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Foundation and Perspectives of the Use of Plant Extracts as Performance Enhancers in Broilers

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ABSTRACT

Feed is responsible for about 70% of broilers production costs, leading to an increasing number of studies on alternative dietary products that benefit bird performance and lower production costs.

Since the 1950s, antimicrobial additives are the most frequently used performance enhancers in animal production and their positive results are observed even in high-challenge conditions. Since the 1990s, due to the ban of the use of some antibiotics as growth promoters and the growing trend of the public to consume natural products, plant extracts have been researched as alternatives to antibiotic growth promoters.

The first study that evaluated the antibacterial activities of plant extracts was carried out in 1881; however, they started to be used as flavor enhancers only during the next decades. With the emergence of antibiotics in the 1950s, the use of plant extracts as antimicrobial agents almost disappeared.

There are several studies in literature assessing the use of plant extracts, individually or in combination, as antimicrobials, antioxidants, or digestibility enhancers in animal feeds. Research results on the factors affecting their action, such as plant variety, harvest time, processing, extraction, as well as the technology employed to manufacture the commercial product and dietary inclusion levels show controversial results, warranting the need of further research and standardization for the effective use of plant extracts as performance enhancers, when added to animal feeds.

This article aims at presenting plant extracts as alternatives to antibiotics, explaining their main modes of action as performance enhancers in broiler production.

INTRODUCTION

Brazil is the largest exporter and the third largest producer of chicken meat in the world, leading to considerable interest and significant investments in the poultry industry. Data of the 2006 annual report of the Brazilian Association of Broiler Producers and Exporters (ABEF) show that chicken meat ranks third among the agribusiness export product, and the sixth Brazilian product in exports. During that year, more than 9.3 million tons of chicken meat were produced, with 2.7 million tons exported. The domestic market is also promising, with a consumption of 35.68 kg chicken meat per capita per year, which represents an increase of more than 60% in the last 10 years (ABEF, 2008).

Brazilian competitiveness in the global meat market is maintained by genetic improvement, management, health control and nutrition, allowing excellent live performance. One of the most important factors is nutrition, as it represents more than 70% of production costs, and therefore is subject of many scientific studies. Researchers are constantly



seeking alternatives to reduce feed costs and to improve animal performance, as well as to satisfy consumers, who increasingly demand safe food products.

Antimicrobial additives are the performance enhancers most frequently used in animal production since the 1950s (Menten, 2001), allowing higher weight gain and better feed conversion ratio in animals reared under intensive conditions. In the last few years, one of the main concerns of the poultry industry is the ban of antibiotic growth promoters by the European Union. These products are now considered as human health risk factors for their possible role in the emergence of microbial resistance (Brugalli, 2003).

There are few scientific studies on the use of plants or their secondary metabolites for therapeutic, prophylactic, or performance-enhancing purposes in animals. However, the recent restrictions to the use of antibiotic growth promoters have stimulated the search for alternative additives. In this sense, the importance of the possibility of exploring the antimicrobial potential - as well as other properties - of plants and their respective compounds (Scheuermann & Cunha Junior, 2005).

PERFORMANCE ENHANCERS

Definition

Performance enhancers - until recently called growth promoters - are organic synthetic compounds, chemical compounds, or simple inorganic elements used to improve animal growth rate and/or feed conversion ratio. Antibiotics and chemotherapeutic drugs are the most frequently used performance enhancers in animal production and have been successfully used in poultry production, particularly under field conditions with high health challenge (Brugalli, 2003).

The antibiotic concept appeared in 1877 when Pasteur and Joubert observed that *Bacillus anthracis* quickly multiplied in sterilized urine, but did not survive when a common air bacterium was simultaneously added to the urine. This finding was the origin of the concept of antibiosis, an association where an organism harms the growth of another organism (Andrade, 2007).

