

## Enhancing the productive performances and broiler meat quality by phyto-

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review

### Summary

Due to its antioxidant properties, ability to improve taste of feed, digestion function and to boost immune response, phyto-, which include essential oils and their components, have a great potential for use in broiler fattening. Furthermore, because of its antimicrobial properties they could be used as a natural and acceptable replacement for antimicrobial growth promoters. Before the considerable use of phyto- in broiler fattening, the mechanism of its action, compatibility with other feed components, safety and toxicological assessment should be explored more detailed. Despite the fact that phyto- can be applied in poultry production, breeders are still not well informed about their use. The positive effect of oil extract of thyme (thymol and carvacrol) and cinnamon (cinnamaldehyde) added to the chickens feed (at 100 and 200 ppm) demonstrated improved weight gain, feed intake and feed conversion. In addition, larger quantities of essential oils in feed, significantly affected feed conversion ratio, abdominal fat and internal organs percentage (liver, heart and gizzard), while treatments with essential oils of thyme and cinnamon significantly reduced cholesterol and H/L ratio, and raised erythrocytes count (RBC), PCV, Hb and white blood cell count (WBC) compared with the control group. On the international level is necessary to standardize commercially available components of essential oils to improve their everyday use. **Key words:** broilers, essential oils, production performances, growth promoters

### Phyto- - new

improve production qualities of live- compounds derived by cold ex-

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cause a desired animal response, in terms of nutrition, the change in pH and in metabolic functions as well, and have influence in growth performances. Common additives used in poultry nutrition include antimicrobial substances, antioxidants, pH controlling substances and enzymes. Phyto- are relatively young group of additives which, in recent few years, drew attention of the feed producing industry (Hashemi and Davoodi, 2010).

Phyto- as feed additives (Lawrence and Reynolds, 1984), which are also called phytobiotic, are defined as a components derived from plant and incorporated in a feed to

characteristic or food derived from this animals. Compared to synthetically derived antibiotics and inorganic chemical substances, these products originated from plants are natural, proven less toxic, does not produce residues, could become an ideal feed additives and successfully replace antibiotic growth promoters in feed. Considering the origin and processing, Windisch et al. (2007) were classified them into four subgroups:

1. Herbs (flowering, non woody and non persistent plants)
2. Spices (herbs with an intensive smell or taste commonly added to human food)
3. Essential oils (volatile lipophilic

non aqueous solvents).

Phyto- include great number of herbs, spices and their products, which largely consist of essential oils (Hashemi and Davoodi, 2010). Phyto- active compounds are secondary plant ingredients mostly with antimicrobial properties and are called phytochemicals. Their positive effect on growth performances and animal health are mainly consequences of their antimicrobial properties and the ability to stimulate immunity (Yang et al., 2009).

### Essential oils (EO)

Essential oils, as defined by the Encyclopedic dictionary of human

and veterinary medical terminology (2006), are oils from plants and animals, with intensive aroma, terpenic and sesquiterpenic structure. They have a slight bacteriostatic effect. EO can be used in cosmetics, occasionally in medicine as inhalation (eucalyptus oil). They are known as volatile oils as well. Essential oil is named due to characteristic aroma of the plant material from which originated, for example rosemary essential oil (Oyen and Dung, 1999).

### The composition of essential oils

Essential oils are basically composed of two types of components: terpenes and phenylpropanes. Terpenes can be further divided (Bakali and al., 2008), depending on the number of 5 carbon-base (C<sub>5</sub>) isoprene units, to monoterpenes (2 units – 10 C), sesquiterpenes (3 units – 15 C) and diterpenes (4 unit – 20 C). Terpenes derivatives are further divided, depending on the presence

and part of plant used for obtaining (Burt, 2004).

Way of producing oil (cold pressed method, steam distillation, extraction with non-aqueous solvents) will change the active substance and associated compounds within the final product (Windisch et al., 2007).


Since essential oils are very complex mixtures, their chemical composition and concentration may be quite different. For example, thymol and carvacrol are present in thyme in the concentration range 30-60% (Lawrence and Reynolds, 1984), and cinnamaldehyde makes 60-75% of the total cinnamon oil (Duke, 1986). Due to large differences in the composition of essential oils, their biological impact is different, and for this reason some authors, if they wanted to assess their role as an antimicrobial substances in poultry production, mostly selected only some components of essential oil

cause it is considered that the difference in the organoleptic profile implies a difference in the oil and these affect antimicrobial properties. This has been confirmed when essential oils extracted from plant using hexane showed higher antimicrobial activity compared to those obtained by steam distillation (Packiyasothy and Kyle, 2002).

Due to its volatility, essential oils must be stored in hermetically preserved containers and kept in dark place to avoid changes in their structure (Burt, 2004).

### Legal framework for using phyto- in European Union

In contrast to veterinary drugs which are given in prophylactic and therapeutic purposes, according to a specific diagnosis, for a limited time period and respecting withdrawal time, phyto- are products that manufacturer permanently gives

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there are more of 1000 monoterpenes and 3000 sesquiterpenes.

The second components of essential oils are phenylpropanes and they consist of 6-carbon aromatic ring with a 3-carbon side chain (C<sub>6</sub>-C<sub>3</sub> compounds). Just about 50 phenylpropanes are described (Lee, 2002). It is believed that the phenolic components are mainly responsible for the antibacterial properties of essential oils (Cosentino et al., 1999).

Previous studies have shown that the composition of essential oils of some plants, such as *Origanum* species, may be different, depending on differences in breeding, origin, vegetative stage at the time of harvest and the season (Şahin et al., 2004). Also, the composition of essential oil can differ depending on the season of harvest, geographic origin

used different amounts of added essential oils or their components, and even diverse physical form, which made comparison of results even more difficult. Some of them used ppm/100g or % or mg/100g and only in few papers were pointed out that weather the oil or powder form were used, and weather oil or component are derived from seeds, leaf or stalk, and even less frequently is mentioned the method of essential oil extraction.

