

**Beneficial effect of plant extracts in rabbit husbandry**

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**Abstract**

The present study evaluates the effect of plant extracts of oregano and commercial Xtract™ administrations on microbial, biochemical, immunological and nutritional indicators and on *Eimeria* sp. oocyst occurrence in rabbits. Rabbits (5 weeks old, Hy-plus hybrid, n = 66) were divided into experimental group 1 (E1) with oregano extract application, experimental group 2 (E2) with Xtract™ application and control group. Natural substances were administered for the first 21 days. The experiment lasted for 42 days. The antibacterial effect was determined by the decrease of coagulase-positive staphylococci in E1 compared to control at day 42. *Staphylococcus aureus* cells were detected in lower counts in E2 compared to control at day 21. The counts of *Clostridium*-like bacteria were lower in both experimental groups at day 21 compared to day 7 (difference 1.2 and 1.3 log cycles, respectively) and to control (difference 0.5 and 0.3 log cycles, respectively). At day 7, the counts of coliforms in E1 were significantly lower than in E2 ( $P < 0.01$ ). In rabbits fed with oregano, reduction of *Eimeria* sp. oocysts and higher value of phagocytic activity ( $21.6 \pm 0.51$  %) were found compared to Xtract™, and prolonged immunostimulatory effect was noted. In the animals of both experimental groups higher final weight, feed conversion ratio and lower mortality were achieved compared to control. The administration of oregano showed antibacterial, anticoccidial, and immunomodulatory effects. The results showed that oregano administration may be used as an alternative prophylactic measure in rabbits.

*Origanum vulgare*, antimicrobial activity, *Eimeria* sp., animal, immunity

Plant extracts have been used for a wide variety of purposes for millennia (Jones 1996). The antimicrobial activity of plant oils and extracts has formed the basis for many administrations, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans 1997). Oregano (*Origanum vulgare*) is an important member of the *Lamiaceae* (*Labiatae*) family that comprises about 900 species, over 75% of which are concentrated in the East Mediterranean subregion. The major essential oil constituents of oregano are carvacrol, thymol,  $\gamma$ -terpinene and *p*-cymene, range between 80.2% and 98.4% of total essential oils, with carvacrol yielding as much as 95% in oregano samples (*O. vulgare* subsp. *Hirtum*; Goliaris et al. 2002). The essential extracts derived from oregano are known to possess antimicrobial (Lambert et al. 2001) and antioxidant (Sarac et al. 2009) activities. Oregano has been examined as an alternative growth promoter in broiler chickens (Lewis et al. 2003), broiler turkeys and pigs (Marcin et al. 2006a). Enteric diseases frequently occur in rabbits around the weaning period, leading to an extensive use of antibiotics. Therefore, new and safe antimicrobial agents are searched for to prevent and/or overcome infections.

Based on our previous *in vitro* results (Szabóová et al. 2008), it seems that the use of phyto-additives and their extracts in rabbit husbandry offers an acceptable way to improve welfare and health. From this point of view, the objective of our study was to test the effect of oregano plant extract in rabbits as well as to test the effect of commercially used phyto-additive mixture Xtract™ (involving carvacrol, capsicum oleoresin, cinnamaldehyd). Our

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model study was focused on the microbiota in the intestinal tract of rabbits; the impact on nutritional, immunological and biochemical blood indicators; occurrence of *Eimeria* sp. oocysts and oxidative stress.

### Material and Methods

#### Experimental design

Sixty-six rabbits (35 days old, male sex, Hy-plus hybrid) were divided into 2 experimental (E1, E2) and 1 control group (C) of 22 rabbits each. Rabbits were kept in standard cages, two animals per cage. The experiment was performed on the farm of the Institute of Nutrition, Animal Production Research Centre, Nitra, Slovak Republic. All care and experimental procedures involving animals followed the European Commission guidelines (EEC Directive-86/609) with approval of the State Veterinary and Food Administration of the Slovak Republic. All animals were fed the commercial diet for growing rabbits (AnproFeed, VKZ Bučany, Slovakia; Table 1). The animals in group E1 received the oregano plant extract containing carvacrol  $55 \pm 3\%$  (gas chromatography analysis; density:  $0.959 \pm 0.002$  g/cm<sup>3</sup>; refractive index:  $1.515 \pm 0.001$ ; Calendula a.s., Nová Lubovňa, Slovakia) at a dose of 10 µl for animal/day in water. The animals had *ad libitum* access to water during the whole experiment. The dosage of plant extract was based on our previous *in vitro* tests (Szabóová et al. 2008). The feed for rabbits in group E2 was supplemented with commonly fed Xtract™ of 15 g/100 kg feed (Pancosma, Switzerland), a mixture of plant extracts consisting of 5.4% (wt/wt) carvacrol (C<sub>10</sub>H<sub>14</sub>O; from oregano *Origanum* spp.), 3.2% (wt/wt) cinnamaldehyde (C<sub>9</sub>H<sub>8</sub>O, from cinnamon *Cinnamomum* spp.) and 2.2% (wt/wt) capsicum oleoresin (C<sub>18</sub>H<sub>27</sub>NO<sub>3</sub> from Mexican pepper *Capsicum annuum*). Oregano extract and Xtract™ were administered from the beginning of the experiment (day 0, age of rabbits 35 days) in the duration of 21 days (day 21). The experiment lasted for 42 days.

