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Recent advances of using organic acids and essential oils as in-feed antibiotic alternative in poultry feeds

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Abstract: Antibiotics used in animal feeds have recently been strictly regulated to avoid antibiotic resistance in humans. Thus, scientists are compelled to find new feed additives to replace antibiotics in feed. Apart from having a zero effect on human health, new alternatives may even be able to further improve production performance. Essential oils (EOs) and organic acids (OAs) stood out as powerful and useful replacements for both animal production and human population. These are added to chicken diets and drinking water to induce a favourable growth response. Hence, enhancing the nutrient digestibility, performance, and immunity of birds, OAs and EOs are used in livestock production mainly to inhibit the growth of harmful bacteria and simultaneously maintain the balance of intestinal bacteria. This improves the digestion of nutrients and mineral absorption which will eventually lead to better feed efficiency. The addition of OAs and EOs also causes the intestinal lining to thin, which further enhances nutrient absorption and utilization. Not only for poultry production, the effect of OAs and EOs can also improve poultry immunity and antioxidant capacity. However, the effects will vary depending on the type of OAs and EOs because their mode of action is dependent on their pKa value. In the present review, beneficial properties of OAs and EOs, as well as various dose combinations, to promote their optimal use in poultry nutrition and production will be examined. Their effects on supporting protein digestion, faster absorption of minerals, especially microminerals, stimulating growth performance, regulating antioxidant capacity, and improving immune response will be explored.

Keywords: acidifiers; antibiotics; essential oils; poultry production

Antibiotics as growth promoters for animals are widely used in the feed industry for improving animal performance. Antibiotic feed additives have also been used in the poultry industry to improve the growth and productivity of these species for decades. Antibiotics used as growth boosters at non-therapeutic doses have the unintended consequence of causing antimicrobial resistance in poultry (Robinson et al. 2019). Thus, in 2006, the use of antibiotics was officially banned in the European Union due to antibiotic resistance and human health (Abudabos et al. 2017) and then other countries followed and implemented the same practice. So, the urgent action is to discover feed additives which can serve as an antibiotic alternative in poultry production.

As a result, many feed additives were studied, including enzymes, probiotics, prebiotics, essential oils (EOs) and organic acids (OAs), and others. All these compounds can enhance bird production and gastrointestinal health (Sethiya 2016) and they may be superior alternatives to antibiotics (Yang et al. 2018). In recent years, the combination of acidifiers or sim-

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ply OAs and OEs has been widely practiced (Iqbal et al. 2021; Vinolya et al. 2021) and brings remarkable effects. EOs are volatile oils extracted from various plants that have antibacterial, antiviral, and antifungal activities; as well as immunomodulatory, hypolipidaemic and digestive stimulating effects which could be applied to alleviate heat stress in poultry production (Gopi et al. 2014; Micciche et al. 2018). As a result of their effects on improving the digestion process and increasing the growth performance, these EOs have recently been employed in poultry feed (Abu Isha et al. 2018; Torki et al. 2021) to replace antibiotics. Furthermore, EOs also contain antibacterial and antioxidant properties, which were previously measured in the study of Bouhaddouda et al. (2016). Improved body weight gain (Kim et al. 2015), feed conversion ratio (Pirgozliev et al. 2019), enzyme secretion (Zeng et al. 2015) and nutrient digestibility (Basmaciolu-Malayolu et al. 2016; Attia et al. 2019) were all favourable effects of EOs on broiler performance. The use of EOs in laying poultry has resulted in numerous significant improvements in egg production and egg quality (Xianjing et al. 2017; Torki et al. 2021). Scientists are also drawn to other options such as acidifiers or OAs due to their antibacterial properties against various pathogenic microbes (Beier et al. 2019; Yang et al. 2019). Additionally, they lower down the pH in the gastrointestinal system, which leads to improved nutritional utilization in broiler chicks (Kim et al. 2015; Khan and Iqbal 2016; Sugiharto 2016; Yadav et al. 2016). OAs can improve protein digestibility, antibacterial property, pancreatic secretion, and gut morphology (Liu et al. 2017). These OAs have been used as feed additives to minimize the occurrence of pathogens in feed matrices and potentially improve the gastrointestinal tract function in general (Dittoe et al. 2018; Ricke et al. 2020).

In previous studies, the application of OAs and OEs was proved to have brought out many positive effects for the animals. However, the impact of these compounds on poultry production has not been summarized yet. Therefore, this study aims to review the effects of OAs and OEs as effective feed additives, specifically on poultry production, as an alternative to antibiotics.

Essential oil and organic acidifier sources

OAs are weak acids that can only be separated in part. The dissociation constant (pKa), gut pH, and characteristics of the bacteria determine the antimicrobial action of OAs in the gut (Mroz et al. 2006). The lipophilic property of the undissociated OA molecules allows them to enter the semipermeable bacterial barrier. The pKa constant of most OAs having antibacterial action is between three and five. OAs come in a wide range of physical and chemical properties, and many are utilized as feed additives, drinking water supplements, or even simply acidifiers (Khan and Iqbal 2016). OAs are found naturally in various feed types and are frequently employed for feed acidification. Several OAs, such as formic acid, fumaric acid, citric acid, butyric acid, and others have been studied for their performance and health-promoting effects (Yang et al. 2018). By affecting the pH, acidifiers in feed prevent the growth of harmful bacteria. Below pH 5, the growth of the majority of pH sensitive bacteria (such as *E. coli* and *Salmonella*) is reduced while acid-tolerant ones persist (Gao et al. 2021). OAs offer a superior flavour and stronger bacteriostatic effects than inorganic acids (Xiao et al. 2016). The -COOH carboxyl functional group of OAs, consisting of fatty acids and amino acids, is linked to the acidic capacity of OAs. Simple monocarboxylic acids (acetic, propionic, formic, and butyric acids), the hydroxyl group carboxylic acids (malic, tartaric, lactic, and citric acids) and double bond short-chain carboxylic acids (sorbic acids and fumaric) are all examples (Shahidi et al. 2014). Some chemical characteristics of OAs are shown in Table 1.