### Obtaining methods and essential oils maintaining

Steam distillation is the most common way of producing essential oils for commercial purposes. More expensive method, for getting natural organoleptic profile of oil, is the extraction of liquid carbon dioxide at low temperature and high pressure (Moyler, 1998). This is important be-

tanon, 2007; Hashemi and Davoodi, 2010). For this reasons, their use is limited and in the European Union must be proved their identity and traceability throughout the entire production, as well as the effectiveness of nutritional qualities that are listed, including the absence of possible interaction with other additives and safety for animal (tolerance), for users (producers, workers in feed production), for animal product customers and for environment (Windisch et al., 2007).

The European Commission approved the use of some essential oil components that can be used as a flavoring in food, without risk for health for the consumer. On the list are: carvacrol, carvone, cinnamaldehyde, citral, p-Cymene, eugenol, limonene, menthol and thymol. Estragole and methyl eugenol are

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removed from this very list in 2001 because of its genotoxicity.

Safety assessment of new flavors (Commission Decision of 23 February 1999; Commission Regulation (EC) No 1565/2000; Commission Regulation (EC) No.622/2002; Commission Regulation (EC) No 22232/96) can be carried out after comprehensive toxicological and metabolic studies are done and this represent considerable financial expend. It will be economically favorable using the complete essential oil than single components (Burt, 2004).

#### Essential oils as phytofeed additives

Essential oils are qualified with many properties among which on special place are their antioxidant activity and ability to improve digestion function, improve the taste of feed, increase organism immunity and antimicrobial properties. These are the reasons to display them as a natural growth promoters and successful replacement for antimicrobial growth promoters.

The **antioxidant properties** of herbs and spices are mentioned in several papers (eg. Cuppett and Hall, 1998; Craig, 1999; Zheng et al., 2001). From this point of view, especially interesting are volatile oils from the family *Labiaceae* (mint family) in which on the first place is rosemary oil. Its antioxidative activity is consequence of phenolic terpenes, rosmarinic acid and rosmarol. Other members of this family are the thyme and oregano, which contain large amounts of monoterpenes such as thymol and carvacrol (Cuppett and Hall, 1998). Feed additives obtained from plants from this family contain phenolic components that improve oxidative stability in chicken meat products (Young et al., 2003).

Feeding turkeys with different doses of oregano plant (5 and 10 g/

kg feed) and oregano essential oil (100 and 200 mg/kg feed) effected oxidative stability of turkey meat. Results showed improved oxidative meat stability in all treatments. The best results were at 200 mg/kg and 10g/kg, lower at 5 g/kg and lowest at 100 mg/kg (Florou-Paneri et al., 2005).

Many spices, herbs and their extracts are well known in medicine because of their **influence on intestinal microflora**, in laxative and spasmolytic way, on the digestive system and on reducing flatulence. In broilers, the essential oils used as a feed additives, stimulate activity of amylase and trypsin (Lee, 2002), stimulate intestine mucosal secretion (it is considered to have influence on pathogen adhesion and due to that on microbial eubiotic stabilization in animal intestines; Jamroz et al., 2006). Hernandez et al. (2004) reported improved digestibility in broilers when using extract of sage, thyme and rosemary and essential oil components mixtures of carvacrol, cinnamaldehyd and capsaicin.

Taking into account the large number of different groups of chemical components present in essential oils, it is likely that their **antimicrobial activity** is result of more than one specific mechanism and have a several target sites in bacterial cells (Carson et al., 2002) like: the degradation of cell membranes, damaging cytoplasm membrane, membrane protein damage, leakage of cellular contents, coagulation of the cytoplasm and utilization proton motive force (Burt, 2004).

In recent years interest in phyto-gens as a replacement for antibiotic growth promoter is increasingly growing. It is believed that the primary mode of their action is stabilization feed hygiene but even greater benefit is in the control of potential pathogens affecting the ecosys-

tem of gastrointestinal microflora. In this way the animals are less exposed to microbial toxins and other unwanted metabolites derived from microorganisms (e.g. ammonia and biogenic amines), which provides increased intestinal absorption of essential nutrients and better growth of animals within their genetic potential. Their antimicrobial activity results with reduction of immune defense stress at critical stages of animal production (Windisch et al., 2007).

#### Antimicrobial activity of essential oils

An important characteristic of essential oils and their components is hydrophobicity, which allows them to accumulate among bacteria cell membrane lipids and in that way disturb the structure and make it more permeable, especially for ions and protons (Sikkema et al., 1995). This explains the antimicrobial activity of essential oils when terpene lipophilic components and phenylpropenes penetrate and disintegrate bacteria cell membrane structure with result of ions leakage (Helander et al., 1998; Hashemi and Davoodi, 2010).

Despite the results of antibacterial activity of essential oils obtained *in vitro*, it is generally concluded that they needed a higher concentration in order to achieve the same result in food (Snider, 1997). The concentration depends on the types of food within the rang from 10 to 100 times more, with the exception of *Aeromonas hydrophila*, for which is the same concentration *in vitro* and *in vivo*. The difference in concentration *in vitro* and *in vivo* can be explained with the greater availability of food nutrients to enable the bacteria fast recovery than the media used in laboratory conditions (Burt, 2004).

Essential oils with high percentage of phenolic components such as carvacrol, eugenol and thymol, can

achieve the best antibacterial effect on pathogenic bacteria in food (Burt, 2004).

