In: Essential Oils
Editor: Miranda Peters
ISBN: 978-1-63484-351-5

Chapter 10

ESSENTIAL OILS AS GROWTH PROMOTERS IN BROILER CHICKEN

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ABSTRACT

The large production of poultry is in part due to antimicrobials that are used to improve broiler chicken performance, although in recent years this practice has been questioned due to suspected appearance of residues in the meat and resistant microorganisms. Thus, the essential oils emerge as promising substitutes to the usual growth promoters.

The aim of this study was to analyze the state of the art concerning the use of essential oils as additives in broiler diets and such data will be used to conduct further studies in the future.

The databases used were SciELO, Portal Capes, Science Direct and PubMed. 42 papers published between 2005 and 2014 were selected. 27 plant species were tested, and oregano was the most used. The essential oils act in different ways in the organism of the animals, going beyond the antimicrobial activity and showing effect on several productive parameters of the poultry, with results similar or better than those of antimicrobials.

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INTRODUCTION

The Brazilian poultry industry emerged around the 1940's and gradually developed in the following years until the 70s, when there was a restructuring and consequent substantial growth of the sector. The increase in the urban population, among other factors, increased demand for processed chicken meat, and the implementation of the integration system and new technologies in genetics, nutrition, management and sanitation increased supply (Santos et al. 2005; Pinotti and Paulillo, 2006).

However, in order to increase production it was necessary to overcome the susceptibility to pathogenic micro-organisms the animals presented. The development of these bacteria in the intestine causes damage both by competition for food, as well as through damage to the mucosa. To overcome these problems, antimicrobial agents have come to be added to animal feed, for prevention, at doses below those employed for the treatment of diseases (subtherapeutic). Antibiotics act by manipulating the microbiota, ensuring good animal performance and expression of the whole genetic potential. The antimicrobial agents with this function were called growth promoters and, more recently, have been referred to as performance enhancers (Santos et al. 2005).

Growth promoters prevent the development of pathogenic microorganisms in the intestine of treated animals (may inhibit or eliminate), resulting in better digestion and absorption of various essential nutrients and a more stable equilibrium in the microbial population, increasing weight gain, improving feed conversion and decreasing mortality. In general, they act promoting selectivity to beneficial microorganisms in the intestine, although the action mechanism is still not well understood (Santos et al. 2005).

However, over the years there was a concern with the likely side effects that could result from the use of antimicrobials, driven by the development of microorganism resistance in relation to some antibiotics used in human health. Antibiotics in allowed animal feed are those that are not used in the treatment of diseases in humans. However, there are cases where the microorganism that has developed resistance to an antimicrobial agent may also exhibit this characteristic against other bases (cross-resistance), resulting in drug ineffectiveness (Santos et al. 2005; Souza and Silva, 2008).

The literature contains various works denouncing the presence of antibiotic residues and resistant bacteria in products derived from chicken. 60% of the chicken meat samples analyzed by Wouafo et al. (2010) showed *Salmonella* serotypes, almost 90% of them resistant. Furtula et al. (2010) conducted experiments with diets containing growth promoters and when analyzing poultry litter samples, they found residues of antibiotics and multiresistant *Escherichia coli*. Diarra et al. (2007) also tested different promoters and found that all isolated individuals of *E. coli* had some degree of resistance to multiple antibiotics. Osman and Hariri (2013) isolated samples of *Clostridium perfringens* from poultry with necrotic enteritis and showed that all the individuals were resistant to most commonly used antibiotics.

On the other hand Thibodeau et al. (2008) evaluated the effect of Zinc bacitracin and Virginiamycin on the emergence of resistant bacteria at a commercial of broiler chicken breeder. *E. coli* and *Enterococcus* spp. were isolated and tested for the presence of resistance genes. The results showed that the use of growth promoters did not significantly influence the emergence of resistance in this case.

This concern with food security led to the ban on the use of antimicrobials as feed additives in the European Union. In Brazil, however, the use of certain bases is still permitted, though there are many discussions about it. For example, Avilamycin, Zinc bacitracin and Tylosin sulfate are allowed (although they are banned in Europe) and Chloramphenicol, systemic Sulfonamides, Tetracyclines and Oxitetraciclinas are prohibited (Souza and Silva, 2008).

Europe therefore, as a way to protect its consumers, has created new barriers to the entry of poultry products from other countries, making it difficult for Brazilian exports. This means that these producers must adapt to consumer demands to be competitive in the market. However the non-use of growth promoters has a number of difficulties as consequences. Problems arise, from the drop in performance of the animals to increased mortality, resulting in higher production costs and higher cost for consumers (Souza and Silva, 2008).