Antibiotics are presently defined as natural metabolites of fungi, bacteria, or yeast that inhibit bacterial proliferation by direct action, bactericidal effect, or bacteriostatic effect on sensitive bacteria and/or fungi. These effects may be due to interference in cell wall synthesis, changes in the permeability of

the cytoplasmic membrane, interference in chromosome replication, and interference in cell protein synthesis (Mellor, 2000).

Background

In ancient times, fungi were used by the Chinese, Egyptian, and Greek to cure infections, but their mode of action was not known.

Modern research on antibiotics started in 1928 in England, when Alexander Fleming discovered penicillin. After many years of study, Ernst Chain and Howard Florey were able to produce and to purify this antibiotic. The second half of the 20th century was the most prolific for human and animal medicine in terms of new technologies applied to antibiotics.

By the end of the Second World War, large-scale food production was necessary, and extensive animal production was no longer able to supply the increasing demand for food. Animals then started to be intensively raised, and antibiotics started to be added to the feeds as growth promoters. Several studies carried out at that time proved the positive results in growth and productivity of the animals reared with the continuous use of dietary antibiotics (NRC, 1999), allowing better production efficiency, despite the intensive conditions of rearing.

The study conducted by Coates *et al.* (1963) showed that antibiotics did not promote the growth of broilers raised in a germ-free environment as compared to those raised in a conventional environment, leading the authors to conclude that antibiotics suppressed the growth of some microorganisms responsible for growth depression.

A highly competitive industry developed creating products (antibiotics and chemotherapeutics) very efficient as growth promoters and increasingly specific against Gram-negative and Gram-positive bacteria.

Some years after the approval of the first antibiotics for the use in animal diets as growth promoters by the Food and Drug Administration - FDA, some concerns as to their effect on human health aroused. Initially, these concerns were linked to antibiotic residues in meat and their potential risks of causing resistance in human pathogens. Later, the possible resistance to antibiotics developed by bacteria that may survive after being exposed to the drug in the animal was the focus of attention (NRC, 1999).

In the 1960s, the Swann Report was published in the UK, recommending that only antibiotics that had little or no applications as therapeutic agents in humans and animals and did not interfere with the drugs



prescribed for therapeutics inducing the development of more resistant strains should be used as growth promoters (Andrade, 2007).

The phasing out of the use of antibiotic growth promoters in animal feeds started in the European Union in 1994, and as of January 1st, 2006, antibiotics as performance enhancers in animal feeds were completely banned.

In Brazil, the compounds used as performance enhancers follow the regulations of the Ministry of Agriculture and the Codex Alimentarius. These regulations establish that the use of antibiotics in animal feeds should not exceed maximum residue limits (MRL), which is the amount of a chemical substance allowed to be present in 1kg of feed and that, if ingested by an individual during its entire life, does not cause undesirable or toxic effects (Aveworld, 2005).

Mode of action

There are no conclusive results proving that dietary antimicrobials cause a reduction in total microorganism counts in the digestive tract; however, there are evidences that they are capable of selecting microorganisms adapted to the modified environment.

Changes in the microbiota would benefit animals by different mechanisms: nutrient sparing, control of clinical diseases, protective effect against the production of toxins in the gastrointestinal tract, and metabolic effects (Menten, 1995; Mellor, 2000). It is believed that the simultaneous action of some of these mechanisms generate the performance benefits observed in broilers and other domestic animals (Menten, 1995).

Antibiotic performance enhancers in animal production

Antibiotics can be used preventively, therapeutically, or as performance enhancers, promoting positive effects on animal production. During the last decades, several studies (Coates *et al.*, 1963; Visek, 1978; Thomke & Elwinger, 1998a; Thomke & Elwinger, 1998b) confirmed the performance enhancer effect of antibiotics added in subtherapeutic doses to animal feeds.

However, issues such as loss of antibiotic efficiency along time and risk of residues in food of animal origin, with the possible development of bacterial resistance in humans, have concerned consumers (Thomke & Elwinger, 1998b), creating a significant problem for poultry production.