Bacterial sensitivity in food is influenced with intrinsic properties of food such as content of fat, water, protein, antioxidants, preservatives, pH, salt and other additives, and external influences like the temperature, conditions of packaging (vacuum, gas) and by the characteristics of microorganisms (Zaika, 1988). The increased sensitivity of bacteria on effects of essential oils due to the external factors occurs with lowering pH, storage temperature and oxygen in the package (Burt, 2004). Furthermore, it is assumed that large amounts of fat and/or protein in a food protects the bacteria from the effects of the EO, for example, if an essential oil is dissolved in the lipid components of food it will be less available of it for action in the aqueous components (Burt, 2004).

A significant consequence of the phytofeed additives antimicrobial action in animal feed is improved microbiological carcasses hygiene of poultry. This has been confirmed in studies on the effects of oregano essential oil to the total number of bacteria and specific pathogens such as *Salmonella* on chicken carcasses (Ak-sit et al., 2006; Guo et al., 2004).

Major components of essential oils responsible for microbial activity are: carvacrol, thymol, eugenol, p-Cymene, carvone, cinnamaldehyd, terpinene and capsaicin.

#### Carvacrol and thymol

The most dominant component of thyme essential oil is carvacrol and thymol and they also can be found in the essential oil of oregano and wild bergamot. Most researchers were preoccupied with mode of action for carvacrol. Thymol and carvacrol are structurally very similar, with a hydroxyl group at different positions

of phenolic ring and it appears that both increase the permeability of bacteria cell membrane (Burt, 2004). Carvacrol and thymol can lead to the disintegration of the outer membrane of Gram-positive bacteria which result with lipopolysaccharide (LPS) releasing and increasing the permeability of cytoplasmic membrane for ATP.

Juven et al. (1994) set hypothesis about the mode of action of thymol on the *S. typhimurium* and *Staph. aureus*. According to it, thymol binds to a membrane protein with a hydrophobic part of the hydrogen bonds and thus changes the permeability of the membrane. Thymol has a better effect at pH 5.5 than at 6.5, because at lower pH thymol molecule is dissociated and therefore more hydrophobic and it is binding to the cell membrane better.

#### Eugenol

Eugenol forms a major component (up to 85%) of clove oil. On *Bacillus cereus* act by inhibiting the production of amylase and protease, impairs cell wall and leads to degradation of cells, while in *Enterobacter aerogenes* use hydroxyl group to bind proteins and prevent their action (Burt, 2004).

#### p-Cymene

It is a biological precursor of carvacrol. It is hydrophobic and causes swelling of the cytoplasmic membrane in a much greater extent than carvacrol, but it has not an effective antibacterial activity when is used alone, only in combination with carvacrol. This can be explained that it improves carvacrol transport through the cytoplasmic membrane (Helander et al., 1998).

#### Carvone

According to the chemical composition it is terpenoid and it is part of many essential oils, but it can be found in seeds of dill and cumin the

most. In quantities more than the minimum inhibitory concentration disrupts the pH gradient and membrane potential of cells and reduces specific growth of *E. coli*, *Streptococcus thermophilus* and *L. lactis* (Oosterhaven et al., 1995).

#### Cinnamaldehyde

Cinnamon essential oil contains of 60-75% cinnamaldehyde (Duke, 1986). It is well known that cinnamaldehyde inhibits the growth of *E. coli* O157:H7 and *S. typhimurium* in similar concentration as carvacrol and thymol, but not in a way that damages the outer membrane or use an intracellular ATP (Helander et al., 1998). It is believed that cinnamaldehyde is connected through carbonyl groups to proteins and prevents acid decarboxylase activity in *E. aerogenes*.

#### Terpinene

Thyme essential oil contains large amounts of terpinene and in particular terpinene-4-ol 13.15% and  $\gamma$ -terpinene 9.21% (Viuda-Martos, 2007).  $\gamma$ -terpinene failed to prevent the growth of *S. typhimurium* (Juven et al., 1994), while  $\alpha$ -terpinene was effective in 11 of 25 bacterial species according to study Dorman and Deans (2000).

#### Capsaicin

This is the main capsaicinoid in red pepper (*Capsicum spp.*). Stable in water and according to the studies on animals, conducted by Diepvensa (2007), can be absorbed into the bloodstream. It gives irritating and sharp flavor to different types of hot peppers (Al-Kassie et al., 2011). *Capsicum oleoresin* which is obtained by organic extraction from the pepper yield has antibacterial properties and is effective in treating stomach problems (Spices, 2008).

It could be said that certain essential oils have a better antibacterial effect on meat products. Corian-

der, clove, thyme and oregano oil in quantity of 5–20 µl/g can inhibit *L. monocytogenes*, *A. hydrophila* and native spoilage flora in meat products. On the other hand mustard, cilantro, mint and sage oil have low or none antibacterial activity because they are especially inhibited by high fat content in meat products. If cilantro essential oil immobilizes in gelatin, it shows improved antibacterial properties on *L. monocytogenes* in ham (Burt, 2004).

It could be concluded, with regard to the published research, that antibacterial activity in food falls in the following way oregano/clove/coriander/cinnamon > thyme > mint > rosemary > mustard > cilantro/sage. Essential oil components can be ranked as well (in decreasing antibacterial activity): eugenol > carvacrol/ cinnamic acid > basil methyl chavicol > cinnamaldehyd > citral/geraniol (Burt, 2004).

For massive use of essential oils as feed additives, it would be important to know their effect on organoleptic properties of feed (Burt, 2004). According to the results of Hernández and al. (2010), clove essential oil added to broiler feed in amounts of 100 ppm had no effect on meat quality and no adverse effect.

#### Effect of essential oils on growth performances of poultry

The results of many authors (Lee et al., 2003; Hernandez et al., 2004; Basmacıoğlu et al., 2004; Florou-Paneri et al., 2005; Guo et al., 2004) confirm that it is possible to improve growth performances in poultry with feeding them with phyto-gen additives – essential oils or their components. But, great diversity of biological effects that they cause, possibility of failure because of inappropriate phyto-gen use and different success of various daily doses must be taken into account as well (Windisch et al., 2007).