EO components were discovered in the previous millennia, which aided the pharmaceutical industry expansion. EO isolation from plants has been documented for over 5 000 years. Moreover, the repellent and therapeutic effects of aromatic herbs were recognized in the European Union, and the studies of EOs had already been implemented for a long time ago (Zdrojewicz et al. 2014). However, after antibiotics were identified as the most efficient treatment for bacterial infections in the previous years, further scientific research into EOs was put on hold (Zhai et al. 2018). EOs, which have volatile characteristics, are derived from plant materials mostly through steam distillation. EOs have sparked academic and industry interest as one of the potential broiler chicken growth enhancers. EOs are chemically complicated and have varied mixes of ingredients that fall into two categories: terpenoids and aromatic

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Table 1. Chemical characteristics of acidifier compounds

No.	Name	Molecular formula	Molecular weight (g/mol)	pKa	Melting point (°C)	Solubility (mg/ml)	Solubility in water
1	formic acid	CH_2O_2	46.025	3.75	8.3	100	miscible
2	butyric acid	$C_4H_8O_2$	88.11	4.82	–5.7	100	miscible
3	citric acid	$C_6H_8O_7$	192.12	2.97	100	100	very soluble
4	lactic acid	$C_3H_6O_3$	90.08	3.86	16.8	1 000	completely soluble
5	fumaric acid	$C_4H_4O_4$	116.07	3.03 and 4.54	287	< 1	miscible
6	sorbic acid	$C_6H_8O_2$	112.13	4.76	134.5	< 1	slightly soluble

chemicals (Nazarro et al. 2013). Several studies on the medicinal effects and industrial applications of EOs produced from various plant families have been conducted and positive results have been achieved. However, the results were quite different among researchers. This is due to numerous factors that influence the effects of EOs in poultry. The selection and combination of correct EOs to be used play an important part in the effectiveness of the OAs. Furthermore, EO activities are influenced by functional groups, their compositions and synergistic interactions between components. Due to their chemical compositions, oregano, cinnamon, garlic, thyme, peppermint, spearmint, garlic, lemongrass, lavender, sage essential oils are widely utilized around the world (Zhai et al. 2018; Falleh et al. 2020). Table 2 indicates the chemical compositions of some EOs.

Table 2. Chemical compositions of some essential oils

Plant source	Species	Major components	Overall functions	Authors
Cinnamon	*Cinnamomum verum*	cinnamaldehyde (62.09–89.31%); cinnamyl acetate (1.48–2.44); α-muurolene (4.32%); linalool (1.6–4.08)	Antibacterial activity, hypocholesterolaemic, antioxidant, analgesic, antiulcer and anticandidial activities	Vazirian et al. (2015); Ainane et al. (2019)
Lavender	*Lavandula angustifolia*	carvacrol (26.2%), limonene (19.6%), terpinen-4-ol (7.6%), *p*-cymene (4.2%)	Antibacterial activity, antifungal, immunostimulatory, treatment of respiratory disorders and skin diseases	Torki et al. (2021)
Peppermint	*Mentha piperita* L.	menthol (63%), *p*-menthone (19.5%)	Prevent lipid peroxidation, have hypoglycemic effects, antibacterial, antiviral, antiallergic, anti-inflammatory	Falleh et al. (2020); Lyczko et al. (2020)
Sage	*Salvia officinalis* L.	eucalyptol: 85, beta-thujon: 72, camphor: 179 g/kg	Antibacterial, antifungal, antioxidant and anti-inflammatory	Marcin et al. (2016); Swamy et al. (2016)
Oregano	*Origanum vulgare* L.	carvacrol (73.06%), thymol (7.29%), *p*-cymene (3.90%), caryophyllene (3.70%)	Antimicrobial, antioxidant and antifungal effects	Xianjing et al. (2017)
Spearmint	*Mentha spicata*	menthone (21.8%), menthol (38.45%), neo-menthol (4.19%)	Antioxidant capacity, salivary and gastric glands stimulation, improve performance, decrease in pathogenic bacteria	Wu et al. (2019); Torki et al. (2021)
Thyme	*Thymus vulgaris*	alpha-thujene (1.25%), carvacrol (59.29%), gama-terpinene (29.12%); *p*-cymene (3.72%)	Antibacterial and antioxidant activity, anticoccidial and antifungal properties, improve digestion, warming, and increase appetite	Wade et al. (2018); Alsaraf et al. (2020)
Garlic oil	*Allium sativum*	allyl methyl trisulfide (7.9–13.2%), dimethyl trisulfide (4.3–17.4%)	Antibacterial and antifungal activity	Satyal et al. (2017)
Lemongrass oil	*Cymbopogon citratus*	neral (31.5%), geranyl acetate (2.27%), citral (26.1%)	Anti-inflammatory, antifungal, and anti-protozoa properties	Brugger et al. (2019)

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The mechanism of acidifiers and essential oils

The effectiveness of OAs and OEs might be due to their antibacterial and antioxidant properties. Moreover, the method by which the OAs and OEs improve nutrient digestibility is unknown, but it may result in a fall in gastric pH and a decrease in microbial pathogens in the gastrointestinal tract, particularly gram-negative bacteria (Mahfudz et al. 2019). According to the findings of Zeng et al. (2015), it is essential to take a comprehensive approach when determining the mechanism of action of EOs due to their chemical complexities, the vast microbiological diversity of the gut microbiome, and the multiple activities of the gut. The basic mode of action of EOs can be found in Figure 1. Besides, the poorly understood mechanism of action of EOs is the fundamental explanation for the unclear and inconsistent study findings about the effect of EOs on the health of animals and the performance of animal products (Yang et al. 2015).

The EOs are categorized into four basic classes, based on the plant secondary metabolites: nitrogen-containing alkaloids, phenolics, terpenoids and sulphur-containing chemicals (Stevanovic et al. 2018). All these metabolites contain the same fundamental functional groups, such as benzyl rings, alkyls, hydroxyls, steroids, and alcohols (Bakkali et al. 2008). However, the combination of different chemical groups results in the formation of novel molecules with distinctive chemical structures and unique biological activities. Secondary plant metabolites are created in a range of plant cell types and are derived from the nitrogen metabolism through a variety of modifications, including deamination. These alterations take place during the process of producing secondary plant metabolites. Secondary metabolites have a low abundance in comparison with primary metabolites, which are essential photosynthetic products involved in the maintenance of plant life (Bourgaud et al. 2001).

