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Effect of Oregano Essential Oil on Chicken Lactobacilli and *E. coli*

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ABSTRACT. The effect of the oregano essential oil (EO) on lactobacilli and *E. coli* isolated from chicken was showed in the series of *in vitro* and *in vivo* experiments. Minimum inhibitory concentrations of amikacin, apramycin, streptomycin, neomycin against *E. coli* strains increased after oral application of EO.

Some plant essential oils exhibit antimicrobial properties (*see*, *e.g.*, Ferme *et al.* 2004). Recent studies have shown that essential oil of oregano (*Origanum vulgare*) is among the most active against *E. coli* (Hammer *et al.* 1999; Dorman and Deans 2000). The correlation between intensive use of antimicrobials agents and development of resistant bacteria is well documented for pathogenic bacteria but there is less information about commensal (endogenous) bacterial flora (Sáenz *et al*. 2001).

The objective of this study was to determine and compare minimum inhibitory concentration (MIC) values in *E. coli* isolates recovered from fecal samples of chicken fed with oregano, and bactericidal effect of oregano (as antibiotic alternative) against chicken lactobacilli.

MATERIALS AND METHODS

In vivo *trial*. Two group trials (100 in one group) were run with the chicken hybrid Coob 500 American type broilers, on *Zamostie Poultry Farm* (Slovakia), technology Big dutchmen; trial lasted 36 d. Antibiotic-free diet was fortified with oregano (*Calendula*; Slovakia) essential oil (EO). Chemical analysis of this oil (provided by *Calendula*) showed as the main constituent carvacrol 55 ± 3 %; further physico-chemical characteristics were: specific gravity of 0.959 ± 0.002 g/mL), optical slewability 0 ± 1°, refractive index 1.515 ± 0.001. A feed mixture contained 2.5–6.25 mL EO per 5 kg of diet. Fecal samples were collected at the end of trial from both trial groups and *E. coli* strains were isolated.

MIC values. The *MidiTech* colorimetric MIC method (*NCCLS* 1999) was used for routine susceptibility (ampicillin, ampicillin–sulbactam, ceftriaxon, gentamicin, amikacin, apramycin, streptomycin, neomycin, nalidixic acid, ciprofloxacin, enrofloxacin, florfenicol, tetracycline, trimethoprim, sulfometoxazol) testing of isolated *E. coli* strains (Gattringer *et al.* 2002).

The *in vitro* bactericidal effect against five lactobacilli strains were isolated from chicken feces was determined by disc diffusion assay according to Burt and Reinders (2003) with modification. A 16-h lactobacilli culture was diluted in 0.85 % (*W*/*V*) sodium chloride with reference to the McFarland standard (*bioMérieux*, France); 0.1 mL inoculum (corresponding to $\approx 10^5$ CFU) was placed on pre-dried MRS agar plates. Sterile paper disc (diameter 6 mm; *Albet*, Spain) was placed on the plate and 5 or 10 µL of EO were added. The plates were incubated for 2 d in atmosphere of 5 % CO_2 at 37 °C.

MIC (the lowest concentration of EO inhibiting the visible growth of each organism on the agar plate) of EO was determined by broth dilution method (Hammer *et al.* 1999) with modification. Serial dilution of EO (0.001–1 %, *V*/*V*) was prepared in MRS agar; inoculated ($\approx 10^6$ CFU/mL lactobacilli) plates were incubated for 2 d at 37 °C.

Statistical analysis of the MIC values was carried out by one-way analysis of variance of transformed data (binary logarithm of original data).

RESULTS AND DISCUSSION

Oregano EO exhibited a strong bactericidal effect against lactobacilli at both tested doses (Table I). Comparable antimicrobial activity of oregano and thyme aromatic oils against *E. coli* O157:H7 (with ≈3× higher EO concentration) in the agar-diffusion paper-disc test was described by Burt and Reinders (2003).

Table I. The bactericidal effect (inhibition zone in mm; mean ± SD)[a] of essential oil (EO; 5 and 10 μL) from *Origanum vulgare* against four lactobacilli strains

EO	3/1	3/3	2/4	2/5
5	28.7 ± 0.58	25.3 ± 3.1	27.3 ± 3.1	25.7 ± 4.0
10	33.0 ± 1.41	36.5 ± 3.5	37.5 ± 3.5	32.5 ± 3.5

[a]Including the diameter (6 mm) of paper disc.

Table II. Minimum inhibitory concentrations of *Origanum vulgare* essential oil (MIC; %, *V*/*V*) against some chicken lactobacilli

Strain	MIC
1/3	0.075
3/1	0.050
3/3	0.075
2/4	0.075
2/5	0.075

Table III. Changes of antibiotic MIC against *E. coli* strains after feeding with oregano essential oil

Antibiotic[a]	Trial[b]	MIC[c]
Ami	EO	1.50 ± 0.55*
	C	0.67 ± 0.20
Apr	EO	2.83 ± 0.41***
	C	1.67 ± 0.52
Stm	EO	4.17 ± 2.40*
	C	1.50 ± 1.76
Neo	EO	1.33 ± 1.03
	C	0.83 ± 0.75

[a]Ami – amikacin, Apr – apramycin, Stm – streptomycin, Neo – neomycin.
[b]EO – essential oil, C – control.
[c]Geometrical mean ± SD; significant difference between EO and C at $*p < 0.05$, $***p < 0.001$.

Minimum inhibitory concentration of EO against lactobacilli strains was 0.05 % (Table II). Similar activity but for EO concentration >0.05 % was found by Skandamis *et al.* (2001) against pathogenic *E. coli* strains.

EO after oral administration significantly increased MIC values of amikacin, apramycin, streptomycin and neomycin of *E. coli* chicken isolates (Table III). We suppose that the strong bactericidal effect of EO can present a big selective pressure on gut microflora similar to antibiotic feeding, resulting in increased MIC values. Our results are in contrast to similar study of Docic and Bilkei (2003) who found in pigs that oregano feeding caused decrease in MIC values against *E. coli* of ampicillin, enrofloxacin, gentamicin and doxycyclin. (The only difference in our and their experimental design was that we preferred the use of the geometric mean for statistical analysis of MIC; *cf.* Gattringer 2002 and Babák 2004.)

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