriage of pathogens such as macronutrient diet formulation, antibiotics and growth-enhancing additives, phenolic antimicrobial compounds; organic acids and acidified feed, chlorate and nitro-based compounds and others that have been used in food animals worldwide [7].

Under the principle “from farm to fork”, animal feeds are considered to be a sensitive stage at the beginning of the food chain, being the origin of microorganisms present onto carcasses at slaughter and dressing steps. So, animal feed should accomplish the main objective to produce a final meat product with required quality and without health (chemical or microbiological) risk to consumers. It appears that changes in diet and management practices could precipitate increased shedding of pathogens. Feeding cattle barley-based diets [8] or wet distillers grains [9] increased shedding of *E. coli* O157:H7 compared with feeding standard corn diets. There also is significant research on the feeding of probiotics (“good bacteria”) to livestock to competitively exclude the pathogens. A *Lactobacillus* based direct-fed microbial has shown promise in decreasing the shedding of *E. coli* O157:H7 [10]. Sodium chlorate treatment has been shown to reduce populations of *Salmonella typhimurium* and *E. coli* O157:H7 in the intestinal content of swine and cattle [11,12] and work is underway to see if this can be used in the field. From the point of view of the manipulation of ecosystem microbial, antimicrobial specificity based on the Gram negative and Gram positive bacteria reaction could be useful. For example, [13] investigated the effect of thymol *in vitro* on the fermentation of glucose by *Streptococcus bovis* and *Selenomonas ruminantium* and verified that thymol inhibited lactate production, glucose uptake and the growth of the *Streptococcus bovis* and *Selenomonas ruminantium*. Furthermore, the thymol has been reported to be a strong deaminase inhibitor [14]. Some plants and their extracts have been shown to stimulate the growth of certain bacteria, i.e., prebiotic-type effect [15,16]. This effect was corroborated by [17] that observed the oregano essential oils, like antibiotics, modify the gut microflora and reduce microbial load by suppressing bacteria proliferation. There are some claims that oregano oil can replace anticoccidial compounds, not because they inactivate coccidia, but because they increase the turnover of the gut lining and prevent coccidial attack by maintaining a more healthy population of gut cells. This mode of action would increase the animal’s maintenance energy requirement because enterocyte turnover is a major proportion of the basal metabolic rate. In this context, it is very interesting to consider the use of natural plant extracts, essential oils or some of its components as one of the tools for animal nutritionists to reduce the excretion of pathogenic bacteria [18,19].

It has been documented that substantial amounts of antibiotics are administered to food animals for growth promotion, feed efficiency, and prophylaxis in the absence of known disease [20-25]. In Europe, the market of antimicrobials was segmented into antibiotics (e.g., oxytetracycline), antibacterial (e.g., carbadox), anticoccidial (e.g., salinomycin), growth promoters (e.g., monensin) and others that included anthelmintic (e.g., ivermectin) [26]. Despite the beneficial effects of antibiotics as additives in feed, their use worldwide has been questioned, regulated and even prohibited. For example, European Union member nations banned all antibiotic growth promoters in 2006 according to European Parliament and Council Regulation EC No. 1831/2003 as a precautionary principle [27]. On the other hand,

Russia, Mexico, South Korea, Brazil, USA, Australia, Japan, China, and Canada, allow the use of some antimicrobials as feed additives [28].

In the United States alone, it is estimated that 11.2 million kg of antibiotics per year is administered to food animals for nontherapeutic purposes, whereas only 900,000 kg is consumed for therapeutic treatments [29]. An intensive debate, therefore, has raged over past three decades on the impact of the use of antibiotics in food animals on human health [30]. Adverse effects of the extensive use of antibiotics in animal practices include generating antibiotic-resistant organisms and resistance genes [21]. These resistant bacteria can be transferred to humans directly or indirectly via the food chain, which is of special interest and importance to public health in relation to infectious disease [26,31-33]. Antimicrobial resistance is a result of four strategies namely enzymatic inactivation of the drug [34], modification of target sites [35], reduced cellular uptake [36] and extrusion by efflux [37].

Due to the accumulation of antibiotic-resistant bacteria and genes, leading to the treatment failure or longer convalescence in both humans and animals, research on antibiotic replacement is currently emerging [38-40] under the concept “clean, green, and ethical” (CGE) animal production [41].