Therefore, there is a need to seek alternatives, which has boosted studies involving the use of natural additives, such as essential oils, in the role of performance enhancers (Koaiyama et al. 2014).

In this context, this paper aims to review the literature to analyze the state of the art relating to the use of essential oils as additives in the diet of broiler chickens and with such data submit new studies in future addressing the topic.

METHODOLOGY

A quantitative literature review was carried out through the collection of scientific articles published between 2005 and 2014 in four online databases: SciELO, Science Direct, Portal Capes and PubMed. Thirty-six keywords related to the subject, in Portuguese, Spanish and English were entered in the search field. They were: additives; natural additives; alternative; antibiotics; antimicrobials; birds; poultry; carcass; coacervation; performance; diets; antimicrobial effect; encapsulation; herbal extracts; plant extracts; herbal medicines; broiler chickens; intestinal histology; performance enhancer; secondary metabolites; intestinal microbiota; microencapsulation; intestinal morphology; poultry nutrition; essential oils; volatile oils; medicinal plants; growth promoter; residues in meat; bacterial resistance; cross-resistance; spray drying; replacement; subtherapeutic and supplementation.

The articles were evaluated according to the following inclusion criteria:

1) it is a scientific article;
2) covering at least one of the following subjects:
    a) use of antibiotics as a performance enhancer;
    b) resistance of microorganisms to antimicrobial agents and/or residues in meat;
    c) microencapsulation;
    d) use of essential oils from any plant against bacteria isolated from poultry *in vitro* and/or in place of antimicrobials *in vivo*.

Studies dealing with the use of essential oils as additives were organized in spreadsheets, identifying the database where they were was found; title; author; publication year; plant species used; the use (or not) of microencapsulation; type (*in vitro* or *in vivo*); results and conclusion (positive or negative).

RESULTS AND DISCUSSION

Sixty-eight works were found, of which only forty-two met the inclusion criteria. Three of the works had the use of antibiotics as performance enhancers as their central theme; five dealt with the problems with resistance of micro-organisms; only seven addressed microencapsulation, and finally twenty-seven dealt directly with using EOs as additives.

Among the articles, the plant species used varied, with many articles dealing with testing more than one oil simultaneously. Four articles did not mention the plants used, referring to them only as "blends of essential oils."

Oregano was the most cited plant (17 articles), followed by thyme and cinnamon (7), rosemary (5), pepper, sage and clove (4), garlic (3), citrus peel, bay leaves, basil, fennel and ginger (2 each?) and lemon, peppermint, fenugreek, eucalyptus, cumin, copal, cinnamon-of-china, boldo-do-chile, red pepper tree, star anise and rosemary pepper (1 each ?). All these species showed positive results as growth promoters, individually or in association. The main components of the essential oils of these plants are carvacrol and thymol, cinnamaldehyde, eugenol, capsaicin and allicin, but it is not definitively known if the other components present in the oils acted synergistically with the active ingredients.

Plant extracts may have bactericidal or bacteriostatic effect, acting similarly to antibiotics. Their action is due to their chemical components, called active ingredients. These are secondary metabolites produced by plants in order to protect it in adverse situations - stress, environmental factors, predator and pathogen attack - but such metabolites also provide them with medicinal properties. Glycosides, alkaloids, phenolic and polyphenolic compounds, terpenes, saponins, mucilage and flavonoids are some common secondary metabolites (Barreto et al. 2008).

The essential oils are secondary metabolites that are part of the terpene or terpenoid class. They are characterized by their low molecular weight and consequent volatility. Their function in the plant typically consists of attracting pollinators or repel predators because of their strong odor. Their formation is influenced by several factors (time of year, temperature, humidity, exposure to sun and wind) especially when the storage structures of these compounds, of the species concerned, are located in the leaf (Barreto et al. 2008; Bona et al. 2012). The collection time also affects the essential oil characteristics, therefore it is recommended to collect the morning or evening to avoid exposure to the sun (Melo et al. 2011), in addition to observe the time of year, which can affect the plant biomass production and oil yield (Figueiredo et al. 2009).

The action mode essential oils is not yet fully understood. What is suggested is that these substances may increase the palatability of the diet, stimulate the secretion of endogenous enzymes, facilitate digestion, alter intestinal microflora and help in reducing sub-clinical infections, as well as exert antimicrobial activity via cell wall attack and alter the permeability of the cytoplasmic membrane of bacteria and stimulate antioxidant and nitrogen uptake (Bona et al. 2012; Traesel et al. 2011).