Table 1. Ingredients, chemical composition and nutritive value of diets used for rabbits

Ingredients (%)		Composition (g/kg)	
Clover (grass) meal	27.00	Crude protein	197.00
Extracted sugar beet pulp	10.00	Crude fibre	166.50
Barley	15.00	Crude fat	39.00
Oats	13.00	Ash	80.00
Wheat bran	6.00	Organic matter	921.00
Soybean meal	7.50	Starch	178.00
Sunflower meal	14.00	Lysine	7.50
Monocalcium phosphate	0.60	Methionine+cysteine	6.50
Dicalcium carbonate	0.90	Cholinchloride	0.80
Salt	0.30	ME (MJ/kg)	10.0
Carob-breadfruit of <i>Ceratonia siliqua</i>	2.50		
DL-Methionine+wheat meal	0.10+0.10		
Mineral and vitamin premix <sup>1</sup>	3.00		

<sup>1</sup>Premix (per kg diet): vitamin A (10.000 IU); vitamin D3 (2.000 IU); vitamin E acetate (30 mg); vitamin B2 (5 mg); vitamin B6 (2 mg); vitamin B12 (8 mg); Ca (9.25 g); P (6.2 g); Na (1.6 g); Mg (1.0 g); K (10.8 g); Fe (327.5 mg); Mn (80 mg); Zn (0.7 mg); ME – metabolisable energy

#### Bacterial monitoring

Faeces for bacterial monitoring were sampled at the beginning of the experiment at day 7 (1 week of oregano and Xtract™ administration), day 21 (3 weeks of oregano and Xtract™ administration) and day 42 (3 weeks after discontinuation of oregano and Xtract™ administrations). The samples were treated by standard microbiological method (International Organization for Standardization-ISO) using appropriate dilutions in Ringer solution (pH 7.0, Oxoid Ltd., Basingstoke, Hampshire, England). The appropriate dilutions were plated onto M-Enterococcus agar (Becton & Dickinson, Cockeysville, USA) to detect enterococci. Lactic acid bacteria (LAB) were counted using de Man-Rogose-Sharp agar. Baird-Parker agar supplemented with egg yolk tellurite solution (Becton & Dickinson) was used to enumerate coagulase-positive staphylococci (CoPS, including *Staphylococcus aureus*). Mannitol Salt Agar (Difco Laboratories, Detroit, USA) was used for coagulase-negative staphylococci (CoNS). *Clostridium difficile* agar with selective supplement (SR0096E) and 7% (v/v) defibrinated horse blood (SR0050, Oxoid Ltd., Basingstoke, Hampshire, England) was used to count *Clostridium*-like bacteria; to enumerate coliforms MacConkey agar (Becton & Dickinson) was used. The plates were incubated at 30 °C and/or 37 °C

Table 2. Bacteria counts in faeces of rabbits expressed in cfu/g (mean  $\pm$  standard deviation)