The bioactivity of EOs is due to the complex variety of volatile molecules created by aromatic and medicinal plant secondary metabolism. Primary metabolites are involved in the maintenance of plant life (Prakash et al. 2012). As a result of the volatile and reactive nature of EOs, the efficiency of these oils in animals can be affected by the conditions in which they are produced (Maenner et al. 2011). Additionally, the conditions that exist within the animal gastrointestinal systems (Piva et al. 2007) also play a role in the effectiveness of the EOs. Moreover, the growth-promoting property of EOs is primarily associated with their effects on the gastrointestinal tract which include the enhancement of feed palatability, stimulation of digestive fluid secretion, the improvement of intestinal morphology, the stabilization of the intestinal microbiome and the reduction of inflammation (Steiner and Syed 2015). EOs stimulate the formation of diges-

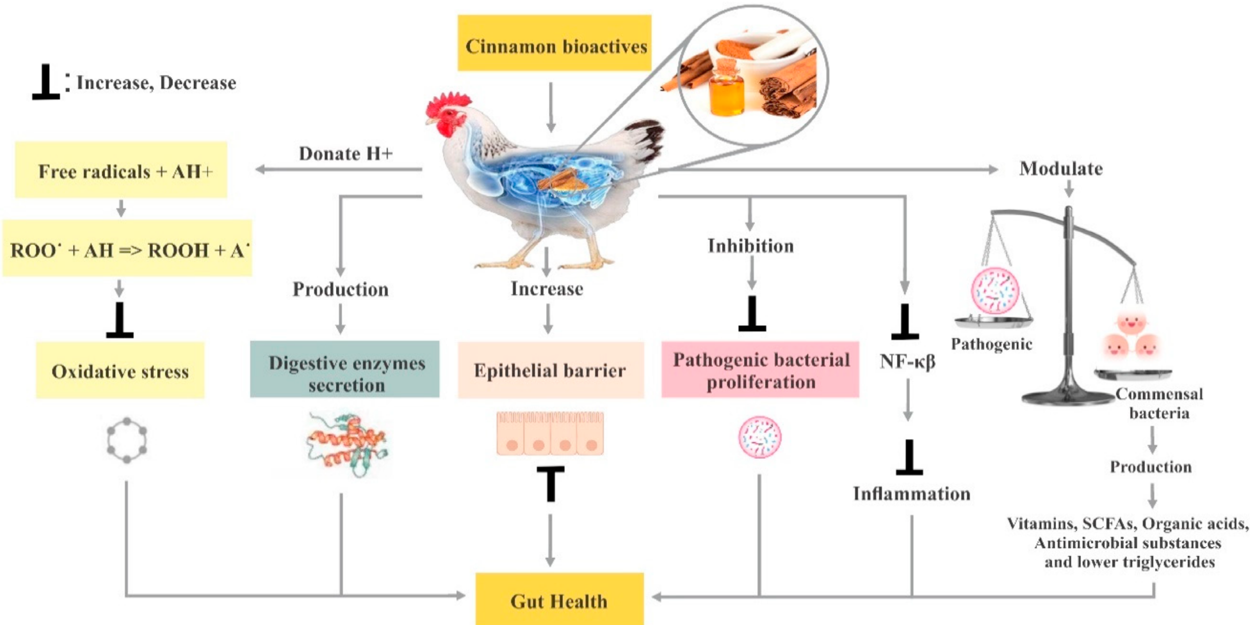

Figure 1. The mode of action of essential oils (case of cinnamon) in poultry (Ali et al. 2021)

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tive fluids, increase blood circulation, function as an antioxidant, lower harmful bacteria levels and may improve the immune state (Brenes and Roura 2010). For pharmacokinetics of EOs, in the digestive tract, EO molecules prefer to interact with food that has been digested. As a result, active compounds could escape to the stomach for solubilization and absorption. In addition, the kinetic rate of the EO component release from fatty acid bonds is determined by the activity of digestive enzymes (Horky et al. 2019). An increase in urine analytes indicates that a large amount of EO compounds is excreted through the kidneys (Schindler et al. 2001). Polyphenols that are not absorbed and are not metabolized leave the body through the faeces (Lin et al. 2019). Moreover, in two hours following the administration the EO active components are at their peak concentration, and five hours later, the chemicals have already been successfully removed from the bloodstream with the exception of carvacrol, thymol, eugenol, which have half-lives between 1.84 and 2.05 hours (Michiels et al. 2008). The toxicity of EOs was also recorded in some studies. The great variety in the active chemical content, which can range from a few to hundreds in one type of oil, complicates the assessment of toxicity (Izgi et al. 2017). The generation of reactive oxygen species, interruption of the cell cycle, mitochondrial damage, DNA aberration, and activation of the NF-kB cascade which induces apoptosis, are the main mechanisms by which EOs exert their deadly effects (Haeseler et al. 2002). For instance, lemon oil is a hallucinogen, while pennyroyal oil causes hallucinations and produces abortions (Laios et al. 2019). The study of Borges et al. (2019) showed that 200 µg/ml of *Piper aduncum* can cause harmful effects on erythrocytes in 24 h, and Barros et al. (2016) also indicated that 22.11 mg/ml of *Achillea millefolium* L. caused detrimental effects on macrophages. As the same function of essential oils depends on pKa, the pharmacokinetics and toxicity of organic acids are also involved with pKa.

OAs inhibit intestinal infections by disrupting bacterial cell membranes and lowering the cytoplasmic pH. Additionally, OAs are available in salt form with comparable bactericidal and bacteriostatic characteristics (Hedayati et al. 2014). OAs diffuse over the cell membrane and dissolve into protons and anions within the cytoplasm (Eklund 1983). The bacterial cell responds by actively expelling protons. This activity depletes the cell energy supply, ultimately culminating in the cell death (Markazi et al. 2019). OAs work by reducing the colonization of pathogenic microorganisms in the intestines (Dittoe et al. 2018). Reduced pathogen colonization correlates with higher body weight, production performance and beneficial bacteria in the gut of chickens (Yegani and Korver 2008). Additionally, decreased quantities of harmful bacteria in the intestine are linked to an anti-inflammatory immune response (Markazi et al. 2019). According to Kim et al. (2015), the mechanism of OAs in animal diets is not well understood. Lack of understanding the function of OAs has limited their application in broiler diets because it has made it difficult to determine how organic acids work in animal diets. On the other hand, a number of different plausible mechanisms have been hypothesized, the vast majority of which are connected to the following: first and foremost, OAs help to reduce the pH value and buffering capacity of the feed, as well as perform antifungal and antibacterial effects; secondly, lowering the pH of the stomach by releasing hydrogen ions, activating pepsinogen to create pepsin, and enhancing the digestion of proteins; thirdly, better use of energy in intermediate metabolism; and finally, inhibition of gram-negative bacteria (Kim et al. 2015). The example of acetic acid mode of action is shown in Figure 2. The short-chain organic acids (C1–C7) have unique antibacterial action; nevertheless, their effect on pH decreases and antimicrobial activity varies according to their dissociation status, which is determined by the pKa value of the acid. Thus, a lower pKa value indicates a stronger acid, indicating its capacity to reduce the pH of the surrounding environment (Kirchgessner and Roth 1991). In ad-

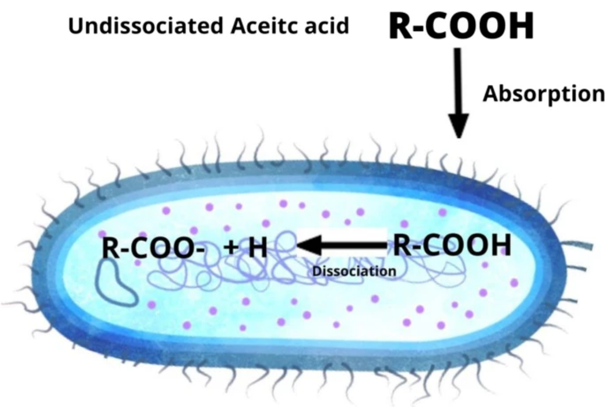

Figure 2. The mode of action of organic acids against bacteria (Khan et al. 2022)

