Antibacterial and antioxidant activity of oregano essential oil

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Abstract
The swine industry is investigating phytonutrients like oregano essential oil (OEO) because of its potent antimicrobial and antioxidant activity. These activities are attributed to OEO's most abundant polyphenols, carvacrol and thymol. Carvacrol and thymol have been shown to permeabilize and depolarize the bacterial cytoplasmic membrane, resulting in cell death. The objective of this study was to quantify the antimicrobial and antioxidant activity of OEO. Antibacterial activity was determined by testing for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of OEO for common livestock pathogens. A standardized microtiter protocol was used. Several bacteria were tested including Salmonella enteritidis, S. typhimurium, S. choleraesuis, Escherichia coli, Klebsiella pnemoniae, Streptococcus suis, and Staphylococcus aureus. Results showed that MICs for both gram-positive and gram-negative bacteria ranged from 1.25 to 10.0 μg/ml. MBCs were identical to the MIC showing bactericidal activity. Antioxidant activity of OEO and vitamin E (positive control) was determined by the oxygen radical absorption capacity (ORAC) value against five oxygen radicals: peroxyl radical, hydroxyl radical, peroxynitrite, superoxide anion, and singlet oxygen. Antioxidant testing showed that OEO had much higher level of total antioxidant activity (2,520,600 trolox equivalents/100g) than natural vitamin E (39,200). These results demonstrate that OEO has high antimicrobial activity for pathogens that cause swine disease. The very high level of antioxidant activity of OEO may protect enterocytes against inflammatory damage caused by reactive oxygen molecules that are released during immune system activation. OEO has several benefits for the swine industry: it is a safe and accepted feed ingredient, it has potent activity against gram-negative and gram-positive bacteria, and it does not leave residues in the environment. Synergistic activity has been demonstrated between OEO and common antibiotics. OEO when used alone or in combination with antibiotics will allow the producer to reduce antibiotic use.

Introduction
Oregano essential oil (OEO) is well known for its antimicrobial properties, as well as its antifungal and antioxidant actions. When harvested at the proper growth stage and steam extracted, oregano essential oil is a mixture of >30 different compounds. The major constituents, carvacrol (55-85%) and thymol (0-5-10%), have the most potent antimicrobial activity due to their phenolic structure. Mechanism studies have shown that carvacrol and thymol kill bacterial cells by altering the permeability of the cell membrane causing leakage of essential cations (1). Selectivity against Gram-negative bacteria but with lesser activity against Gram-positive Lactobacillus and Bifidobacterium has been observed (2). Antimicrobial activity of OEO has been demonstrated in different ways. Activity varies depending on the assay method, the source of oregano essential oil, and bacterial isolates tested.

Antioxidants are compounds that prevent damage to cells and tissues in the body. This is particularly important in the gut, which is continuously exposed to dietary and environmental challenges that can cause tissue damage. The gut is the first line of defense against enteric pathogens. When bacteria or viruses invade gut cells, the body responds with inflammation. Inflammation produces "reactive oxygen intermediates" or ROI's, which destroy disease-producing organisms. However, these ROI's are quite toxic and often cause unintended tissue damage. Antioxidants neutralize ROI's and other molecules that damage host tissues.

Oxygen radical absorbance capacity (ORAC) is a method of measuring antioxidant capacities in biological samples. A wide variety of foods have been tested using this methodology, with certain spices, berries and legumes rated very highly. Correlation between the high antioxidant capacity of fruits and vegetables, and the positive impact of diets high in fruits and vegetables, is believed to play an important role in defense against many health conditions.

The objective of this study was to determine MIC and MBC of OEO for several swine and livestock pathogens using a standardized microtiter protocol. In addition, the antioxidant activity of OEO was determined by ORAC value and compared with vitamin E, a well known antioxidant which is routinely added to livestock feeds and topical products.

Materials and Methods

MIC and MBC were performed for a wide range of livestock and poultry pathogens at the University of Minnesota Udder Health Laboratory using a microtiter assay method following Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines (3).

OEO (Ralco Nutrition, Inc., Marshall, MN) was prepared as a stock solution and serially diluted by two-fold dilutions in Mueller Hinton broth from 20 µg/ml to 0.039 µg/ml. Positive (no oregano oil) and negative (no bacteria) controls were included in each microtiter plate. Pure cultures of each bacterium were prepared on agar plates. A standard bacterial suspension was prepared (i.e. 0.5 McFarland standard), bacteria were added to microtiter plates, and plates were sealed and incubated at 37°C. Each microtiter plate was run in duplicate. After 18h, wells were scored for bacterial growth (i.e. turbidity). The MIC was the lowest concentration of OEO showing no bacterial growth. After scoring for growth, one loopful of broth from each clear well was streaked onto a blood agar plate. Plates were incubated at 37°C and examined for bacterial growth after 18h. The MBC was determined as the lowest oregano essential oil dilution showing no growth on plates.

