

Chapter

Oregano Essential Oils as a Nutraceutical Additive in Poultry Diets

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Abstract

Oregano essential oils (OEOs) are volatile compounds obtained from the leaves of the oregano plant (*Origanum vulgare*) through a process of steam distillation. Its major bioactive compounds include carvacrol and thymol. The OEOs from *Origanum* spp. have been considered to be the best ones because they have a higher content of carvacrol; however, in tropical America and Africa ecosystems, chemotypes with a high thymol content are found as *Lippia organoides*. Carvacrol and thymol are responsible for the strong antimicrobial, antioxidant, and anti-inflammatory effects of OEO. They have been studied for their potential effects on the function of intestinal barrier, which plays a crucial role in maintaining gut health. These compounds may help reduce inflammation in the gut, protect and preserve intestinal integrity, control the growth of harmful bacteria in the gut, and improve the poultry's productive performance. The OEO has potential as a natural alternative to antibiotics for improving intestinal health and barrier function in poultry. However, more research is needed to fully understand the mechanisms underlying these effects and determine the optimal dosage and duration of OEO supplementation.

Keywords: *Lippia organoides*, *Origanum*, thymol, carvacrol, growth promoter, nutraceuticals

1. Introduction

The *Origanum* genus covers a wide range of more than 60 plant species, most of which belong to the *Lamiaceae* and *Verbenaceae* families; among these species, *Origanum vulgare* L., *Lippia graveolens* Kunth, and *Lippia organoides* Kunt are considered species of economic importance with a wide range of applications [1, 2]. Oregano essential oils (OEOs) are volatile compounds obtained from oregano leaves by steam distillation; they are composed of more than 50 phytochemically different molecules, such as terpenes, phenols, alcohols, organic acids, aldehydes, and ketones. Those metabolites are responsible for the medicinal properties of OEO, including antibiotic and anti-inflammatory properties that can help strengthen the immune system of birds [3]. The OEO may improve feed intake, the growth rate of broilers, and the overall body condition and quality of meat carcasses [4, 5]. Oregano is also

known to fight off bacteria that frequently make poultry sick, such as *Escherichia coli*, *Salmonella*, and *Clostridium* [2, 6]. The OEO is now used commercially by producers in place of antibiotic feed. Due to its antibiotic properties, oregano has been found to improve intestinal functions and alter the gut microbiota in broiler chickens and laying hens. Another application of OEO has been the improvement of intestinal antioxidant capacity, immunity, and intestinal microbiota in birds [7, 8]. Recently, the effects of oregano essential oil (OEO) on intestinal health and barrier function in poultry have been investigated. OEO has been shown to improve the barrier function of intestinal epithelium and consequently strengthen immune defense against pathogens in laying hens [8]. This mechanism appears to be based on regulating intestinal bacteria and inflammation. OEO has been shown to improve intestinal antioxidant capacity, immunity, and gut microbiota in yellow-feathered chickens and, therefore, improve growth performance, antioxidant status, and intestinal health in broilers. In pigs, the OEO has also been found to improve intestinal morphology and expression of tight junction (TJ) proteins associated with modulation of selected intestinal bacteria and immune status [9]. Furthermore, feeding OEO to pullets during rearing can help improve flock uniformity, reproductive fitness, and feed efficiency [8]. The OEO is known for its potent antimicrobial properties, which are primarily attributed to its major bioactive compounds, including carvacrol and thymol. These compounds possess significant biological activities and have been extensively studied for their antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [10].

The previous evidence suggests that OEO has potential as a natural alternative to antibiotics for improving intestinal health, barrier function, and productive performance in the poultry industry. This chapter presents some experiences that we have had, as well as those of other authors, about the uses and applications of the different OEO chemo types for poultry production.