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dition to that, OAs in their undissociated form increase the acidity of the cellular pH, impairing the bacterial cellular metabolism (Stefanello et al. 2017). OAs were employed in poultry production in three ways: (1) solidified and added to poultry feed. This inhibits the growth of mildew in feed and lowers the pH of the bird crops; (2) sprayed on chicken litter. This inhibits the bacteria responsible for uric acid degradation, hence decreasing the quantity of ammonia released. (3) Injected into water to destroy germs. This aids chlorine in eliminating bacteria and lowers the pH in the bird crops. OAs and their salts limit the growth of stomach and gut bacteria by lowering the pH and interfering with the microbial cell anion and proton balance (Hajati 2018).

The mechanism of action of OAs and OEs on microorganisms is also mutual. Particularly, EOs reduce the permeability of bacterial cell membranes, thereby increasing the bactericidal effect of OAs. Moreover, OAs work mainly in the stomach while EOs work mainly in the small intestine of animals. These differences in the mechanism and site of action are relevant for the synergies between OAs and EOs in the diets of poultry without growth-promoting antibiotics (Stefanello et al. 2017).

However, the application of OAs and EOs still has a restriction on gastrointestinal characteristics and microbial ecosystems of birds. Before using, we have to know which OAs or which plants can be supplemented to the diet depending on their pKa, if the application of OAs and EOs is not to destroy all the beneficial bacteria in the body (Hajati 2018). Besides, the primary impediment to using OAs and EOs as animal antibiotics is that the active ingredients enter the intestines at concentrations below the inhibitory threshold.

Essential oils and acidifiers in antimicrobial activity

Beneficial bacteria live in a lower pH environment than pathogenic bacteria. Thus, the addition of OAs and EOs to lower the pH will inhibit groups of pathogenic bacteria and create favourable conditions for the growth of beneficial bacteria. In the presence of OAs, the quantity of harmful bacteria, particularly total aerobes, coliforms, and *E. coli* is decreased (Youssef et al. 2017). Furthermore, acidifiers have been shown to have antibacterial effects on microorganisms when applied to the diet (Kim et al. 2015; Khan and Iqbal 2016). According to Markazi et al. (2019), OAs have the potential to change the bacterial cell membrane by interfering with bacterial nutrition transportation and energy metabolism. Moreover, OAs diffuse through the cell membrane and dissolve within the cytoplasm to form protons and anions, which are toxic to cells (Eklund 1983). Bacterial cells have to react to these protons and anions by expelling them out of the cells to protect themselves; and this activity makes cells lose energy (Mitchell and Moyle 1969). Consequently, due to this activity, the cell energy reserves are depleted, which ultimately leads to the cell death. A change in feed pH can prevent the growth of harmful bacteria and reduce microbial competition for host resources; both of which is detrimental to harmful microbes. Hassan et al. (2010) found a reduction in intestinal *Salmonella* spp. and *E. coli* in the intestinal microbiota of broilers after integrating a combination of OAs or salts into the feeding regime of the birds. The increase in beneficial bacteria is most likely due to the inhibition of pathogenic bacteria, which results in greater available resources for alternative microorganisms in the intestines. Fortunately, good bacteria such as *Bifidobacteria* and *Lactobacillus* are less sensitive to pH than *Salmonella*, making them more resistant to the effects of OA supplementation (Kim et al. 2005). In the study by Emami et al. (2017), the colonization of beneficial bacteria in the intestines of broilers was inhibited by *E. coli* when formic and propionic acids were provided in greater quantities than in the control group. According to Markazi et al. (2019) the OAs, such as those mentioned above, can decrease the growth of *Salmonella* bacteria. A drop in *E. coli* populations was observed in broilers given rations supplemented with OAs while an increase in *Lactobacillus* populations was observed in the same rations (Nguyen et al. 2018). OA supplementation significantly enhanced the performance of laying hens, modulated the anti-*Salmonella* immune response, and significantly decreased *Salmonella* infection. The addition of acidifying products to the diet of layer hens increases their resistance to *Salmonella*. Hence, it may be a useful addition to the diet of layer hens in some instances. Furthermore, OAs were used as disinfectant and microbial control agents when sprayed over the litter material, where organic acids block microbes that aid in uric acid break-

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down, limiting the ammonia output (Hajati 2018). According to Pateiro et al. (2021), the most common mechanism of antimicrobial activity of EOs is its membrane disruption effect, which is analogous to the function of OAs in nature. The accumulation of bioactive chemicals in the cytoplasmic membrane phospholipid bilayer leads to the degradation of cytoplasmic membranes, an increase in permeability and fluidity, the leakage of intracellular contents, the disruption of embedded proteins and ultimately the death of the cell. The ultimate factor that leads to the breakdown of the cytoplasmic membrane is the accumulation of bioactive chemicals within the phospholipid bilayer of the membrane (Huang et al. 2014; Calo et al. 2015; Pateiro et al. 2021). The hydrophobicity of a substance is linked to its antibacterial activity, which in turn disturbs the permeability of cell membranes and the homeostasis of cells. It is possible that this will lead to the loss of biological components, the introduction of new substances, or even the death of cells (Brenes and Roura 2010; Solorzano-Santos and Miranda-Novales 2012; O'Bryan et al. 2015). In relation to this, OAs must pass through the bacterial cell membrane to change the metabolism, according to Stefanello et al. (2017). Since one of EO actions is to disrupt the bacterial cell membrane, more OAs can permeate into the bacterial cytoplasm. OAs in their undissociated form can lower intracellular pH and disrupt the bacterial metabolism, leading pH-sensitive bacteria including *E. coli*, *C. perfringens* and *Salmonella* to death. As a matter of fact, gram-negative bacteria have more hydrophilic elements in their outer membrane than gram-positive bacteria. Thus, it is important to note that they are more resistant to the activities of EOs than gram-positive bacteria (Seow et al. 2014). It should be noted that the antibacterial activity of EOs is not dependent on a single mechanism and that it differs depending on the components of different microorganisms (Pateiro et al. 2021). In order to suppress fungal growth, EOs work in a variety of methods: disruption and change of cell membranes, inhibition of the cell wall construction, dysfunction of the fungal mitochondria, blockage of efflux pumps and the production of reactive oxygen species (Nazzaro et al. 2013). The minimal inhibitory concentration of EOs, ranging from 0.12 to > 2.0 vol% and 0.25–5.0 l/ml, was found to be efficient in inhibiting the growth of bacteria in the laboratory (Mucha and Witkowska 2021). Many *in vitro* tests have been conducted to investigate the antibacterial action of EOs and the studies concluded that thymol and carvacrol exhibit significant antimicrobial activity against pathogenic bacteria such as *Escherichia coli* and *Salmonella typhimurium* (Zhai et al. 2018). When used alone or in combination in *in vivo* investigations, EOs showed the significant growth inhibition of *Clostridium perfringens* and *E. coli* in the hindgut and proved to be more effective at alleviating intestinal lesions and weight loss than in the challenged control birds (Jerzsele et al. 2012). Furthermore, the dosage of EOs according to Vinolya et al. (2021) is critical in sustaining the population of lactic acid bacteria in the intestinal gut. The synergistic effects of OAs and EOs against pathogenic bacteria have been proposed to be explained by the following three hypotheses: (1) the membrane-damaging effect of essential oils may make bacteria more sensitive to an acidic environment; (2) at low pH, the hydrophobicity or antilisterial activity of essential oils increases, allowing them to more easily dissolve in the lipids of the target bacteria cell membrane, and (3) organic acids appear to be particularly active in the feed, crop and gizzard (Langhout 2000; Karatzas et al. 2001).