The relationship of growth performances of broilers and phyto-gens added into feed is still subject to the criticism and therefore Cross and al. (2007) concluded that quality and quantity of active chemical substances in plant extracts significantly affect the response in poultry. The factors that can influence phyto-gen additive activity in feed depend on part of the plant used, physical form of phyto-gen additive, genetic variation of the plant, plant age, used doses variety, extraction method, harvest time and compatibility with other components in feed. This can explain why the difference in body mass and feed conversion ratio is present when using different phyto-gen in broiler feed (Yang et al., 2009). Their efficiency depends on some internal and external factors such as nutritional status of animals, exposure to infection, composition of feed and state of environment (Lee, 2002).

According to some studies, the effect of essential oils on growth performances of poultry can be either positive or with no importance (Bassett, 2000; Langhout, 2000; Botsoglou et al., 2002). In positive impact, growth was higher compared to a same meal in experimental then in control group. If there was no impact it is explained by the fact that the poultry properties have been superior and there was no space for greater improvement, poultry was healthy and there was a special care taken in keeping poultry, cleaning and disinfection (Botsoglou et al., 2002). This suggested that the impact of the essential oils in diet is expressed when poultry is not kept in optimal conditions or when low quality feed is used and/or less clean environment (Lee, 2002).

After oral, pulmonary or dermal absorption, essential oils are metabolized and eliminated from the body through the kidneys in the glucuronide form or exhalation as CO<sub>2</sub>,

within 24 hours. Due to very rapid metabolism and excretion their retention in the tissues as a residue is unlikely. However, if poultry is constantly nourished with essential oils, some of their ingredients can be deposited in different tissues depending on their dose in feed, but with negligible effect on the organoleptic properties of poultry meat. It is not well investigated whether essential oils cause negative effects on human health if they are consumed through food but thymol, carvacrol and cinnamaldehyde were labeled as GRAS (generally recognized as safe) by the Flavor and Extract Manufacturers' Association and the Food and Drug Administration (FDA) which indicated their safe use (Lee, 2002).

Positive action of plant extracts on the poultry digestion is manifested in reducing pH value in the ileum and increasing the number of *Lactobacillus* spp. and bifidobacteria which lead to better intestine microflora balance and provide optimal conditions for the protection against pathogenic microorganisms (Tekeli et al., 2006; Vidanarachchi et al., 2006).

Lee et al. (2003) compared the effects of carvacrol and thymol in a concentration of 200 ppm in feed. Carvacrol reduced the feed gain ratio and feed intake, but improved feed conversion, which means there were a better feed utilization and/or changes in the carcass composition. It is possible that carvacrol effect on feed intake by changing poultry appetite (Lee et al. 2003). Similar results had Al-Kassie et al. (2009) who investigated the effects of cinnamon and thyme to increase the weight of live animals and to improve poultry health, as well as improve other properties such as utilization and feed intake. They have proved a positive effect of oil extracts of thyme (thymol and carvacrol) and cinnamon (cinnamaldehyde) added to the

chickens feed (á 100 and 200 ppm) to weight gain, feed intake and feed conversion ratio. This was particularly manifested in treatments with more quantity of the EO. In addition, larger amounts of EO in feed significantly affected the feed conversion ratio, the amount of abdominal fat and the size of internal organs (liver, heart and gizzard). The same study demonstrated that treatment with the thyme and cinnamon EO can significantly reduce the amount of cholesterol in serum, the H / L ratio, while there was a significant increase in the number of red blood cells, hematocrit, white blood cells and hemoglobin compared with the control group (Al-Kassie, 2009).

The amount of 2 g / kg cinnamon in feed gave satisfactory results in the growth of chickens and its use as a potential alternative to antimicrobial growth promoters is possible (Toghyani et al., 2011). Red hot pepper, which ingredient capsaicin is associated with antimicrobial activity on bacteria and positive effect on digestion, is added to poultry feed in order to determine the impact on body weight, daily gain and feed conversion. Concentrations of 0.25%, 0.50%, 0.75% and 1% showed a significant effect of pepper added in all treatments, compared to the control group. Significant differences between experimental treatments and controls occurred in hematological measurements involving the erythrocyte and leukocyte number, hematocrit, the H / L ratio, the amount of glucose and cholesterol, which all values were reduced compared to the control group (Al-Kassie, 2009). In studies involving the combination of oregano essential oil, hops extract and Mannan-oligosaccharide have shown that essential oils in combination with other additives can have a positive effect on increasing body weight of fattening chickens (Bozkurt et al., 2009).

Mode of action of essential oils

extracts within the bounds of doses to be applied for growth enhance, Kim et al. (2010) attempted to clarify in the study of the impact of carvacrol, cinnamaldehyde and *Capiscum* oleoresin on a change in gene expression of intestinal intraepithelial lymphocytes in chickens. The feed contained mentioned additives in amounts as follows: 5 mg / kg, 3 mg / kg and 2 mg / kg. Intestine mucosal layer plays an important role in immune defense against pathogens that are introduced with feed, but also comes in direct contact with feed and nutrients. The experiment showed that carvacrol changed the expression of 74 genes, cinnamaldehyde of 62 and *Capiscum* oleoresin of many as 254 genes. There has been increased lipase activity in the chicken pancreas and intestinal wall, which indicates that their role in the mechanism of lipid metabolism is important. Also, the results show that several genes that are associated with already known ability to stimulate immunity in the case of bacteria or fungi infection in poultry changed to better. Authors concluded that studied phyto-gens influenced significantly on the immune response, metabolism and physiology of the host in a way that changed the expression of genes important for resistance to pathogens. This contributed to the further development of the feeding poultry strategy, in order to stimulate changes in host immunity in normal conditions and during illness.