Several studies have demonstrated the effectiveness of essential oils *in vitro* and *in vivo*. In the study of Santurio et al. (2007) the essential oils from oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) and cinnamon (*Cinnamomum zeylanicum*) showed antimicrobial activity against strains of *Salmonella enterica* of poultry origin. The results show that oregano and thyme essential oils are effective against *Salmonella enterica*.

Barreto et al. (2008) found similar performance and organ morphometry parameters for poultry fed oregano, cloves, cinnamon and red pepper extract (microencapsulated extracts with 20% essential oil) and those fed Avilamycin. Only treatment with bell pepper presented worse morphometry results than those of the control.

Scheuermann et al. (2009) show that the use of essential oils (commercial mixture, plus capsaicins and saponins) in broiler diets improves efficiency in the use of calcium, phosphorus, energy and amino acids. This makes it possible to reduce these levels in the feed and thereby reduce costs.

According to Silva et al. (2009) oregano has an effect similar to that of Avilamycin + Salinomycin regarding the intestinal morphology of animals challenged with *Eimeria tenella*.

The essential oils of oregano, and garlic (individually or associated) can replace growth promoters, showing similar effects on performance variables and *Clostridium* counts (Kirkpinar et al. 2010).

Rizzo et al. (2010) worked with three combinations of phytogenic additives: 1) plant extracts of cloves, thyme, cinnamon and pepper; 2) synthetic essential oils of oregano and cinnamon and pepper extract oleoresin; 3) eucalyptus oil, chinese cinnamon essential oil-, boldo, Chile bilberry leaves and fenugreek seeds, compared to antibiotics. The use of the plant extracts did not differ from results obtained from the use of antibiotics for performance, carcass characteristics and use of protein and dietary energy.

Santurio et al. (2011) analyzed the *in vitro* antimicrobial effect of essential oils from the spices - oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum zeylanicum*), Mexican oregano (*Lippia graveolens*), ginger (*Zingiber officinale)*, sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*) and basil (*Ocimum basilicum)* - against *E. coli* samples isolated from poultry and cattle. The authors concluded that oregano, Mexican oregano, thyme and cinnamon oils were effectively bactericidal and oregano oil presented the greatest antimicrobial activity.

Silva et al. (2011) found that 0.4% of red mastic essential oil promotes improvement in intestinal absorptive surface of broilers. Evaluating the intestinal mucosa characteristics is important because some microorganisms can cause injury. In such cases there are alterations in the mucosa structure (villi and crypts) that harm cellular turnover and nutrient absorption, impairing animal performance.

Traesel et al. (2011) concluded that the essential oils of oregano (*Origanum vulgare*), sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*) and pepper (*Capsicum frutescens*) extract used to substitute antimicrobials showed antioxidant activity, providing lower plasma lipid peroxidation and therefore less oxidative damage in broilers.

Working with essential oils of oregano, rosemary and a mixture of oils *in vitro* and *in vivo*, Mathlouthi et al. (2012) noticed that these oils have activity against the most common bacteria of poultry and it is possible to use them to substitute the usual antibiotics, since they an effect similar to that of Avilamycin.

Good results were found by Bonn et al. (2012) with the essential oils of oregano, rosemary, cinnamon and pepper extract in the control of *Clostridium perfringens* in poultry challenged with *Eimeria* or *Salmonella Enteritidis*. The oils promoted control of bacteria, reduction in lesions, increase in the villus/crypt ratio and the number of CD3+ (T-lymphocytes) cells in the duodenum of the animals.

Agostini et al. (2012) showed that 100 and 200 mg/kg of essential oil of clove improves feed efficiency and can modulate the microflora and intestinal epithelium.

Amerah et al. (2012) used xylanase and a mixture of essential oils (containing thymol and cinnamaldehyde), associated or individually, and showed improvement in performance and a 77% reduction of *Salmonella* vertical transmission. The best results occurred in the treatment in which the xylanase and the oil blend were associated.

Betancourt et al. (2012a) obtained higher body weight and economic efficiency, besides a lower negative impact on the weight of broilers challenged with *Coccidia* receiving essential oil of rosemary-pepper.

In another study, Betancourt et al. (2012b) tested several varieties of oregano, analyzing ileal digestibility of protein, fat and energy in addition to intestinal and histomorphology performance variables. Treatments with oregano were inferior to those with the antibiotic only in the ileal digestibility of protein, showing that it may be used as a promoter. They also perceived that there is a negative correlation between the consumption of carvacrol and body weight, and another correlation, positive, with the consumption of thymol.