Role of essential oils and acidifiers in poultry intestinal health

Intestinal structural integrity is critical for maintaining nutrient absorption and intestinal health since it serves as the largest barrier of the body between the internal environment and external environment. The villi and the crypts are two major components of the small intestine, and the geometry of these two structures serves as an indicator of the absorptive capacity of the small intestine (Heydarian et al. 2020). Enterocytes with shorter villi reach the apex early, at a stage when their enzyme secretory capacity is less developed, resulting in decreased digestive and absorptive efficiency (Broom 2017). The internal epithelium turnover is a dynamic balance between the generation of enterocytes in the crypts and their eventual desquamation from the villus, which occurs in the intestine. The villus height (VH) to crypt depth (CD) ratio is a metric that can be used to assess intestine health and function, and it is readily available (Su et al. 2021). The high VH/CD ratio

https://doi.org/10.17221/99/2022-CJAS

has evolved as a result of both rising values of VH and falling values of CD. This ratio indicates mature enterocytes at the villus tips, balanced enterocyte migration, and sloughing in broiler chickens. This is owing to the fact that the VH/CD ratio is directly tied to VH and CD equilibrium (Mustafa et al. 2021). The combination of OAs and EOs has brought many positive results in the intestinal system since they synergistically function inside the poultry body. In the study of Vinolya et al. (2021), the combination of OAs and EOs improved the villus height in both duodenum and jejunum in broiler chickens. The study on Japanese quails using commercial products (consisting of OAs: acetic acid, formic acid and butyric acid, as well as EOs: thymol, β-cymene, carvacrol and borneol) (Matty and Hassan 2020) which resulted in the improvement of intestinal morphology such as the crypt depth, the villus length and width, the villus/crypt ratio, the thickness of the intestinal wall, goblet cell percentage and the appearance of the intestinal surface area clearly showed that OAs and EOs are useful in preserving the intestinal mucosa (Stefanello et al. 2017). The study of Basmaciolu-Malayolu et al. (2016) noted that the combination of OAs and EOs was more useful than the individual component supplements in modulating intestinal flora and improving histomorphology.

Firstly, the increase in VH and CD in the jejunum was recorded in the diet with EO supplementation, which is consistent with earlier studies by Kishawy et al. (2019) and Barbarestani et al. (2020). The biological effects of EOs alter the interactions between phytochemicals and their bioavailability in the gastrointestinal system of animals, according to Stevanovic et al. (2018). For example, the use of 400 mg/kg of feed containing volatile oil blends of oregano, rosemary and fennel in broiler chickens improved the intestinal microbial balance, decreased the quantity of coliform bacteria, and increased the *Lactobacillus* spp. counts in the gut (Cetin et al. 2016). According to Irawan et al. (2021), the use of EOs enhanced the ratio of VH to CD by 2.67 to 9.10 when compared to the control. Previous research on various kinds of EOs revealed that EOs have a powerful function, whether taken as supplements or in combination with other EOs. It was discovered that thyme oil enhanced the intestinal barrier which protects against the passage of hazardous compounds from chicken feed, among other things. A favourable effect on intestinal morphology was demonstrated by the use of carvacrol in feeds, when the length of poultry intestinal villi was increased (Zhang et al. 2021). Functional oils comprising cashew nutshell and castor oil were found to greatly improve the thickness of the *jejuna muscularis mucosae* by 28% in four days and significantly lower the crypt depth of turkey by about 21% in 11 days in a study conducted by Ferket et al. (2020). In comparison with the other research studies, the inclusion of these functional oils in the diet for 21 days considerably enhanced the height of the villi. Moreover, when EOs were used to promote goose growth in the study by Olmez et al. (2020), statistical examination of intestinal CDs in all groups revealed that the control group without essential oils substantially differed from the 0.1% and 0.2% essential oil groups. Further details on the effects of EOs on intestinal morphology are shown in Table 3.

Additionally, the digestive tract of chickens is quite short, particularly in immature birds, and the pH of the small intestine is highly sensitive to changes in external environmental conditions, such as temperature and humidity (Gadde et al. 2017). The use of OAs helps prevent harmful bacteria from colonizing the epithelium, consequently lowering inflammation and infection in the intestinal mucosa and enhancing villus height and secretion, digestion, and nutritional absorption in the gut (Thanh et al. 2009). Furthermore, mixed OAs are able to assist the digestive tract in maintaining a suitable level of acidity, which not only serves as one of the primary mechanisms of mixed OAs in their action on the body of the bird, but also is one of the primary benefits that mixed OAs provide (Suiryanrayna and Ramana 2015; Liu et al. 2018). OAs have been added to poultry diets all over the world to improve the feed nutritional value. The suppression of pathogens present in the intestinal environment, as well as the formation, development and repair of the intestinal wall, are all crucial functions of these OAs (Stefanello et al. 2017). Birds have larger intestinal villi, which results in a faster growth rate. In response to the occurrence of rapid growth or stimulation by microbes, deeper crypts suggested a quicker rate of cellular turnover than it would be expected. Supplementation of OAs lowers CD and raises the VH/CD ratio, indicating a beneficial effect of OAs on intestinal health as reported in the study of Mohammadagheri et al. (2016). In the small intestine, dietary supplementation of low amounts

Czech Journal of Animal Science, 68, 2023 (4): 141–160

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Table 3. The effect of essential oils (EO) and organic acids (OA) on intestinal morphology