A large number of authors who studied essential oil antimicrobial activity, agrees that EO have better influence on Gram-positive than Gram-negative bacteria (Burt, 2004), but Wilkinson et al. (2003) concluded just opposite. One of the most sensitive bacterial species is Gram-negative *A. hydrophila* and the most resistant Gram-negative is *P. aeruginosa* (Wan et al., 1998). Early mentioned differences in the essential oils com-

position (plant parts, harvest time, geographic origin), are sufficient to lead to different levels of sensitivity among Gram-positive and Gram-negative bacteria (Burt, 2004).

In recent studies, the essential oils showed good antimicrobial properties *in vitro* as well in feed on *Campylobacter jejuni* in raw poultry meat. The coriander essential oil gave the best result (Rattanachai-kunsopon and Phumkachorn, 2010). The concentration of coriander oil in feed was 17 times higher than concentration *in vitro*. Its ability to inhibit the growth of Gram-negative *C. jejuni* opens the possibility that similar results could be achieved with other Gram-negative bacteria (Langhout, 2000).

If essential oils are used as mixtures, their action can be divided, according to their impact, on the cumulative effect, antagonistic or synergistic effect. The cumulative effect is defined as action that is equal to the sum of individual effects, synergistic when the action is greater than the sum of individual actions and antagonistic if the total effect would be less than the sum of individual actions. This is important when a mixture of essential oils is used, because knowing their properties so far, a synergistic effect is present when carvacrol and p-Cymene act on *B. cereus*, a cumulative effect is present when carvacrol and thymol from oregano essential oil act on *Staph.aureus* and *Pseudomonas aeruginosa*. On the other hand, different combination mixture of cilantro, coriander, dill and eucalyptus may have synergistic, antagonistic or cumulative effects (Burt, 2004).

Synergistic effect is possible when essential oils are combined low pH and low water activity (a<sub>w</sub>) or if some other additives are added. Sodium chloride has synergistic effect if applied with mint essential oils and

clove powder and antagonistic if applied with carvacrol, p-Cymene and cinnamaldehyde. Sodium nitrite combined with oregano essential oil, as well as nisine with carvacrol or thymol also acts synergistically (Burt, 2004). Burt (2004) states some interesting combinations that have a positive impact on the antibacterial activity of essential oils. So carvone effect better on *L. monocytogenes* at an increased temperature of 45°C for 30 minutes while thymol and carvacrol better operate under the higher hydrostatic pressure. Antibacterial activity increased in case of oregano and thyme essential oil effect on *S. typhimurium* and *Staph. aureus* as well as oregano effect on *L. monocytogenes* in vacuum packing. Clove and coriander oil acted lethally on *A. hydrophila* packed in a vacuum at a storage temperature of 2-10°C. Packing in modified atmosphere (40% CO<sub>2</sub>, 30% N<sub>2</sub>, 1 30% O<sub>2</sub>) with oregano essential oil added contributed to the lower initial contamination and reduced the total microbial count that cause spoilage in ground beef.

Garcia et al. (2007) suggest combined use of organic acids and essential oils. In their study they added to feed 5,000 ppm of formic acid; 10,000 ppm of formic acid; 200 ppm of plant extract based on a blend of oregano, cinnamon and pepper essential oil; and 5,000 ppm of hydroalcoholic plant extract from sage, thyme and rosemary leaves. In all treatments apparent ileum digestibility improved as well as feed conversion ratio, except in the group with oregano, thyme and sage added. Authors explained that the use of hydroalcoholic plant extract from sage, thyme and rosemary leaves were inappropriate for this research.

Dušan et al. (2006) published the research results of the antimicrobial properties of EO on *E. coli* and possible adverse effect on the cells of the small intestine for four types of

essential oils: oregano, thyme, clove and cinnamon, each at two doses (medium 0.01% and high 0.05%). They used gas chromatography oil analysis to determine the proportion of major components in the oils and found 55% carvacrol in oregano oil, 24% thymol in thyme oil and eugenol in proportion of 85% in clove oil and 77% in cinnamon oil. Undoubtedly antimicrobial activity occurred with all essential oils in high doses, as well as in proportional doses of their components in feed, except for thyme which can be explained by the fact that the other components in thyme, not thymol, are responsible for antimicrobial activity. However, high doses also had a strong cytotoxic effect on Caco-2 cells of the small intestine. Cytotoxicity of essential oils in medium dose was relatively small and antimicrobial effect on pathogenic *E. coli* is partial. Appropriate essential oils doses which shows good antibacterial effect doesn't have so much adverse effect on the small intestine cells (Dušan et al., 2006).

Isabel and Santos (2009) used a mixture of clove and cinnamon essential oils in the amount of 100 ppm in broiler feed and increased quantity of breast meat as well as feed conversion ratio as compared to the control group. These results showed a potential advantage over organic acid salts (calcium propionate and calcium formate) which were expected to be used instead antimicrobial growth promoters.

Rosemary essential oil added 0.5% in feed improved broiler growth performances as well as feed conversion ratio during the fattening period. Obtained results were better than the group with antibiotic added in the feed. It can be concluded that use of 0.5% rosemary essential oil in broilers feed has the potential of growth promoters in poultry fattening (Fotea et al., 2009).

Because of their properties to inhibit the growth of pathogenic bacteria like *S. typhimurium*, *E. coli* and *L. monocytogenes* and to prevent the transmission of foodborne diseases, the use of essential oils in broiler feed is very interesting. From the commercial point of view capacity of EO to extend the validity of sensory characteristic of products is very valuable and added advantage is that the spices of their origin are traditionally and seasonally used in food. There is a possibility to use essential oils that are not traditionally related to specific food if concentration in which antibacterial effect is evident will not cause undesirable changes in taste and smell.