Erhan et al. (2012) found similar performance parameters among chickens fed diets with antibiotics and diets with essential oil of pennyroyal. There was also a decrease in the *E. coli* count and increase in the number of beneficial intestinal bacteria.

The association of essential oils of oregano, fennel and citrus peel presents high potential to replace antibiotics, improving performance and health status (improved feed efficiency and survival rate, reduced cholesterol, improved HDL concentration, did not affect bacteria in the ileum, reduced ammonia in the ileum and increased villus height in the duodenum) as well as meat quality (Hong et al. 2012).

Siva et al. (2012) used oregano essential oil and ginger extract compared to antibiotics (Bacitracin and Colistin) and found similar performance parameters and intestinal morphology for all groups.

The essential oil of peppermint and prebiotics (fructooligosaccharides) were compared with virginiamycin (growth promoter) by Emami et al. (2012). Most of the variables worsened with the use of essential oils and prebiotics, which does not make the essential oils in question good alternatives to antibiotics. Wirth et al. (2012) concluded that oregano and thyme can be used as additives improving the performance of animals tested.

The essential oils of thyme and star anise were tested by Cho et al. (2013) and were seen to promote performance improvement, reduction in total blood cholesterol and inhibition of *C. perfringens* and *E. coli* proliferation in the small and large intestine, achieving better results than those of Avilamycin.

Karadas et al. (2014) showed improved performance and improvement in hepatic concentrations of carotenoids and coenzymes in chickens supplemented with a commercial blend of essential oils (including carvacrol, cinnamaldehyde, and Capsicum oleoresin).

Khattak et al. (2014), using a commercial blend of essential oils based cumin, bay leaves, basil, lemon, oregano, sage and thyme, achieved better performance parameters and carcass yield.

The essential oils of oregano and garlic improve the chicken meat characteristics and do not change the carcass yield compared to antibiotics (Kirkpinar et al. 2014).

Table 1. Species of plants tested, sensitive and resistant microorganisms, activity as performance enhancer and primary action of essential oils in the organism

Species	Antimicrobial activity[1]	No activity[2]	*in vivo* Activity	Principal action mechanisms
Rosemary	*Salmonella, E. coli, Listeria, Clostridium, Eimeria*	*	Yes	Hinders meat oxidation; performance; carcass yield[3]
Rosemary-pepper	*	*	Yes	Performance
Garlic	*Clostridium*	*	Yes	Performance; carcass yield; hinders oxidation and increases succulence of the meat
Star anis	*E. coli, Clostridium*	*	Yes	Performance; cholesterol levels
Mastic	*	*	Yes	Performance; intestinal morphometry
Chile Bilberry	*	*	Yes	Performance
Cinnamon	*Salmonella, Clostridium, Eimeria*	*E. coli*	Yes	Performance; carcass yield
Chinese cinnamon	*	*	Yes	Performance
Citrus peels	*	*	Yes	Performance; cholesterol levels
Cumin	*	*	Yes	Performance; carcass yield
Copaiba	*	*	Yes	Performance; carcass yield
Clove	*	*	Yes	Performance; carcass yield; modifications in microbiota
Fennel	*	*	Yes	Performance; cholesterol levels
Eucalyptus	*	*	Yes	Performance
Fenugreek	*	*	Yes	Performance

Koaiyama et al. (2014) tested three combinations of essential oils: 1) rosemary, cloves, genbibre and oregano; 2) cinnamon, sage, white thyme and copaiba oleoresin; and 3) association with 50% of each of the combinations on performance and carcass yield. Mixtures 2 and 3 (association) had potential as a performance enhancer, improving weight gain, feed conversion and carcass and cut yields.

Studies show that essential oils used as additives, alone or in association, promote similar results and sometimes higher results than the antimicrobial growth promoters in various variables. These data demonstrate the feasibility of replacing such additives. Among the works assessed, there was only one work in which the results were negative (Emami et al. 2012), in which the authors suggest further studies, since the use of peppermint, in the form of essential oil, had not been reported in the literature, plus the fact that the variation of environmental conditions changes the composition of the oils, which may have caused the absence of antimicrobial activity in this case.

Table 2 summarizes the results identified in the work, demonstrating species, micro-organisms against which the antimicrobial activity was reported (or disproved), and the occurrence of positive results in *in vivo* tests and the main action mechanisms in the organism of the animals.