Bacterial resistance may occur naturally when bacteria are not sensitive to antibiotics, or be acquired,

when sensitive bacteria become resistant to antibiotics. This resistance may be caused by loss of membrane permeability, active exclusion of the antimicrobial compound, changes in the binding sites, changes in the membrane receptor, overproduction of target enzymes, alternative metabolic pathways, mutations, genetic transference or efflux (Aveworld, 2005).

Another cause of bacterial resistance is the reduction of the number of antibiotics used in shuttle programs due to the ban of the use of some antibiotics in animal feeds. In addition, in the last few years, few new antibiotics have been discovered (Cowan, 1999). The lack of available options for shuttle programs caused the rapid loss of efficacy of some of those antibiotics (Aveworld, 2005).

Plant extracts

Definition

Plant extracts are derived from plant products, individually or in combinations used as spices and flavoring agents in foods. These plant products may be classified as herbs or spices. Herbs are the dried leaves of aromatic plants, usually found without stems, whereas spices are the dry parts of the plants, except for the leaves (Peter, 2001).

Background

Plant extracts are important components of traditional medicine, and its use follows the history of mankind, not only in disease treatment, but also in cooking, religious rituals, and even in mummification processes.

Records with more than 4,000 years make references to plants, such as the poppy (*Papaver somniferum*), whose alkaloids were used as narcotics, and belladonna (*Atropa belladonna*), whose liquid extract was used to dilate pupils (then considered a feminine beauty mark) and to produce poisons. Morphine, derived from opium extracted from poppies, is currently used as a narcotic, whereas atropine and scopolamine, both extracted from belladonna, are used in ophthalmics and also as antispasmodic agent (Carvalho, 2005).

The records on studies with plant extracts date from only 2,000 years ago, when essential oils were obtained by distillation. After the 13th century A.D., essential oils started to be manufactured by chemists, and their pharmacological effects, described in pharmacopoeias. However, their use was only disseminated in London,



and consequently to the rest of Europe, in the 14th century (Burt, 2004).

In 1881, De La Croix carried out the first study on the antibacterial properties of plant extracts (Boyle apud Burt, 2004), and the use of essential oils to provide flavor to foods was disseminated in the following decades. In 1945, with the development of the synthetic pharmaceutical chemistry and the emergence of antibiotics produced by bacterial fermentation, there was a marked decrease in the research of medicinal plants (Scheuermann & Cunha Junior, 2005), and the use of plant extracts as antimicrobials almost disappeared (Cowan, 1999).

In the 1990s, due to ban of the use of some antibiotics as growth promoters, and the increasing public trend to consume natural products, plant extracts re-emerged as alternatives for the replacement of antibiotics in alternative therapeutics.

The use of plant extracts

In the last few years, the consumer trend of seeking for natural and high-quality products led to the development of research involving chemical and pharmacological studies of medicinal plant, aiming at obtaining new products with therapeutic properties (Cechinel Filho, 1998). The number of studies on the use of plant extracts in animal feeding also significantly increased in Europe in the search of alternatives to antibiotics as performance enhancers. In Brazil, however, this subject has been only recently called the attention of researchers, and consequently only few studies have been carried out.

Plant extracts are typically used in animal feeds in the form of oil-resin or essential oils. Oil-resin is obtained by percolation, with the use of solvents, and results in a liquid or paste consisting of resin and chemical and organic substances, which confer specific color and viscosity to the extract. An essential oil is a homogenous liquid consisting of several chemical substances, and it is obtained by steam distillation. Essential oils are the most frequently used in animal diets.