2. Oregano essential oils composition

When working with locally grown OEO, there is a wide range of secondary metabolite compositions that determine its wide variation in the biological activity. In our studies, 54 compounds were identified in the volatile fraction of *Origanum* and *Lippia* genus essential oils. Carvacrol and thymol and their precursors p-cymene and α -terpinene were the majority of phenolic monoterpenoid compounds found. *Origanum vulgare* L. ssp. *Hirtum*, grown in Greece, showed the highest value of carvacrol (90.3%), in contrast to the major volatile compounds found in *O. vulgare* L. Grown under greenhouse conditions was thymol (21.51%). Carvacrol showed the lowest value (4.3%). In contrast, *Lippia origanoides* Kunth, an endemic species of the Patia region [2], presented the highest value of thymol (78.7%). *L. origanoides* is very important for Colombia because it is an endemic species of arid tropical lands [11]. *L. origanoides* is a native species of oregano adapted to arid conditions, dry soil in the Patia region of Colombia's southwest [12]. These conditions allow that *Lippia origanoides* Kunth is a unique species because of its high thymol content (close to 80%), low variation in metabolites, and low percentage of precursor compounds. *Origanum majorana* was another species cultivated under a greenhouse. It was represented by the bicyclic monoterpenoids sabinene and cis- and trans-sabinene hydrates (17.1%), with a high level of precursors α -terpinene and terpinen 4-ol (20.0%), very low thymol (10.0%), and carvacrol (3.7%), with the presence of sesquiterpenoid compounds such as germacrene and bicyclgermacrene, acyclic monoterpenoid b-myrcene, and

sesquiterpenoid trans- β -caryophyllene [2]. In contrast, in other tropical areas, it has been found that carvacrol is the major component, together with α -terpinene, thymol, and p-cymene, in oils from *L. origanoides* leaf hat [13]. In contrast, in our study, carvacrol was present only as a trace constituent (0.9%), but thymol contents ranging from 1.4 to 74.4% are reported [14] due to new chemotypes found. It can be concluded that the crop, the weather, the species, and the seasonal conditions can affect secondary metabolite production [15].

Despite the great variability, European and Greek oreganos have been the most evaluated and processed to be distributed on a commercial scale throughout the world because they have the highest content of carvacrol [13]. However, to tropical ecosystems such as America and Africa ecosystems, where *Lippia* genus native species with high thymol content is found, it might be important. Additionally, carvacrol has been shown to have more negative effects than thymol [16], raising the question of whether a high carvacrol content in the essential oil is a good indicator of its quality.

3. Antimicrobial activity of OEO

The OEO's antimicrobial activity has been attributed to its compounds such as carvacrol and thymol, which are the main constituents responsible for the strong antimicrobial effects of oregano essential oil [2, 6]. They exhibit broad-spectrum activity against various bacteria, fungi, and even some viruses. These compounds disrupt the cell membranes and inhibit the growth and proliferation of microorganisms [10, 17]. The OEO damage disrupts in-membrane integrity, which affects pH homeostasis and equilibrium of inorganic ions [18]. Carvacrol has been shown to alter the electrolyte balance because of decrease in the intracellular potassium, increase in the extracellular potassium, and consequently the bacterial membrane potential [19]. But the effect of OEO goes beyond the bacterial membrane because a transfer of monoterpene to inside of bacteria and the interaction with their components is also suggested [20]. Another mechanism of antibacterial action has been found for the OEO, the inhibition of quorum sensing (QS) in *Escherichia coli* and *Pseudomonas aeruginosa*. The QS is a mechanism that modulates the expression of genes that allow to recognize the bacterial population density through the accumulation of specific signaling molecules to ensure the survival and pathogenesis [21]. The OEO has proven to have a wide spectrum of antibacterial activity and is one of the most inhibitory species. However, there are few references that compare the antimicrobial activity of *Origanum* gender species versus *Lippia origanoides* as well as the antibacterial activity against pathogenic and beneficial bacterial strains to animal health and production.

Our studies compared the minimum bactericidal concentration (MBC) of carvacrol, *O. vulgare* spp., and *L. origanoides*. Carvacrol presented the lowest value of MBC against *E. coli* (0.006 mg/mL) and *Salmonella typhimurium* (0.098 mg/mL), followed by *L. origanoides* against *E. coli*, and *O. vulgare* L. ssp. Hirtum and *O. vulgare* L. against *S. typhimurium*. The highest bactericidal activity against *S. enteritidis* was presented with carvacrol, *O. vulgare* L. ssp. Hirtum (high carvacrol), and *L. origanoides* (high thymol). In contrast, the lowest bactericidal activity was presented against beneficial bacteria with a higher value of MBC when they were compared with pathogenic bacteria. Except to *Bifidobacterium breve*, while carvacrol presented the highest bactericidal activity (1.56 mg/mL), *L. origanoides* had the lowest activity (50 mg/mL). The *O. majorana* essential oil showed the lowest bactericidal activity against pathogenic and beneficial bacteria. The higher bactericidal activity against pathogenic bacteria