Poultry breed	Amount of supplement	Age (days)	Criteria	Control	Addition	Reference
Essential oils						
Arbor Acre broilers	200 mg commercial EO/kg feed	21	V/C	4.38	5.62	Su et al. (2021)
		42	V/C	5.42	7.98	
Pekin ducks	100 mg oregano/kg feed	14	V/C	4.80	5.40	Ding et al. (2020)
		35	V/C	4.55	5.76	
Broiler chickens	250 g oregano and laurel per ton	42	VH (μm) in duodenum	1 804.4	1 889.2	Giannenas et al. (2016)
			VH jejunum	1 532.6	1 641.6	
			ileum VH (μm)	932.3	1 029.5	
Arbor Acre broilers	200 mg oregano per kg	42	C/V in duodenum	< 8	> 8	Zhang et al. (2021)
Vencobb 400 broiler chicks	500 mg/kg commercial EO	39	VH in duodenum	1 247	1 343	Parveen et al. (2020)
Japanese quails	400 ppm of *Mentha pulegium*	35	duodenum VH (μm)	993.3	1 087.3	Dehghani et al. (2018)
	200 ppm of *Mentha pulegium*		VW in duodenum (μm)	93.3	96.0	
	200 ppm of *Mentha pulegium*		CD (μm)	84.0	89.3	
Quails	palmarosa oil 100 μg/kg diet	35	CD	74.25	94.24	Kurekci et al. (2021)
Organic acids						
Ross 308	2 g/kg organic acids combined with 2 g/kg probiotics	35	VH (μm)	560.35	627.78	Rodjan et al. (2017)
			CD (μm)	82.58	93.07	
Arbor Acres male chicks	6 000 mg/kg	21	ileum V/C	5.18	6.73	Ma et al. (2021)
Male Arbor Acres broiler chicks	0.3% OA, mainly based on acetic acid, propionic acid, formic acid, and ammonium formate	42	VH (μm)	584.13	766.86	Dai et al. (2021)
Arbor Acres broiler	0.50% formic acid	42	VH (μm) in doudenum	45.55	66.30	Ghazala et al. (2011)
	0.50% fumaric acid		VH (μm)		67.50	
	0.25% acetic acid		VH (μm)		66.40	
	2.0% citric acid		VH (μm)		60.00	
Ross 308	2 g/kg organic oil blend	42	VH ileum	669	814	Basmaciolu-Malayolu et al. (2016)
Arbor Acre broiler chicks	0.4% OA (formic acid, propionic acid)	35	VH:CD	6.90	8.95	Agboola et al. (2015)
Japanese quails	3 g/kg organic acid blend	35	VH jejunum	374.3	380.6	Ustundag and Ozdogan (2019)
			VW jejunum	77.4	80.9	
			VH ileum	327.7	337.6	
			VW ieum	81.5	82.6	
Ross 308	0.1% organic acid	35	VH jejunum	44.86	47.35	Sureshkumar et al. (2021)

C = crypt; CD = crypt depth; V = villus; VH = villus height; VW = villus width

https://doi.org/10.17221/99/2022-CJAS

of mixed OAs (3 000 mg/kg) and high amounts of mixed OAs (6 000 mg/kg) increased the expression of tight junction proteins. The broilers fed low amounts of mixed OAs performed better than the broilers fed high amounts of mixed OAs (Rodjan et al. 2017). Another example given by Ma et al. (2021), the CD, VH, and VH/CD in the jejunum, duodenum and ileum of 21-day-old and 42-day-old broiler chicks were enhanced by supplementation of low and high quantities of mixed OAs, respectively. Mixed OAs, which are substrates for the tricarboxylic acid cycle, have the potential to stimulate energy metabolism by supplying energy directly to the intestinal epithelial cells, allowing for faster renewal and proliferation of intestinal epithelial cells and increasing the height of intestinal villi or all of these things have the potential to save a significant amount of time in comparison with the energy that is provided by the glycolytic pathway (Rodjan et al. 2017).

Effects on poultry growth performance

The modern broiler business requires a lower feed conversion ratio and increased production levels, which can be attained to some extent by the use of particular feed additives (Table 4). As one of the functions of organic acidifiers and essential oils, growth performance was improved by improving intestinal health and reducing harmful bacteria in the poultry body. The improvement in poultry performance is primarily attributable to an increase in nutrient digestibility. OAs, in conjunction with EOs, improved the digestibility of gross energy and ether extract in broilers, whereas the OAs enhanced the intestinal villus integrity as a mechanism of action (Vitor et al. 2012). Basmaciolu-Malayolu et al. (2016) also showed that OAs and EOs had a beneficial influence on body weight gain, feed conversion ratio, and apparent digestibility of nutrients. The high digestibility may be a result of a better intestinal mucosa shape and/or enhanced intestinal digestive activity. In the jejunum of broilers fed OA-supplemented diets, Yang et al. (2018) found an increase in VH and in the ratio of VH to CD in absorption efficiency after 21 days. The enhanced performance may be attributable to the availability of OAs in water and feed, which boosts endogenous gastrointestinal tract enzyme production and has a positive influence on the gastrointestinal tract passage rate and nutrient digestibility in broilers (Khan and Iqbal 2016). OAs have developed as growth promoters and can be employed in place of antibiotics in the large array of feed additives available (Fascina et al. 2012). Additionally, low pH in the diet and digestive tract acts as a microbial barrier, reduces buffering capacity, and improves nutrient digestibility, resulting in enhanced growth performance (Pearlin et al. 2020). Moreover, as the explanation about the functions of OAs above, an increase in broiler weight and feed conversion ratio may be a result of the positive effect of organic acids on gut microflora and their bactericidal action, as organic acids interfere with bacterial cell membranes and macromolecules of cells, impairing energy metabolism and nutrient transport (Elnaggar and Abo El-Maaty 2017). In case of essential oils, EOs have been shown to promote digestive enzyme activity and increase nutrient digestibility (Jang et al. 2004). Improved growth performance and digestibility may also be connected with an improved state of the intestinal health (Stefanello et al. 2017) or stimulating digestion, regulating gut flora, and enhancing the release of various endogenous digestive enzymes are all probable explanations for higher performance owing to EOs (Popovic et al. 2016). However, there were various inconsistent results in the effect of OAs and EOs. The discrepancies between studies may have been related to or caused by the physiological state of animals, active component concentrations, the kind, origin and level of inclusion of EOs, diet composition, infection, and ambient circumstances of the trial in some researches.