Essential oil analyses show that they consist mainly of terpenes, which are present as hemiterpenes, monoterpenes, or sesquiterpenes and their derivatives (Trombetta *et al.*, 2005). In addition, oxygenated components (alcohols, esters, aldehydes, ketones, etc.) may be present, as well as small amounts of non-volatile residues (paraffin, wax, etc.) (Losa, 2001). Essential oils may also contain phenolic products, classified as phenylpropanoids (Peres, 2007). According to Langhout (2005), more than 2600 different components present

in essential oils have been identified, and part has been synthetically produced.

The part of the plant from where the essential oil is derived depends on the plant species. For instance, in plants of the family Lamiaceae, essential oils occur in the glandular trichomes, whereas in plants of the family Apiaceae, they are stored in the oil channels. Essential oils may also be stored in the leaves (lemon grass, eucalyptus, laurel), flowers (orange tree), bark or stem (cinnamon), fruits (anise), and other parts of the plants.

The components of the essential oils are produced by the plant secondary metabolism. This means that they are not always required by the plant life cycle, but they play important roles in the interaction of the plant with the environment, such as defending the plant against the attack of pathogens, attracting beneficial organisms, and protecting against abiotic stresses, *i.e.*, those associated with temperature changes, soil water content, exposure to ultraviolet rays, and mineral nutrient deficiencies (Peres, 2007). Therefore, the composition and concentration of secondary metabolites in a plant may vary according plant species, weather changes during plant development, plant origin, harvest time, type of material (fresh or dried) (Janssen *et al.*, 1987; Cechinel Filho, 1998), extraction/distillation method and storage conditions and period (Huyghebaert, 2003; Janssen *et al.*, 1987).

According to Kamel (2000), the positive effects of plant extracts on animal nutrition are associated to the composition of its active principles and secondary components. Active principles are chemical components present in the entire plant or in specific parts that confer some therapeutic activity to medicinal plants (Martins *et al.*, 2000).

The substance that constitutes the active principle of an essential oil may be found in different plants, and often in different concentrations. Thymol, for instance, despite being found in the essential oils of thyme (41%) and oregano (10%) (Kamel, 2000), can only be considered an active principle in thyme, where as in oregano, the active principle is carvacrol, which is present in 60% of this plant essential oil.

The presence of several active principles in plant extracts is explained by the fact that the plants develop several metabolites with complementary functions in the defense against pests and diseases (Peres, 2007). Although a plant may contain hundreds of secondary metabolites, only those present in higher concentrations are usually isolated and studied (Cechinel Filho, 1998).



Plant extracts present some advantage as compared to traditional antimicrobials because the latter have a single active principle. Therefore, the presence of several compounds in one product may have a synergistic beneficial effect (Peres, 2007) in the improvement of animal performance. When added to animal diets, the active principles of plant extracts are absorbed in the intestine by the enterocytes and quickly metabolized by the body. The products of this metabolism are transformed in polar compounds by conjugation with glucuronate, and excreted in the urine. Other active principles can be eliminated during respiration as CO₂. The rapid metabolization and short half-life of active principles suggests that the risk of accumulation of these compounds in the tissue is negligible (Kohlert *et al.*, 2000).

Species such as *Hypericum perforatum* (St. John's wort), *Allium sativum* (garlic), *Origanum majorana* (marjoram), *Origanum vulgare* (oregano), *Mentha piperita* (peppermint), *Rosmarinus officinalis* (rosemary), *Thymus vulgaris* (thyme), *Juniperus communis* (juniper), *Capsicum annuum* (chili pepper) and *Allium cepa* (onion) called the attention of animal nutrition researchers, as the active principles of these plants may be beneficial for animals (Kamel, 2000). Considering the wide variety of existing plants, the biggest challenge in the use of plant extracts as alternative to the use of antibiotics is the identification and measurement of the effects of the different components present in essential oils on the animal.