was associated with the content of phenolic components carvacrol or thymol with respect to species such as *O. majorana* with a high content of sabinyl compounds. This study found that the OEO had a selective bactericidal effect against pathogenic and beneficial bacteria. The beneficial bacteria *Lactobacillus* and *Bifidobacterium* were less sensitive to OEO than the pathogens *Salmonella* and *E. coli* to both carvacrol and OEO high carvacrol and high thymol. These results give to OEO a potential use to maintain the intestinal eubiosis [12]. Other studies also concluded that the essential oil of *Lippia origanoides* H.B.K. presented antimicrobial action against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* strains [22]. The OEO from *Lippia origanoides* high thymol grown in Brazil also demonstrated antimicrobial activities against the foodborne bacteria *Bacillus cereus*, *B. subtilis*, and *Salmonella typhimurium*, but not against *Pseudomonas aeruginosa* [23]. It can be concluded that *L. Origanoides* presents a potential for the development of new phytotherapeutics.

4. Benefits as an additive in poultry feed

The food-animal industry has used antibiotics in the feed for decades. The use of antibiotics in subtherapeutic doses in animal feed has been questioned due to its possible relationship with resistance in bacteria that has an impact on human and animal health and the antibiotic resistance is one of the top health challenges of this century [24]. In recent years, there has also been a growing interest in antibiotic-free broiler meat production, therefore, it is necessary to generate nonantibiotic alternatives for their use in feed and animal nutrition. Bioactive compounds derived from medicinal plants have been proposed as natural sources of phytochemicals with antibacterial, anti-inflammatory, and antiviral properties, among others, which are ideal for use as feed additives in food animal production [25, 26]. Within these phytochemicals, oregano essential oils have been evaluated and used as additives in poultry feed. Various studies have verified the positive effects of OEOs on feed intake, metabolism, digestive secretions, and growth, among others [4, 27, 28], as well as nonculturable pathogens [29]. Both studies and commercial products with OEO have been based on essential oils from Greek oregano species (*Origanum* spp.) with high carvacrol content. It has been proposed that the effectiveness of these OEOs may be the result of their antimicrobial activity as presented back. Our studies on *Lippia origanoides* Kunth, also commonly named oregano, have shown that AEO rich in thymol can be a viable natural additive to produce chicken meat with a possible anticoccidial effect. We found that the inclusion level of AEO depends on the broiler's health status. This species, native to tropical America, was analyzed by gas chromatography, and 22 compounds were identified in the volatile fraction of the essential oil. We established that thymol was the major secondary metabolite (78.7%) in *L. origanoides* Kunth with a very low level of precursors such as p-cymene (6.6%), α -terpinene (2.7%), and carvacrol (0.9%); however, this type of oregano has been little studied. Our studies included the effect of OEO from *L. Origanoides* Kunth on cecal microbial diversity and composition in broilers challenged with attenuated coccidia oocyte vaccine (2 $\times$) and found that supplementation with 100 ppm of AEO reduced the negative impact of the challenge with attenuated oocysts of coccidia. A significant interaction was identified, because 65 ppm was OEO level that maximized the body weight in non-coccidian-challenged chicken groups, but 147 ppm was required for the coccidian-challenged group. The OEO supplementation to coccidia-challenged broilers

improved the body weight (9.3%) and the feed conversion ratio (FCR) (-6%). It was shown that AEO rich in thymol can be a viable natural additive to produce chicken meat without antibiotic [30].

Average daily gain (ADG) and feed conversion ratio (FCR) were better in broilers fed with OEO than those fed with a control diet during days 1–21 and days 22–42 of age [3]. Although there is no agreement on the inclusion levels, 150 or 300 mg/kg of OEO has proven to be effective in increasing the average daily feed intake and average daily gain [31]. Other studies raised higher levels of 150–750 ppm of OEO in broiler diets and concluded that during the grower period, the OEO-supplemented groups showed a better conversion of feed, with linear and quadratic performance. A linear increase in body weight, body weight gain, the protein efficiency ratio, the relative growth rate, and better conversion was observed at 750 ppm of OEO dietary level compared to the control groups. With the broken-line regression model, it was found that 580 ppm of OEO optimizes the final body weight and the feed conversion [32].