According to the changes of population eating habits which lead to the reduced use of artificial and synthetic additives, it is possible that there will be increase in demand for essential oils which could be a motivation to bioengineers to produce plants that have a greater capacity for essential oil synthesis. For this reason it is certainly necessary to standardize the essential oil components that are commercially available at the international level (Burt, 2004).

### Conclusion

The use of essential oils in broiler nutrition is a new category among feed additives. Although there are many well-known and positive effects of their use, it still should investigate action mechanism of essential oil components on proteins which are incorporated in cell membrane as well as on membrane phospholipids. Further research should give answer about essential oils changing influence on the growth performances of poultry and what are the circumstances for their negative result. Effect on gut micro flora and ingestion physiology is often inconsistent. For this reason it is necessary to study their mode of action, in particular taking into account their

compatibility with other factors, such as other components of food, hygiene standards and/or keeping animal conditions.

### References

- Aksit, M., E. Goksoy, F. Kok, D. Ozdemir, M. Ozdigan (2006): The impacts of organic acid and essential oil supplementations to diets on the microbiological quality of chicken carcasses. *Arch.Geflugelkd.* 70,168-173.
- Al-Kassie, G. A. M., Mamdooh A. M. Al-Nasrawi, Saba J. Ajeena (2011): The Effects of Using Hot Red Pepper as a Diet Supplement on Some Performance Traits in Broiler. *Pakistan Journal of Nutrition*, 9, 842-845.
- Al-Kassie, G. A. M. (2009): Influence of two plant extracts derived from thyme and cinnamon on broiler performance. *Pakistan Vet. J.* 4, 169-173.
- Anonymous (1996): Parliament and of the Council on 28 October 1996. 1999/217/EC: Official Journal L084, 27/03/1999, pp.1-137.
- Anonymous (1999): Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European.
- Anonymous (2000): Commission Regulation (EC) No1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No2232/96 of the European Parliament and of the Council: Official Journal L180 19/07/2000, pp.8-16.
- Anonymous (2002): Commission Decision of 23 January 2002 amending Commission Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. 2002/113/EC: Official Journal L49 20/02/2002, pp.1-160.
- Anonymous (2002a): Commission Regulation (EC) No.622/2002 of 11 April 2002 establishing deadlines for the submission of information of chemically defined flavouring substances used in or on foodstuffs: Official Journal L95 12/04/2002, pp.10-11.
- Anonymous (2006): Enciklopedijski rječnik humanog i veterinarskog medicinskog nazivlja, Hrvatska akademija znanosti i umjetnosti, Leksikografski zavod Miroslav Krleža. Zagreb 2006.
- Bakkali, F., S. Averbeck, D. Averbeck, M. Idaomar (2008): Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46, 446-475.
- Basmacoğlu, H., Ö. Tokusoğlu, M. Ergül (2004): The effect of oregano and rosemary essential oils or alpha-tocopheryl acetate on performance and lipid oxidation of meat enriched with n-3 PUFAs in broilers. *South African Journal of Animal Science* 2004, 34 (3), 197-210.
- Bassett, R. (2000): Oregano's positive impact on poultry production. *World Poultry*. 16,31-34.
- Botsoglou, N.A., P. Florou-Paneri, E. Christaki, D.J. Fletouris, A. B. Spais (2002): Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *Br. Poultry. ci.* 43,223-230.
- Bozkurt, M., K. Küçükylmaz, A. U. Çeltik, M. Çinar (2009): Effect of dietary mannan oligosaccharide with or without oregano essential oil and hop extract supplementation on the performance and slaughter characteristics of male broilers. *South African Journal of Animal Science*, 39 (3).
- Burt, S. (2004): Essential oils: their antibacterial properties and potential applications in foods—a review; *International Journal of Food Microbiology* 94, 223-253.
- Carson, C. F., B. J., T. V. Mee Riley (2002): Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time kill, lysis, leakage and salt tolerance assays and electron microscopy. *Antimicrobial Agents and Chemotherapy*, 6, 1914-1920.
- Castanon, J. I. R. (2007): History of the use of antibiotic as growth promoters in European poultry feeds. *Poult.Sci.* 86,2466-2471.
- Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzede, E., Palmas, F. (1999): In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. *Letters in Applied Microbiology* 29, 130-135.
- Craig, W.J. (1999): Health promoting properties of common herbs. *Am. J. Clin. Nutr.* 70, 491-499.
- Cross, D. E., McDevitt R. M., Hillman K., Acamovic T. (2007): The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *Br. Poultry. Sci.* 4, 496-506.
- Cuppert, S. L., C. A. Hall (1998): Antioxidant activity of Labiatae. *Adv. Food Nutr. Res.* 42:245-271.
- Dorman, H. J. D., S. G. Deans (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oil. *Journal of Applied Microbiology*, 88, 308-316.
- Diepvens, Kristel, Klaas R. Westerterp, Margriet S. Westerterp-Plantenga (2007): Obesity and thermogenesis related to the consumption of caffeine, ephedrine capsaicin, and green tea. *Am J Physiol Regul Integr Comp Physiol* 292: R777-R785.
- Duke, J. A. (1986). CRC handbook of medicinal herbs. CRC press, Florida, 1986.
- Dušan, F., M. Sabol, K. Domaracká, D. Bujáková (2006): Essential oils—their antimicrobial activity against *Escherichia coli* and effect on intestinal cell viability. *Toxicology in Vitro*, 20, 1435-1445.
- Florou-Paneri, P., G. Palatos, A. Gavaris, D. Botsoglou, I. Giannenas, I. Ambrosiadis (2005): Oregano Herb Versus Oregano Essential Oil as Feed Supplements to Increase the Oxidative Stability of Turkey Meat. *International Journal of Poultry Science*. 4 (11), 866-871.
- Fotea, L., E. Costăchescu, G. Hoha (2009): The effect of essential oil of rosemary (*Rosmarinus officinalis*) on the broilers growing performance. *Animal Science Faculty, University of Agricultural Sciences and Veterinary Medicine, Iasi Romania*, 2009.
- Garcia, V., P. Catala-Gregori, F. Hernandez, M. D. Megias, J. Madrid (2007): Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology and meat yield of broilers. *J. Appl. Poultry Res.*, 16, 555-562.
- Guo F. C., B. A. Williams, R. P. Kwakkel, H. S. Li, X. P. Li, J. Y. Luo, W. K. Li, M. W. Verstege (2004): Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science* 2, 175-182.
- Hashemi, S. R., H. Davoodi (2010): Phytochemicals as a New additive in Poultry Industry. *Journal of Animal and Veterinary Advances* 9 (17), 2295-2304.
- Helander Ilkka M., Hanna-Leena Alakomi, Kyösti Latva-Kala, Tiina Mattila-Sandholm, Irene Pol, Eddy J. Smid, Leon G. M. Gorris, Atte von Wright (1998): Characterization of the Action of Selected Essential