MODE OF ACTION OF PLANT EXTRACTS

Antimicrobial activity

Antimicrobial activity is one of the intrinsic effect of plant extracts, as several studies published in scientific literature show clear evidence of *in vitro* antimicrobial, antifungal, and antiviral activities of plant extracts against pathogens that attack animals (Mitsch *et al.*, 2004; Santúrio *et al.*, 2007), food (Juven *et al.*, 1994; Helander *et al.*, 1998), and humans (Didry *et al.*, 1994).

The essential oils of laurel, cinnamon, thyme, and clove (Smith-Palmer *et al.*, 1998) as well as of oregano, basil, coriander, and other plants (Elgayyar *et al.*, 2001) were tested against bacteria and fungi present in foods, and the results demonstrated the important antimicrobial potential of oregano essential oil, even against Gram-negative bacteria, which are typically very resistant to the action of essential oils. According to Smith-Palmer *et al.* (1998), the essential oils of oregano, cinnamon, and thyme are able to maintain

the same bacteriostatic capacity at 4-35°C against *Listeria monocytogenes*, a Gram-positive bacterium whose control is of utmost importance for the food industry. However, when tested against different *Salmonella* serovars of poultry origin (Santúrio *et al.*, 2007), only oregano essential oil presented high activity, causing bacterial death in 50% of the studied serovars, whereas thyme and cinnamon essential oils presented moderate activities. It must be noted that the results of the *in vitro* antimicrobial activity of a essential oil may vary as a function of the methodology used for its determination, and therefore further *in vivo* trials are warranted to validate the results obtained (Hammer *et al.*, 1999).

The antimicrobial action of oregano essential oil against *Salmonella* spp. was also observed by Peñalver *et al.* (2005) in a study with intestinal bacteria of poultry and swine. That essential oil presented minimum inhibitory concentration of 0.25-1% for *Salmonella* (*S. enteritidis* and *S. enterica* in poultry, and *S. choleraesuis* and *S. typhimurium* in swine) and of 4% for *Escherichia coli* poultry and *Escherichia coli* ETEC. Minimum inhibitory concentration (MIC) is used to evaluate the antimicrobial activity of a product and its has different definitions in literature (Burt, 2004). In the above study, MIC was defined as the lowest concentration that is capable of inhibiting the visible growth of the studied microorganism in an agar plate.

In practice, plant extracts should be included in feeds at much higher doses to produce the same bactericidal or bacteriostatic effect observed *in vitro*. Therefore, *in vivo*, the mode and site of action of the active components of essential oils depend on their structure, metabolism, and inclusion level (Brugalli, 2003). This stresses the need to carry out detailed studies of plant extracts to be added to animal diets.

The combination of active principles of some plant extracts apparently affects *Clostridium perfringens* proliferation in the intestine of broilers. A significant reduction of *C. perfringens* colonization was observed in the intestine of broilers fed diets containing mixtures of thymol, eugenol, curcumin and piperin, or thymol, carvacrol, eugenol, curcumin and piperin (Mitsch *et al.*, 2004). The *in vitro* effects of essential oils and the effects of digestive enzyme stimulation, intestinal flora stabilization, and inactivation of *C. perfringens* toxins may favor a reduction of clostridium colonization in the intestines. This fact stresses the importance of conducting studies to evaluate performance parameters of poultry fed plant extracts, as necrotic enteritis - disease caused by *C. perfringens* - damages



the intestinal mucosa, which results in lower feed intake, mortality, and consequently, worse performance.

Most essential oils exert their antimicrobial effect by damaging the bacterial cell wall, denaturing and coagulating proteins. They change the permeability of the cytoplasmic membrane to hydrogen (H⁺) and potassium (K⁺) ions, causing the interruption of essential cell processes, such as electron transport, protein translocation, phosphorylation steps and other enzyme-dependent reactions, resulting in loss of chemiosmotic control of the affected cell, leading to cell death (Dorman & Deans, 2000).