Use of natural antimicrobials such as extracts from spices and/or herbs for the purpose of food preservation and/or the substitution of antibiotics have arisen. Before the advent of readily-available antibiotics in the 1950s, antimicrobial plant derivatives were commonly used [42]. These bioactive compounds act as plant survival mechanisms and defense mechanisms in response to environmental stressors, pathogen attack, competing plants and herbivory [43]. Phytochemicals have a large variety of principle actives and their effects expected are several as act on the improvement in the palatability of feed, feed intake, feed digestibility, stimulate the pancreatic secretions to increase endogenous enzyme activity, gut development/health, antimicrobial/antiviral, antioxidative and anti-inflammation effects and immune system that could benefit performance and health of farm animals [17,41,44-54]. Therefore, phytochemicals could be used to replace some antibiotic growth promoters [55,56]. However, to be most effective as growth promoters, these herbal antimicrobial compounds must be supplemented to the feed in a more concentrated form than found in their natural source [57]. Their application in food animal production has been limited.

According to Liu [58] the phytochemicals can be classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds or organosulfur compounds. The most studied of the phytochemicals are the phenolics and carotenoids. Among them, the most studied are the phenolics and carotenoids. Phenolic compounds and phenols could be found in a wide range of plant materials (by-products, fruits, leaves, seeds) and plant extracts (essential oils, infusions) [59]. The antimicrobial activity of plant extracts is due to their chemical structure, in particular to the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components [56,59-61]. Usually, the phytoconstituents with phenolic groups as oils of clove, oregano, rosemary, thyme, sage and vanillin are the most effective [62-65]. They are more inhibitory against Gram-positive than Gram-negative bacteria [66,67]. Currently, the only way to ensure controlled dosage is to use characterized dried plant material, plant extracts or isolated bioactive secondary metabolites. These sources could be incorporated as natural additives into supplemental concentrate feeds [26]. Besides, the lipophilic nature of these compounds also presents a challenge in effective delivery to

the animal gut and this can partially be resolved by microencapsulation and combination with other compounds (synergistic effect) [56].

As with antibiotics, continued use of these plant-based antimicrobials may result in the development of resistance in some pathogenic bacteria [57]. At present there is not sound evidence indicating that the use of natural antimicrobials from plants can induce resistance to those compounds in microorganisms but considering its action mechanism it can be thought that they could behave as antibiotics. In general, it is well documented that the use of inactivation treatments at sub-lethal doses produce cellular damage that after being repaired can give to the cells new abilities or some resistance to the killing agent. Nevertheless, according to several studies, the use of natural antimicrobials can induce resistance or reduce resistance to some antibiotics [57,68-70]. Moreover, the use of carvacrol and citral on *Listeria monocytogenes* produced some changes on virulence of the microorganisms as indicated by Silva [71]. On the other hand, in the literature there are also reports that phytochemicals are capable of inhibiting processes related with quorum sensing (QS), which is responsible for microbial cell genes expression, especially the virulence factors [72,73], without encouraging the appearance of antimicrobial resistance [72]. That way, more research is necessary to evaluate this potential risk for animal production and to human health even though phytochemicals are generally recognized safe (GRAS).

In this scenario, there is potential for isolating antimicrobial compounds that exhibit mechanisms unrelated to conventional antimicrobial compounds. Compounds feeding stuffs supplementation with these natural plant materials with possible or tested antimicrobial capability could be an alternative to reduce the final microbial counts at faeces level, with the subsequent impact on food safety not only along the food chain, but also with implications in the international trade [74,75].

However, understanding the potential for novel antimicrobial compounds in foods and feeds will require the physiological examination of foodborne pathogen response under experimental conditions comparable to the environment where the pathogen is most likely to occur.

Research on the foodborne zoonotic agents *Salmonella typhimurium*, *Listeria monocytogenes*, *E.coli* O157:H7 and *Campylobacter jejuni* is extensive and should provide a model for detailed examination of the factors that influence antimicrobial effectiveness. Analysis of pathogen response to antimicrobials could yield clues for optimizing hurdle technologies to more effectively exploit vulnerabilities of *Salmonella* and other foodborne pathogens when administering antimicrobials during food and feed production.

Although further research must be carried out to understand all the mechanisms and potentials of those active molecules there is little doubt that animal performance can be improved through their use. Feed additives can help in a variety of ways to reduce the risk of *Salmonella* infections. Essential oil compounds antibacterial properties support good flock health and could contribute to consumer food safety.

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