Stefanello et al. (2017) found that challenged broilers fed diets supplemented with a blend of OAs and EOs exhibited an overall improvement in body weight gain and feed conversion ratio when compared to the control group. Moreover, this improvement in broiler growth performance can be explained in part by the observed increase in ileal digestibility in the study. The results of Stefanello et al. (2017) also showed that the ileal digestibility of dry matter and ileal digestible energy increased by 3.2% and 106 kcal/kg, respectively, when compared to the control treatment. And also, the high digestibility may be a result of a morphologically more normal intestinal mucosa and/or a more active intestinal digestive system. The addition of OAs and EOs may boost the weight of broilers during the starter phase.

Czech Journal of Animal Science, 68, 2023 (4): 141–160

https://doi.org/10.17221/99/2022-CJAS

Table 4. The effect of essential oils and organic acids on growth performance of poultry

Breeds	Age	Types	Amount	Effects on performance	Reference
Oragnic acid					
Male Arbor Acres broilers	1 day	commercial acidifiers	3 kg/ton	Increased average daily gain, decreased feed conversion ratio	Ding et al. (2017)
Broiler chicks	360 days	organic acid blend	1.5 ml/l	Improved average of body weight gain, body weight, and feed conversion ratio	Mustafa et al. (2021)
Arbor Acres broiler chicks	1 day	mixed organic acids	0.3% at 0–21 days; 0.2% at 21–42 days	Improved growth performance, intestinal morphology	Dai et al. (2021)
Broiler chicks from Ross strain	22–42 and 7–42 days	Orgacids® (combination of six acids)	3 g/kg	Body weight, body weight gain, and feed conversion ratio improved	Khooshechin et al. (2015)
Ducklings (Cairina Moschata)	7 days	formic and citric acid	0.1% and 0.3%	Had significantly greater body weight, body weight gain, economical efficiency and better feed conversion	Elnaggar and Abo El-Maaty (2017)
Japanese quail	1 week	fumaric acid	15 g/kg feed	Increased growth performance live body weight, body weight gain, and feed conversion ratio	Reda et al. (2021)
Arbor Acres broilers	1 day	phosphoric acid	0.1 g/kg or 0.2 g/kg	Improved performance, and meat quality	Gao et al. (2021)
Essential oils					
Arbor Acres broilers	1 day	thyme oil (*Thyme vulgaris* L.)	1 g/kg	Improved performance	Youssef et al. (2017)
Japanese quails	7 days	palmarosa oil, lemon myrtle oil	100 μg/kg diet	Changes in pH after 24 h and colour the quail's breast muscle	Kurekci et al. (2021)
LSL-Lite laying hens	42–56 weeks	lavender essential oil, mint essential oil	250 mg/kg; 250 mg/kg	Feed conversion ratio, egg production and egg mass improved	Abu Isha et al. (2018)
Hy-Line Layers	30 weeks	oregano essential oils	150 mg/kg	Improved egg quality and hen's performance	Xianjing et al. (2017)
Arbor Acre broilers	1 day	EO blend (thymol, carvacrol and cinnamaldehyde)	200 mg/kg	Improved broiler performance	Su et al. (2021)
Ross 308	1–42 days	oregano essential, laurel essential oils	25 mg/kg combination of 25 mg/kg oregano and 2.5 mg/kg laurel essential oils	Improved growth performance	Giannenas et al. (2016)
Japanese quails	1 day	lemongrass essential oil	300 mg/kg diet	Increased body weight, body weight gain, decreased feed intake	Alagawany et al. (2021)

https://doi.org/10.17221/99/2022-CJAS

Particularly, in the study of El-Shenway and Ali (2016), quail chicks can get the benefits from the addition of OAs and EOs to their diet. An increase in bioavailability of nutrients in quail chick feed can be achieved by enhancing the digestion process of most of these components in the diet. El-Shenway and Ali (2016) also showed that the blend of OAs and EOs decreased the feed intake of quails in the starter period. Vinolya et al. (2021) also showed that short-chain fatty acids with EOs improved the body weight of Vencobb 400 broilers. In particular, Matty and Hassan (2020) showed that the performance of Japanese quail chicks was improved by increasing the final weight and total weight gain, decreasing total feed consumption and improving feed conversion ratio. Al-Mashhadani et al. (2011) recorded similar results to those in Matty and Hassan (2020), when they showed an improvement in the performance of Japanese quails fed a blend of OAs and EOs.

In summary, the results between various studies were quite different and inconsistent due to the range of inclusion levels, active ingredient compounds, EO types used, basal feed composition, diseases, environmental conditions and herb origin. It was also stated by Iqbal et al. (2021).

Antioxidant capacity of essential oils and organic acids

Free radicals (superoxide, hydrogen peroxide) are created during normal metabolic activity and removed by a number of methods. However, stress conditions result in antioxidant shortage and an overabundance of free radicals. These radicals induce tissue damage, as well as fatty acid lipid peroxidation (LPO). LPO is an irreversible reaction that starts with polyunsaturated fatty acid oxidation and progresses through autocatalytic chain reactions, causing damage to a range of biological components. As a result, it is the most sensitive oxidative stress indicator (Sies 1997). It is widely accepted that the primary cause of meat quality degradation during storage is lipid oxidation. The most critical factor in the development of lipid oxidation is the availability of oxygen. Meanwhile, protein oxidation increases the toughness of meat products by oxidizing the protein thiol and forming myosin heavy chain disulphide cross-links (Nieto et al. 2011). Furthermore, antioxidant compounds added to animal feeds may help control and reduce oxidative rancidity in meat (Hashemipour et al. 2013). Additionally, there have been rejections of synthetic food additives from a lot of consumers in recent years. Thus, there is growing interest in research studies evaluating natural additives as the potential antioxidant. Since EOs are able to donate hydrogen or electrons to free radicals, in addition to the fact that they are able to delocalize the single unpaired electron that is contained inside the aromatic structure (Fernandez-Panchon et al. 2008), EOs are effective antioxidants that protect other biological molecules from the effects of oxidation. Additionally, natural antioxidants such as EOs or their components can be used to boost the antioxidative status of chicken meat (Adaszynska-Skwirzynska and Szczerbinska 2017) which can be influenced by adding EOs to the diet or by incorporating them into meat to alter the fatty acid profiles and the oxidative stability of the meat (Wenk 2003). Total antioxidant capacity (TAC), both enzymatic and non-enzymatic, measures the overall effectiveness of antioxidant defence systems. Several *in vitro* investigations have shown that plant extracts and EOs have antioxidant effects (Yu et al. 2018). Most researched EOs have variable antioxidant capabilities in the literature, indicating the need for further research into various EO combination types and delivery routes in order to ensure EO efficacy via a synergistic or additive mechanism. The presence of phenolic OH groups in the chemical structure of these EOs acts as a hydrogen donor, engaging with peroxyl radicals during the early step of lipid oxidation and therefore preventing hydroxy peroxide production (Lee et al. 2013). Supplementation of star anise EO resulted in increased total antioxidant capacity in laying hens (Alhajj et al. 2017) and broilers (Ri et al. 2017). Furthermore, the effects of thyme EO on the activity of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), which protect tissues from oxidation, and the enzyme glutathione peroxidase (GSH-Px), which protects intracellular lipids from peroxidation, revealed that thyme EO significantly increased the hepatic activity of all three enzymes, as well as the serum activity of CAT and GSH-Px (Gumus et al. 2019). The study of Oladokun et al. (2021) showed an increase in the weight of immune organs such as liver and bursa. The tendency of increased weight was in line with the increasing amount of EO supplement. In the study of Ding et al. (2020), the effects of dietary supplementation of EOs on serum, hepatic and jejunal SOD, malondialdehyde (MDA) and TAC of ducks were detected.