We compared OEO from four chemotypes against antibiotics in broiler diets and, contrarily, *O. majorana* with slow carvacrol, thymol, and antibiotics presented higher values of energy and fat ileal digestibility and a higher body weight. Interestingly, a negative correlation between the body weight and carvacrol intake ($r: -0.55$) and a positive correlation with thymol intake ($r: 0.46$, $p 0.05$) have been reported. These results found a possible negative effect of high carvacrol intake and different responses of broilers as a function of OEO composition [33]. The impact of OEO on digestive efficiency had been more consistent, with a greater amylase enzyme activity, a quadratic elevation in chymotrypsin, and higher chymotrypsin and lipase activity, which would be expressed in a better efficiency in the use of food in broilers, greater average daily gain (ADG) and a lower fructose-to-glucose (F/G) ratio [8], a linear increase in villus height to crypt depth ratio, a quadratic decrease in feed conversion ratio with a lower feed conversion ratio (200 ppm) than the control during weeks 1–12 of the trial, and higher eggshell thickness at the end of weeks 4, 8, and 12 [8]. With a level of 275 ppm of OEO in the diet of laying hens, it improved the body weight, lowered the triglycerides, and raised the high-density lipoprotein levels [34]. The OEOs, or secondary metabolites, have been very widely used in the poultry industry in mixtures with other types of essential oils with beneficial effects on body weight and feed conversion ratio values when evaluated in coccidia-challenged broiler chickens [35, 36]. Coccidia is an important parasite because it generates a high economic impact and welfare and becomes a challenge for the poultry industry. On the other hand, the use of drugs as feed additives is being withdrawn, thus, these OEO mixtures as well as *L. origanoides* essential oils are a potential option as a natural alternative to antibiotics for improving the poultry productive performance. However, more research is needed to fully understand the mechanisms underlying these effects and determine the optimal dosage and duration of OEO supplementation.

5. Effects on gut microbiota

The gut microbiota is considered one of the key elements contributing to the efficiency, welfare, and health of birds. The evaluation of gut microbiota changes in response to the use of feed additives is a key indicator to evaluate the efficiency of natural alternatives to the use of feed antibiotics in poultry production. The latest generation molecular techniques have contributed to characterizing the microbial

communities in a complex environment such as the digestive tract [33, 37, 38] and have also allowed them to transcend to the animal's metabolome. In our studies, a positive correlation was found between body weight and OEO from *L. origanoides* supplementation with the Firmicutes:Bacteroidetes ratio and Firmicutes levels in cecal content. Quite fewer Bacteroidetes bacteria than those of Firmicutes have been found in fat broilers [39], and a similar relationship was found in obese humans experiencing insulin resistance [40]. The addition of 200 ppm of dietary OEO increased the abundances of Burkholderiales, *Actinobacteria*, Bifidobacteriales, Enterococcaceae, and Bacillaceae, whereas it decreased *Shigella* abundance in the ileum [8].

We also explored the effects of OEO from three chemotypes through the use of denaturing gradient gel electrophoresis (DGGE) technology on broiler chickens at high altitude, *Origanum vulgare* L. (OL), *O. vulgare* L. ssp. Hirtum (OH), and *O. majorana* (OM) from a greenhouse of Sabana de Bogotá and *O. vulgare* L. ssp. Hirtum (OG) from Greece. Bacterial community DNA revealed two main clusters: OEO-treated chicks and nontreated control chicks. These results indicate that all treatments with OEO had some effect on the gut microbial communities' changes. A reduction of about 50% in mortality caused by Ascites with *O. Majorana* essential oil and an average of 68% of all additive-supplemented groups compared to controls indicate a possible association of pulmonary hypertension syndrome with the use of OEO and gut microbiota [41].