Changes in the permeability of cell wall membrane of bacteria are due to the lipophilic characteristic of essential oils, which accumulate in the membrane. The external membrane of Gram-negative bacteria contains lipopolysaccharides, forming a hydrophilic surface. This hydrophilia creates a barrier to the permeability of hydrophobic substances, such as essential oils (Dorman & Deans, 2000). This explains the resistance frequently observed in Gram-negative bacteria to the antimicrobial effect of some essential oils (Chao *et al.*, 2000).

It is thought that the changes in membrane permeability may not be the direct cause of bacterial cell death, as the studies conducted by Trombetta *et al.* (2005) revealed that monoterpenes can cross the lipid bilayer, penetrate the cell, and interact in specific sites, exerting their antimicrobial activity intracellularly.

Plant extract antimicrobial activity results obtained in *in vitro* experiments, together with the reemergence of the interest in natural therapeutics and food safety concerns, demand further studies to demonstrate the efficacy of plant extracts in improving animal performance.

Antioxidant activity

The antioxidant activity of plant extracts is mainly related to the presence of phenolic compounds. However, compounds such as flavonoids (present in oregano and thyme) and terpenoids (e.g., thymol, carvacrol, and eugenol) also present antioxidant activity. This substance may intercept and neutralize free radicals, preventing the propagation of the oxidation process (Hui, 1996).

Rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) are the herbs with the highest antioxidant potential (Madsen *et al.*, 1997), and its protective effect on meats was demonstrated. Other plant extracts, such as Cretan dittany (*Origanum dictamnus*), cultivated in

Greece, and mate (*Ilex paraguariensis*), a well-know Brazilian herb also cultivated in Uruguay, Paraguay, and Argentina, also show antioxidant potential when added as ground dried extract to chicken meat (Racanicci *et al.*, 2004; Racanicci *et al.*, 2008).

Some essential oils, such those of clove, thyme, and sage, despite presenting antioxidant action, should not be added in large amounts to foods, as they may negatively affect food flavor and aroma (Madsen *et al.*, 1997)

Aiming at solving these problems, recent research studies have assessed if this same antioxidant effect can be obtained by the supplementation of plant extracts or essential oils in animal feeds, as the use of synthetic antioxidants in animal nutrition is restricted (Valenzuela *et al.*, 2003).

Recent studies show that the addition of oregano essential oil to broiler feeds improved the oxidative stability of chicken meat (Botsoglou *et al.*, 2002a; Botsoglou *et al.*, 2002b; Botsoglou *et al.*, 2003a), turkey meat (Botsoglou *et al.*, 2003b) and rabbit (Botsoglou *et al.*, 2004).

According to Lopez-Bote *et al.* (1998), the supplementation of sage and rosemary oil-resin in broiler diets increased the oxidative stability of thighs and breast meat. In addition, Luna *et al.* (2007) showed that thymol and carvacrol, present in oregano essential oil, were able to replace synthetic antioxidants in broiler diets, promoting similar protection against lipid peroxidation of breast meat samples, probably because this meat has low fat content.

Therefore, it is suggested that plant extracts, when directly added to feeds, may influence the oxidative stability of meat after slaughter.

Effects of plant extracts on digestibility

During the digestive process, the presence of enzymes is essential for the digestion of nutrients. It is believed that some plant extracts may stimulate the production of saliva, and of gastric and pancreatic juices, favoring the secretion of enzymes, and improving nutrient digestibility (Mellor, 2000).

Aromatic compounds, such as capsaicin, active principle of the chili pepper (*Capsicum annum*), have shown efficient stimulation of pancreatic and intestinal enzymes in monogastric animals. Consequently, they promote a reduction of the intestinal viscosity, making the digestive process more efficient (Brugalli, 2003).

In addition to capsaicin, other plant active components, such as eugenol and cinnamaldehyde, active principles of clove and cinnamon, respectively,



also have digestion-stimulating properties (Kamel, 2000).