https://doi.org/10.17221/99/2022-CJAS

These findings are consistent with those of Seven et al. (2009), who found that utilizing phenolic-rich plant extracts reduces MDA levels in heat-stressed broilers. Furthermore, Marcincak et al. (2008) found that adding oregano EO to broiler meals delayed lipid oxidation as compared to a control diet. Oregano EO has two primary phenols: carvacrol and thymol, which have antioxidant properties (Yanishlieva et al. 1999). Supplementation of a phytogenic product to a broiler diet containing an equal blend of thymol and carvacrol at four concentrations boosted SOD activity in a dose-dependent manner, according to Hashemipour et al. (2013).

In acidifying products, antioxidant capacity was also recorded in some studies. This indicates the positive effect of acidifiers. This can be linked to the dietary supplementation of OA, which prevents the conversion of oxymyoglobin to methaemoglobin and hence improves the redness of meat (Millar et al. 2000). SOD and GSH-Px are the most powerful enzymes against reactive oxygen species because they scavenge free oxygen radicals and these enzymes can remove lipid hydroperoxide from the body, reducing the damage caused by organic hydrogen peroxide (Shirani et al. 2019). Ma et al. (2021) found that supplementing mixed OAs to diets increased the amount of SOD and CAT in the serum of 21- and 42-day broilers, as well as the levels of TAC, SOD, and CAT. Furthermore, Abudabos et al. (2017) observed that dietary organic acid increased TAC and decreased serum H_2O_2 concentrations to minimize oxidative stress in 42-day broilers. Broilers fed an OA blend also recorded the decrease in total antioxidant capacity. It is noteworthy to emphasize that OA can help poultry recover after suffering from infectious diseases by decreasing total antioxidant capacity through the OA supplementation (Abudabos et al. 2017). Furthermore, organic acids deliberated in this trial resulted in boosted total antioxidant capacity, improved immunological responses of broiler chicks, particularly to *Salmonella*, and reduced oxidative stress conditions in broilers by lowering the quantity of H_2O_2 in the blood, according to Abudabos et al. (2017).

The role of essential oils and acidifiers in immunity

OAs and EOs have bactericidal and bacteriostatic properties to help strengthen the animal immune system by suppressing the pathogen population in animals. B cells produce immunoglobulins (Ig), which govern humoral immunity. Infection and oxidative stress are common immunological stressors that cause them to be produced (Alp et al. 2012). Some researchers found increased levels of IgG and IgM after EO supplementation (Nadia et al. 2008; Abdel-Ghaney et al. 2017; Sulaiman and Tayeb 2020) while others found no effect of EOs on immunity. An age-dependent immunological response to EO and OA supplementation may exist in birds, as shown by the explanation provided by Movahhedkhah et al. (2019). This may serve as a basis for future research. Preventing the spread of harmful bacteria and altering the bacterial habitat of the intestine were prioritized in order to promote overall health and immunity, as well as productivity. EO improved the feed efficiency, which resulted in better performance by bolstering the immune system, regulating intestinal microflora, enhancing the secretion of endogenous digestion enzymes and triggering antioxidant, antibacterial and antiviral properties (Saeed et al. 2018; Kishawy et al. 2019; Mahgoub et al. 2019; Abo Ghanima et al. 2020). The immunomodulatory effects were clearly described in the research study of O'Bryan et al. (2015). The EOs of capsicum and cinnamaldehyde alter the expression of genetic information in a variety of ways. Changes in the genes involved in avian metabolism and bird immunity were triggered by capsicum oleoresin. Moreover, cinnamaldehyde affected gene expression in inflammation, antigen presentation and humoral immune response (O'Bryan et al. 2015).

The effect of OAs on immune response was also recorded in various research studies conducted in various poultry species. Firstly, Emami et al. (2017) conducted research on the influence of three commercial OAs on the immunity and intestinal morphology of broiler chickens that had been challenged with *Escherichia coli* K88. According to the findings of the authors, adding dietary supplements of these OAs can improve the ileal morphology and immunity of broilers that have been exposed to disease challenges. Secondly, Lee et al. (2017) carried out an experiment to assess the beneficial effects of OAs on the immunological responses of broiler chickens to viral antigens (H_9N_2). They found that the percentage of $CD4^+$, $CD25^+$ T-cells was higher in the group that was given a H_9N_2 vaccine and a diet supplemented with OAs. This is

https://doi.org/10.17221/99/2022-CJAS

attributable to the potential induction of regulatory T cells by feed additive. The percentage of these cells was higher in the experimental group than in the control group. Thirdly, in the study conducted by Liu et al. (2017), the researchers used 450 heads of Cobb 500 chicks that were only one day old in order to explore the potential benefits of a protected OA and EO combination product administered at 0.30 g/kg. When compared to the control group, the authors found that the supplementation of the product led to improvements in the CD and VH of the jejunum, as well as an increased spleen index at 42 days. This was the case regardless of whether the supplementation was given orally or intravenously. OAs were able to reverse the detrimental effects of the *S. typhimurium* challenge and boost the immunological response of the body (Abudabos et al. 2017). In addition to this, the trypsin and chymotrypsin activities of the digestive tract as well as the secretory IgA concentration of ileal mucosa were improved (Liu et al. 2017).

Conclusion

OAs and EOs help improve the digestive system and the ability to absorb nutrients in feed. Feed with functional oils could serve as a partial alternative to replace antibiotic growth promoters. OAs and EOs, through their antioxidant-regulating mechanism, can improve poultry health under stress conditions, as well as boost immunomodulatory functions of the body. Improvements in feed hygiene, lowering of gastric pH, pathogen inhibition without affecting beneficial bacteria, improved nutrient digestibility, enhanced growth performance, antioxidant capacity, and immunity are just a few of their apparent effects. The application of OAs and EOs in feed can be a potential approach to create a new and safe feed additive for both humans and animals. However, the application of OAs and EOs to animals, especially to poultry, should focus on pKa and also plants.

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Conflict of interest

The author declares no conflict of interest.

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