Table 2. Species of plants tested, sensitive and resistant microorganisms, activity as a performance enhancer and primary action of essential oils in the body C*ontinuation*

Species	Antimicrobial activity [1]	No activity[2]	*in vivo* Activity	Principal action mechanisms
Ginger	*	*Salmonella*	Yes	Performance; carcass yield; Intestinal histomorphometry
Peppermint	*	*	No	Negative results
Lemon	*	*	Yes	Performance; carcass yield
Bay	*	*	Yes	Performance; carcass yield
Basil	*	*	Yes	Performance; carcass yield
Oregano	*Salmonella, E. coli, Clostridium, Eimeria, S. aureus*	*	Yes	Performance; carcass yield; cholesterol levels; intestinal morphometry; hinders oxidation and increases succulence of the meat; histointestinal morphometry; nutrient digestibility
Mexican oregano	*E. coli*	*	Yes	Antimicrobial activity
Red pepper	*Clostridium, Eimeria*	*	Yes	Performance
Red bell pepper	*	*	Yes	Performance; avoid meat oxidation
Pennyroyal	*E. coli*	*Salmonella*	Yes	Performance
Sage	*	*Salmonella*	Yes	Hinders meat oxidation
Thyme	*Salmonella, E. coli, Clostridium,*	*	Yes	Performance; cholesterol levels

[1]Microorganisms sensitive to essential oils, cited in the articles selected.
[2]Micro-organisms resistant to essential oils, cited in the articles selected.
[*]Sensitivity not mentioned in selected articles.
[3]Performance and carcass yields were equal or above those with antibiotics.

The principal microorganisms sensitive to the oils were *Salmonella* spp., *E. coli* and *Clostridium*, besides *Eimeria* spp., *Listeria* spp. and *Staphylococcus aureus* (antimicrobial and anticoccidial effect), also mentioned in the selected articles. As to the benefits of the oil use, as well as their already known bactericidal activity, the most frequently reported actions were: effect on performance and carcass yield (in some studies these parameters improved; in others they were only similar to treatments with the performance enhancers and nevertheless the results were considered positive: the aim of the studies was to verify the possibility of substituting antibiotics, so it is enough that the oils do not worsen the characteristics). A decrease in meat oxidation was also reported, as were a positive effect on the intestinal morphometry and microflora; improvement in flavor characteristics and succulence of the meat; reduced cholesterol and increased nutrient digestibility.

Microencapsulation of Oil

Essential oils are very volatile substances. To work around this problem they can be protected by microencapsulation (Peng et al. 2014; Souza et al. 2008). Among the works mentioned with essential oils as additives five cite the technique as the procedure performed or as a suggestion (Barreto et al. 2008; Betancourt et al. 2012a; Rizzo et al. 2010; Scheuermann et al. 2009; Traesel et al. 2011).

Microencapsulation is essentially a process in which microscopic droplets or particles of the substance (in this case the oil) are enclosed by a layer of encapsulating material, creating tiny capsules (Fernandes et al. 2014). It is used for the purpose of masking unpleasant taste and odor or reduce volatilization and reactivity; increasing the concentration and stability of the substance against harsh environmental conditions (light, oxygen, and pH extremes); it lowers costs; increases facility of storage and protects the substance against the more aggressive processing methods (Comunian et al. 2013; Fernandes et al. 2013; Peng et al. 2014, in addition to promoting the slow release of the encapsulated substance (Alvim and Grosso, 2010; Dima et al. 2014), which can contribute to the oils passing through the stomach intact and be released properly in the intestine.

In the process oils are processed from a liquid form into a dry powder or they remain in liquid form, but are more stable. The most widely used techniques for the microencapsulation of essential oils are spray drying (Fernandes et al. 2014; Souza et al. 2008) and coacervation (Comunian et al. 2013; Peng et al. 2014).

CONCLUSION

There are numerous reports in the literature containing residues of antibiotics and/or resistant bacteria in chicken derived products, though not all present results showing that the resistance was generated by the use of growth promoters and not antibiotics for therapeutic use.

The use of phytotherapics as growth promoters has been shown to be an excellent alternative to common antibiotics, whose use has been questioned in recent years. Most of the related research demonstrates the antimicrobial effect and efficacy of the essential oils in poultry diets with results similar to those obtained with the use of antimicrobials, indicating the feasibility of replacing these drugs.

The literature review, although still small, shows very interesting results for use in this type of research and can be used as a basis for conducting further studies in the area.

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