It is also reported that OEO supplementation increases the abundances of *Ruminococcus*, *Bifidobacterium*, and *Enterococcus* as well as its Spearman's correlation positively correlated with the messenger RNA (mRNA) expression of mucins. Moreover, the relative abundance of *Enterococcus* was positively correlated with amylase activity [42]. The OEO supplementation enriched the genera *Propionibacterium*, *Brevinema*, and *Corynebacterium*, whereas the genus *Vibrio* was enriched in the control with no OEO supplementation in *Cyprinus carpio* [3].

6. The OEO and tight junctions as a barrier between intestinal cells

The intestinal barrier is formed by a layer of epithelial cells with intercellular junction complexes that form a regulated, selectively permeable barrier between luminal contents and the underlying tissue compartments. These include tight junction (TJ) proteins, such as claudins (CLDN), occludins (OCL), and *zonula occludens* (ZO), which form the continuous intercellular barrier between epithelial cells required to regulate the selective permeability across the intestinal epithelium to a barrier function [43, 44]. Although there is limited research about oregano's effects on tight junctions in birds, some studies have investigated the potential benefits of its active compounds on intestinal barrier function. Dietary OEO (150–300 mg/kg) increased the content of secretory immunoglobulin A and the relative expression of Claudin 1, Mucin 2, and Avian β -defensin 1 in yellow-feathered chicken's ileum [31]. The OEO has been shown to improve intestinal morphology and expression of tight junction proteins, which are associated with the regulation of selected intestinal bacteria and immune status in pigs supplemented with 25 mg/kg of OEO for 4 weeks. These results show that OEO promotes intestinal barrier integrity, probably through modulation of intestinal bacteria and immune status in pigs [9]. The OEO supplementation in pig decreased serum endotoxin levels, increased the villus height and expression of the TJ zonula occludens-1 (ZO-1), signaling pathways and expression of inflammatory

cytokines such as mitogen-activated protein kinase (MAPK), protein kinase B (Akt), and nuclear factor kappa B (NF- κ B) in the jejunum. A lower population of *Escherichia coli* in the jejunum, ileum, and colon, and inactivation of inflammation were observed too. The supplementation of OEO in production hens' late phase resulted in a quadratic reduction in the mRNA expression of interleukin 1 (IL-1), tumor necrosis factor alpha (TNF- α), interferon gamma (IFN- γ), and toll-like receptor-4 (TLR-4), but a linear and quadratic increase of ZO-1 expression in the ileum. It was observed that the addition of 200 mg/kg of OEO maximized the results [8]. The effect of OEO on sheep was studied too and it showed that this effect reduces the serum interleukin 2 (IL-2) and TNF- β levels as well as mRNA levels of NF- κ B, p65, toll-like receptor-4 (TLR-4), and interleukin 6 (IL-6) in the jejune mucosa [42]. All these studies show that the integrity of the intestinal barrier was improved by OEO treatment.

As mentioned earlier, carvacrol and thymol, two major components of oregano essential oil, have been studied for their antimicrobial and anti-inflammatory properties. These compounds have been shown to inhibit the growth of certain pathogenic bacteria and reduce inflammation in the gut. By modulating the gut microbiota and reducing inflammation, oregano compounds might indirectly support the integrity of tight junctions. Additionally, oxidative stress has been implicated in the disruption of tight junction proteins, potentially compromising the intestinal barrier. The OEO antioxidant effects could counteract the oxidative stress, thus reducing the oxidative stress, and may help protect tight junction integrity [45]. However, it's important to note that the research on oregano's effects on tight junctions and the intestinal barrier is limited, and more studies are needed to fully understand its mechanisms and potential benefits.

7. The OEO antioxidant properties

The potent antioxidant properties of OEO have also been attributed to these phenolic compounds: carvacrol, thymol, and rosmarinic acid. They scavenge free radicals, neutralize oxidative stress, and protect cells from damage caused by reactive oxygen species (ROS). The OEO supplementation in broiler chickens improved the antioxidant indices in serum, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glutathione reductase (GR) on day 21 and the activity of total antioxidant capacity (T-AOC) of birds on day 42 [3]. Similar results were observed in jejunum, decreasing serum oxidative stress parameters, increasing the activity of T-AOC, and decreasing of the level of malondialdehyde (MDA) in serum and jejunum. The oregano leaf-flower oils were shown by the 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay that they had the strongest antioxidant activities, while the stem oils had the weakest, and although the essential oils of each part showed strong antioxidant activities, their antioxidant capacities were much lower than those of the synthetic antioxidant butylated hydroxytoluene (BHT) [7]. In contrast, oregano essential oils from *Lippia origanoides* Kunt and butylated hydroxytoluene (BHT) had similar antioxidant capacities, which can be attributed to the synergistic action of OEO phenolic compounds [46]. Even more, it is reported that the radical scavenging activity of the essential oil from *Lippia origanoides* was higher than that of BHT but similar to that of α -tocopherol [45]. And *Lippia berlandieri* Schauer demonstrated the preservation of ground beef quality to be similar to that of BHT [47].