### Erhöhung der Herstellungsqualität und Fleischqualität von Broilern durch Nutzung von ätherischen Ölen

#### Zusammenfassung

Phytogene, zu denen auch ätherische Öle und deren Komponenten gehören, haben ein großes Potential im Broilermarkt, dies wegen ihrer Antioxiideneigenschaften, ihres Einflusses auf die Verbesserung des Nahrungsgeschmacks, ihrer Wirkung auf die Verbesserung der Verdauungsfunktionen und wegen der Vergrößerung der Immunantwort des Organismus. Hierzu tragen ihre antimikrobiellen Eigenschaften bei, wobei sie als natürlicher und annehmbarer Ersatz für antimikrobielle Wachstumsförderer angesehen werden. Wirkungsmechanismus, Kompatibilität mit anderen Nahrungskomponenten, Bewertung von Sicherheit und toxykologischer Wirkung – sind Gebiete, die im voraus genau untersucht werden sollen, bevor Phytobiotika massenartig im Geflügelmast angewendet werden. Zur Zeit besteht das Interesse für ihre Anwendung, jedoch sind solche Erzeugnisse den Geflügelzüchtern nicht genug bekannt. Bewiesen ist ein positiver Einfluss von Ölextrakten von Tymian (Tymol und Karvakrol) und Zimt (Cinnamaldehyd), zugefügt in das Geflügelfutter (je 100 und 200 ppm), auf die Vergrößerung der Körpermasse, Futteraufnahme und Futterkonversion. Außerdem hat eine größere Menge von ätherischen Ölen in Nahrung (EU) bedeutend das Randmaß, die Menge des abdominalen Fetts und die Größe der inneren Organe (Leber, Herz, Magen) beeinflusst, während die Behandlung mit EU Tymian und Zimt das Quantum von Cholesterin im Serum gesenkt haben, auch das Verhältnis von Heterophylen und Lymphozyten (engl. H/L ratio), während die Erythrocytenzahl, Hematokrit, Leukozyt und Hemoglobin in Bezug auf die Kontrollgruppe erhöht wurden. Um die Anwendung von ätherischen Ölen in Zukunft zu optimieren, ist es notwendig, auf der internationalen Ebene die kommerziell zugänglichen Komponenten der ätherischen Öle zu standardisieren.

**Schlüsselwörter:** Broiler, ätherische Öle, Herstellungsbesonderheiten, Wachstumsförderer

### Elevamento di caratteristiche produttive di qualità del pollame broiler usando gli oli eterici

#### Summario

Per le sue caratteristiche antiossidative, per l'influenza sul miglioramento del sapore, l'azione di miglioramento di digestione e per la capacità di aumento della risposta immunologica del organismo, i fitogeni, tra i quali oli eterici e i suoi componenti, hanno un grande potenziale per l'applicazione di allevamento arricchito dei broiler. A questo contribuiscono ancora di più le loro caratteristiche antimicrobiche, e perciò sono ritenuti un cambio naturale e accettabile per i promotori di crescita antimicrobici. Il meccanismo d'azione, la compatibilità con gli altri componenti del cibo, la valutazione di sicurezza e di tossicità sono le aree che bisogna esaminare più dettagliatamente prima dell'uso di fitobiotici nell'allevamento del pollame. Per il momento c'è l'interesse di applicarli, però gli allevatori ancora non conoscono bene i prodotti di questo tipo. Ci sono delle prove dell'influenza positiva degli estratti di oli di timo (timoli e carvacroli) e di olio di canella (cinnaldechidi) aggiunti al cibo di polli (100 e 200 ppm) sull'aumento del peso corporeo, il consumo e la conversione del cibo. A parte questo, una quantità notevole di oli eterici del cibo ha avuto un'influenza evidente sul randman, sulla qualità del grasso addominale e sulla grandezza degli organi interni (fegato, cuore, stomaco), mentre i trattamenti con gli oli eterici di timo e di canella hanno notevolmente fatto diminuire la quantità di colesterolo nel siero di sangue, poi il rapporto eterofili-linfociti (in ingl. H/L ratio), ma hanno fatto aumentare il numero di eritrociti, ematocrito, leucocite ed emoglobina in paragone con il gruppo di controllo. Affinché l'uso di oli eterici abbia un successo quanto più grande, è necessario stabilire al livello internazionale gli standard di componente accessibile commerciale.

**Parole chiave:** il pollame broiler, oli eterici, specifiche produttive, promotori di crescita

Oil Components on Gram-Negative Bacteria. *J. Agric. Food Chem.* 9, 3590–3595.

Hernandez, F., J. Madrid, V. Garcia, J. Orengo, M.D. Megias (2004): Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poultry Science*, 2, 169–174.