Antioxidant activity of OEO and vitamin E, a well known antioxidant, was assessed using the oxygen radical absorptive capacity (ORAC). ORAC values are expressed as trolox equivalents (TE). Trolox is a vitamin E analog (Hoffman LaRoche) with high antioxidant activity. ORAC values are given as micromoles TE/g or micromoles TE/100 g. Anti-oxidant activity was measured against five (5) oxygen radicals: (1) peroxyl radical; (2) hydroxyl radical; (3) peroxynitrite; (4) superoxide anion; and (5) singlet oxygen. Results are given as a sum of the activity against these five ROI's (total ORAC).

Results

MIC and MBC

Table 1 shows that MIC for all pathogens ranged from 1.25-10.0 µg/ml. MBC ranged from 1.25-10 µg/ml, confirming prior reports that OEO is bactericidal for the bacteria tested. The killing activity of OEO has been attributed to the action of carvacrol on the bacterial membrane (1).

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of oregano essential oil for common livestock pathogens.

Bacteria	Source	MIC (µg/ml)	MBC (µg/ml)
E. coli K88	Swine	2.5	2.5
E. coli F88	Swine	2.5	2.5
E. coli O157:H7	Cattle	2.5	2.5
E. coli	Mastitis (cow)	2.5	2.5
E. coli	Mastitis (cow)	5	5
Klebsiella pneumoniae	Mastitis (cow)	5	5
Klebsiella pneumoniae	Mastitis (cow)	2.5	2.5
Streptococcus suis	Swine	5	5
Listeria monocytogenes	Cow	5	5
Salmonella typhimurium	Swine	5	5
Salmonella choleraesuis	Swine	5	5
Salmonella enteritidis	Poultry	5	5
Staphylococcus aureus	Mastitis (cow)	5	5
Streptococcus agalactiae	Mastitis (cow)	5	5
Streptococcus uberis	Mastitis (cow)	5	5
Pseudomonas aeruginosa	Human	10	10
Bacillus cereus	Environment	1.25	1.25

Total ORAC values for Ralco's OEO oil and common high antioxidant foods are given in Table 2. Results are given as ORAC value per 100 grams. Results showed that OEO has the highest ORAC value of the tested ingredients as well as one of the highest ORAC values in the databases listed.

Table 2. Antioxidant activity for oregano essential oil, vitamin E and other foods with known antioxidant activity.

Substance	Total ORAC (µmoles TE/100 g)
Red grapes – raw[a]	1,206
Raspberries - raw[a]	4,882
Red wine[a]	5,034
Cranberries – raw[a]	9,584
Dark chocolate candy[a]	20,823
Natural vitamin E	39,200
BHT[b]	72,000
Cinnamon[a]	267,536
Oregano essential oil	2,520,600

[a] ORAC values were obtained from one of these websites: http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/ORAC/ORAC07.pdf
[b] http://www.nutritiondata.com

Discussion

In this study, we used a validated microtiter assay to investigate the antibacterial activity of OEO. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for several swine and other livestock pathogens, including Escherichia coli, Salmonella enteritidis, S. typhimurium, Klebsiella pnemoniae, and Staphylococcus aureus. Results showed that MIC and MBC ranged from 1.25-10.0 µg/ml, with the MBC equal to the MIC in all cases demonstrating bactericidal activity. Similar bactericidal action of OEO for Clostridium perfringens and Candida albicans has been shown previously.

Several of the bacteria in this study are zoonotic pathogens (Salmonella spp., E. coli O157:H7, L. monocytogenes) or have zoonotic potential (4). S. aureus is a difficult pathogen to eliminate, whether it causes mastitis in dairy cows or skin, bone or systemic infections in humans. Staphylococci sequester in fibrin-like clots, making elimination by antibiotics difficult. Methicillin-resistant S. aureus (MRSA) has been in the popular press recently because of its increasing spread in humans outside of the hospital setting and possible association with swine, poultry and other livestock. Infections caused by MRSA are difficult to cure because of its resistance to oral antibiotics. Results from this studies and others (5) show that S. aureus is effectively killed by OEO at concentrations similar to other pathogens.

These results demonstrate that OEO has high antimicrobial activity for pathogens that cause swine disease. The very high level of antioxidant activity of OEO may protect enterocytes against inflammatory damage caused by reactive oxygen molecules that are released during immune system activation. OEO has several benefits for the swine industry: it is a safe and accepted feed ingredient, it has potent activity against gram-negative and gram-positive bacteria, and it does not leave residues in the environment. Synergistic activity has been demonstrated between OEO and common antibiotics. OEO when used alone or in combination with antibiotics will allow the producer to reduce antibiotic use while maintaining protective gut health.

References

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