Different mixtures of plant extracts have shown positive effects on animal diet digestibility. The inclusion of graded levels of a mixture containing clove, thyme, and oregano essential oils, with the active principles eugenol and carvacrol in newly-weaned piglet diets increased apparent dry matter digestibility as compared with diets with or with no addition of antibiotic (Oetting *et al.*, 2006). García *et al.* (2007) observed that finisher broiler diets supplemented with a mixture of sage, thyme, and rosemary or with a commercial product containing capsaicin, cinnamaldehyde, and carvacrol present similar ileal dry matter and protein digestibilities as the diet supplemented with antibiotic, and significantly higher than the control diet.

When evaluating the same mixture of plant extracts in the grower and finisher broiler diets, Hernández *et al.* (2004) demonstrated that the grower diet presented better dry matter digestibility when the mixture containing sage, thyme, and rosemary plant extracts was added. As to crude protein digestibility, the diets containing antibiotic or a commercial product with capsaicin, cinnamaldehyde, and carvacrol presented the best results, which were observed only during the finisher phase.

Similar results to those obtained by Hernández *et al.* (2004) for crude protein digestibility was reported in a grower broiler diet with the addition of the same commercial product containing plant extracts with capsaicin, cinnamaldehyde, and carvacrol (Rostagno *et al.*, 2001). The improvement in amino acid digestibility may be related to capsaicin, the active principle of the plant extract used in the experiment. According to Platel & Srinivasan (1996), capsaicin stimulates the action of proteases, particularly of trypsin and chemotrypsin, in laboratory animals.

Plant extracts as performance enhancers

As a function of plant extract properties and potential, several products are being tested as animal performance enhancers when added to feeds. The use of individual plant extracts to replace antibiotic performance enhancers was evaluated in studies conducted with broilers by Barreto (2007) and with newly-weaned piglets by Costa *et al.* (2007). Broiler performance was not statistically different when the control treatment (with no addition of performance enhancers) was compared to the treatments with antibiotics or with plant extracts. However, when clove and oregano extracts were included in piglet diets, they

promoted similar performance as to that obtained with the use of antibiotics.

Although the active principles are responsible for the main effect on the animal, research studies show the presence of a synergistic effect among plant primary and secondary components. The secondary components would enhance the effects of the primary components (Kamel, 2000).

Some researchers believe that the administration of combinations of essential oils derived from different plants in animal diets promote better performance results (Langhout, 2000). The synergistic effect of essential oil components was observed *in vitro* in a mixture of eugenol and cinnamaldehyde. Differently from the results observed when these two compounds were individually added, their mixture was able to completely inhibit the growth of four bacteria for a period longer than 30 days (Moleyar & Narasimham, 1992).

Studies carried out with broilers seem to confirm the *in vitro* findings. In a trial reviewed by Losa (2001), it was observed that the inclusion of a mixture of plant extracts reduced in 70% the number of broilers infected with *Clostridium perfringens*, improving their performance as compared to the control treatment with zinc bacitracin.

Other results report significant improvement of feed conversion ratio, weight gain, and carcass yield when broilers were fed a diet containing 48 ppm of a combination of oregano, laurel, sage, fennel, Myrtus, and citrus as compared to birds fed a control diet or one containing 10 ppm avilamycin (Alçiçek *et al.*, 2003). Studies carried out with plant extract mixtures containing capsaicin, cinnamaldehyde, and carvacrol (Rostagno *et al.*, 2001) or 100 ppm oregano, cinnamon, thyme, and chili pepper essential oils (Zhang *et al.*, 2005) in substitution of a antibiotic performance enhancer showed similar feed conversion ratio results as compared with the diet containing antibiotics.

Those studies show that plant extracts and their active principles may be used as an alternative to the use of antibiotic performance enhancers in animal nutrition. However, further studies on the effects of those compounds, their combinations, and dietary inclusion levels are needed to allow the animal industry to safely adopt them and to obtain the best cost/benefit in animal production.

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