Hernández, P., V. Juste, C. Zomeño, J. R. Moreno, P. Peñalver (2010): Effect of Dietary Clove Essential Oil on Poultry Meat Quality. <http://www.docstoc.com/docs/25009266/Effect-of-Dietary-Clove-Essential-Oil-on-Poultry-Meat>.

Isabel, B., I. Y. Santos (2009): Effects of dietary organic acids and essential oils on

growth performance and carcass characteristics of broiler chickens. *J. Appl. Poult. Res.* 18, 472–476.

Jamroz, D., T. Wiertelicki, M. Houszka, C. Kamel (2006): Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *J. Anim. Physiol. Anim. Nutr. (Berl)* 90, 255–268.

Juven, B. J., J. Kanner, F. Schved, H. Waislowicz (1994): Factors that interact with antibacterial action of thyme essential oil and its active constituents. *Journal of applied bacteriology*, 76, 626–631.

Kim, D. K., H. S. Lillehoj, S. H. Lee, S. I. Jang, D. Bravo (2010): High-throughput gene expression analysis of intestinal intraepithelial lymphocytes after oral feeding of carvacrol, cinnamaldehyde, or *Capicum oleoresin*. *Poultry Science Association Inc.* 89, 68–81.

Langhout, P. (2000): New additives for broiler chicken. *World Poultry*, 16:22–27.

Lawrence, B. M., R. J. Reynolds (1984): Progress in essential oils. *Perfumer and Flavorist* 9, 23–31.

Lee, K.W (2002): Essential oils in broiler nutrition, Utrecht, The Netherlands.

Lee, K.W., H. Everts, H. J. Kappert, K.-H. Yeom, A. C. Beynen (2003): Dietary Carvacrol

Lowers Body Weight Gain but Improves Feed Conversion in Female Broiler Chickens. *J. Appl. Poult. Res.* 12, 394–399.

Moyler, D. (1998): CO<sub>2</sub> extraction and other new technologies: an update on commercial adoption. International Federation of Essential Oils and Aroma Trades—21st International Conference on Essential Oils and Aromas. IF-EAT, London, pp. 33–39.

Oosterhaven, K. B. Poolman, E. J. Smid (1995): S-carvone as a natural potato sprout inhibiting, fungistatic and bacteriostatic compound. *Industrial Crops and Products*, 4, 23–31.

Oyen, L.P.A., N.X. Dung (1999): Essential-oil plants. Backhuys Publishers, Leiden, 1999.

Packiyasothy, E.V., S. Kyle (2002): Antimicrobial properties of some herb essential oils. *Food Australia* 54 (9), 384–387.

Rattanachalunsoopon, P., P. Phumkha-chorn (2010): Potential of Coriander (*Coriandrum Sativum*) Oil as a Natural Antimicrobial Compound in Controlling *Campylobacter jejuni* in Raw Meat. *Biosci. biotechnol. Biochem.* 74, 31–35.

Şahin, F., M. Güllüce, D. Daferera, A. Sökmen, M. Sökmen, M. Polissiou, G. Agar, H. Özer (2004): Biological activities of the essential oils and methanol extract of *Origanum vulgare* spp. *vulgare* in the Eastern Anatolia region of Turkey. *Food Control* 15, 549–557.

Sikkema, J., J. A. M. De Bont, B. Poolman (1995): Mechanisms of membrane toxicity of

hydrocarbons. *Microbiological reviews* 2, 201–22.

Snider, O. P. (1997): Antimicrobial effect of spices and herbs. Hospitality institute of technology and management; St. Paul, Minnesota, 1997.

Spices B. (2008): Pages 1–52 in Spice India. Niseema Printers&Publisher, Cochin, India, 2008.

Tekeli, A., L. Celik, H. R. Kutlu, M. Gorgulu (2006): Effect of dietary supplemental plant extracts on performance, carcass characteristics, digestive system development, intestinal microflora and some blood parameters of broiler chicken. Proceedings of XII European Poultry Conference, 10–14 september 2006., Verona, Italy.

Toghyani, M., M. Toghyani, A. Gheisari, G. Ghalamkari, S. Eghbalsaidi (2011): Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. *Livestock Science*, 138, 167–173.

Vidanarachi, J. K., L. L. Mikkelsen, C. Constantinoiu, P.A. Iji, M. Choct (2006): Plant extracts from Australian and New Zealand native plants as prebiotics in broiler chickens. 05–06 April 2006, Australian Veterinary Poultry Alliance Annual Meeting, Holiday Inn, Gold Coast, Australia, pp. 22–24.

Viuda-Martos, M., Yolanda Ruiz-Navajas, Juana Fernández-López, J. A. Pérez-Álvarez

(2007): Chemical Composition of the Essential Oils Obtained From Some Spices Widely Used in Mediterranean Region. *Acta Chim. Slov.* 54, 921–926.

Wan, J., A. Wilcock, M. J. Coventry (1998): The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. *Journal of Applied Microbiology*, 84, 152–158.

Wilkinson, J. M., Michael Hipwell, Tracey Ryan, Heather M. A. Cavanagh (2003): Bioactivity of *Backhousia citriodora*: Antibacterial and Antifungal Activity. *J. Agric. Food Chem.* 51, 76–81.

Windisch, W., K. Schedle, C. Piltzner, A. Kroismayr (2007): Use of phytogetic products as feed additives for swine and poultry. *Journal of Animal Science* 86, 140–148.

Young, J. F., J. Stagsted, S. K. Jensen, A. H. Karlsson, P. Henckel (2003): Ascorbic acid, alpha-tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality. *Poult. Sci.* 82:1343–1351.

Zalika, L. L. (1988): Spices and herbs: Their antimicrobial activity and its determination. *J. Food Safety*, 9, 97–118.

Zheng, W., Shioy Y. Wang (2001): Antioxidant Activity and Phenolic Compounds in Selected Herbs. *J. Agric. Food Chem.* 49, 5165–5170.

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