8. Anti-inflammatory properties

The anti-inflammatory effect of OEO has been verified; as mentioned before, the OEOs inhibit the production of pro-inflammatory cytokines and enzymes, thereby reducing inflammation in various tissues and organs, which has been demonstrated in pigs [9]. The OEO downregulates the expression of TNF- α , interleukin 1 β (IL-1 β), interleukin 6 (IL-6), IFN- γ , and monocyte chemoattractant protein-1 (MCP-1) and inhibited the activation of c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase 1/2 (ERK1/2), and protein kinase B (Akt) in pig jejunum. The greater inactivation of inflammation was reflected by the mitogen-activated protein kinase (MAPK), protein kinase B (Akt), and nuclear factor kappa B (NF- κ B) signaling pathways and expression of inflammatory cytokines in the jejunum; downregulated the relative expression of tumor necrosis factor α (TNF- α) and transforming growth factor β (TGF- β) as well as a significant increase in nitric oxide, viability, and differentiation into neutrophil-like cells.

9. Other applications

Carvacrol and thymol have demonstrated cytotoxic effects against various cancer cell lines, including breast, colon, and prostate cancer cells. This activity has been explained by the management of inflammatory conditions of OEO. The OEO metabolites induce apoptosis (programmed cell death), inhibit tumor growth, and show promise as adjuvants in cancer treatment. Furthermore, it has been suggested that OEO oil metabolites, particularly carvacrol, may have antidiabetic effects. They help regulate blood sugar levels, improve insulin sensitivity, and inhibit certain enzymes involved in carbohydrate metabolism [10]. However, further research is needed to establish their clinical significance in diabetes management.

Other applications in ethnopharmacological studies have been found to suggest *L. organoides* for disorders of the genitourinary system used specially by quilombola women from Oriximiná. These applications have also been found to demonstrate an antispasmodic action, analgesic and antimicrobial uses of other species of *Lippia* genus rich in thymol and carvacrol [48]. In addition, oregano has been found to modulate innate immunity parameters in *Lumbricus terrestris* [21]. It has also been shown to improve immune response, activity of digestive enzymes, and intestinal microbiota of the koi carp, *Cyprinus carpio* [49].

10. Conclusions

The European oreganos are *Origanum* spp. have been considered the best ones because they have a higher content of carvacrol; however, in tropical America and Africa ecosystems, chemotypes with a high thymol content, such as *Lippia Organoides* spp., it has been shown that they have functional effects on poultry health and production.

Oregano essential oils have many benefits for poultry, including strengthening the immune system, inhibiting pathogenic bacterial growth, improving intestinal integrity, improving egg quality, and promoting growth performance. It is important to note that the effectiveness of oregano may vary depending on the dosage and form of administration.

Our studies found that *Lippia origanoides* Kunth is a promising species due to its high thymol content (close to 80%), low variation in active compounds, and low percentage of precursors. It also demonstrated strong antimicrobial activity against pathogenic bacteria and the lowest antimicrobial activity against beneficial bacteria. Additionally, the OEO supplementation of coccidia-challenged broilers improved their productive performance, showing that *L. Origanoides* EO high thymol can be a viable natural additive to produce chicken meat.

Consequently, OEO has potential as a natural alternative to antibiotics for improving intestinal health and barrier function in poultry. However, more research is needed to fully understand the mechanisms underlying these effects and determine the optimal dosage and duration of OEO supplementation.

In human health, it is necessary to investigate other applications of the OEO for anticancer, anti-inflammatory, and antidiabetic properties.

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Conflict of interest

The author declares no conflict of interest.

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