	Day 0*		Day 7		Day 21		Day 42		
	E1	E2	C	E1	E2	C	E1	E2	
Enter	3.3 $\pm$ 1.2	4.1 $\pm$ 0.8	3.6 $\pm$ 0.7	2.5 $\pm$ 0.9	3.0 $\pm$ 0.5	3.1 $\pm$ 0.6	2.9 $\pm$ 0.5	4.3 $\pm$ 0.8	3.6 $\pm$ 0.3
CNS	3.5 $\pm$ 0.3	3.3 $\pm$ 0.5	3.7 $\pm$ 0.5	2.5 $\pm$ 0.2	3.4 $\pm$ 0.3	2.3 $\pm$ 0.7	3.2 $\pm$ 1.0	2.7 $\pm$ 0.4	3.0 $\pm$ 0.5
CPS	1.6 $\pm$ 0.5	3.5 $\pm$ 0.7	3.7 $\pm$ 0.5	2.1 $\pm$ 0.9	3.0 $\pm$ 0.6	2.6 $\pm$ 1.0	1.8 $\pm$ 1.0	3.0 $\pm$ 0.9	3.0 $\pm$ 1.5
<i>S. aureus</i>	< 1.0	1.5 $\pm$ 0.8	1.6 $\pm$ 1.0	< 1.0	1.4 $\pm$ 0.5	2.1 $\pm$ 1.4	< 1.0	< 1.0	< 1.0
LAB	2.6 $\pm$ 1.1	4.1 $\pm$ 0.8	4.1 $\pm$ 0.8	3.0 $\pm$ 0.6	3.5 $\pm$ 0.4	3.6 $\pm$ 0.7	1.7 $\pm$ 0.8	3.1 $\pm$ 1.2	3.5 $\pm$ 0.3
CB	1.3 $\pm$ 0.4	2.8 $\pm$ 0.8	2.5 $\pm$ 0.5	1.6 $\pm$ 0.9	1.8 $\pm$ 0.9	2.1 $\pm$ 0.9	1.8 $\pm$ 0.8	2.9 $\pm$ 0.6	2.9 $\pm$ 0.4
Coliforms	1.6 $\pm$ 1.0	1.8 $\pm$ 1.0 <sup>a</sup>	< 1.0	1.6 $\pm$ 0.9	1.8 $\pm$ 0.9	2.1 $\pm$ 0.9	1.8 $\pm$ 0.8	2.9 $\pm$ 0.6	2.9 $\pm$ 0.4

E1- experimental group of rabbits with oregano administration; E2- rabbits with Xtract<sup>TM</sup>; C- control rabbits; <sup>ab</sup>*P* < 0.01; Day 0 - mixed sample, Day 7 - one week of extracts administration, Day 21 - three weeks of extracts administration, Day 42 - three weeks after stopping the administration of extracts; Enter - enterococci, CNS - coagulase-negative staphylococci, CPS - coagulase-positive staphylococci, *S. aureus* - *Staphylococcus aureus*; LAB - lactic acid bacteria, CB - *Clostridium*-like bacteria; <sup>a</sup>mixed sample

for 24–48 h depending on the bacterial genera and the counts of bacteria isolated from faeces were expressed as log<sub>10</sub> of colony forming units (CFU) per gram  $\pm$  SD.

Selected animals in each group were killed at days 21 (3 weeks of oregano and Xtract<sup>TM</sup> administration) and 42 (3 weeks after stopping of oregano and Xtract<sup>TM</sup> administration) by cutting the jugular vein and the carotid artery after electroanaesthesia (90 V for 5 s) and caecal contents were collected to count bacteria. The samples of caecal contents were treated and bacteria expressed as described.

Biochemical and immunological indicators, lactic acid and volatile fatty acids measurement, nutritional variables

Biochemical and immunological indicators were examined at the beginning of the experiment (day 0), at days 21 and 42. Serum concentrations of total proteins (g/l, TP 245) and total lipids (g/l, TL 100), cholesterol (mmol/l, CH 200), glucose (mmol/l, GL 2623), calcium (mmol/l, CA 590) were measured using commercial kits Randox (Randox Laboratories Ltd., UK) and the level of glutathione peroxidase (GPx, U/gHb, RS 504) was determined using a Ransel standard set (Randox Laboratories Ltd., UK).

The phagocytic activity (PA) was assessed by direct counting procedure using microspheric hydrophilic particles (MSHP). Ingestion of MSHP by polymorphonuclear cells (PMN) was determined using a modified test described by Vetvicka et al. (1982). Blood smears were prepared and stained in accordance with May-Grünwald and Giemsa-Romanowski (Hrubiško 1981).

Lactic acid (g/100g) and volatile fatty acid values (acetic, propionic and butyric acids in mmol/l) were determined using gas chromatography (glass column with inner standard: isocaproic acid- column SP 1200 H<sub>3</sub>PO<sub>4</sub>; for lactic acid column contained 10–15% dimethylstearamid) from the samples of caecal content at days 21 and 42.

Nutritional variables such as weight (g), weight gain (g), feed conversion (g/g), feed intake (g/day) and mortality (n) were checked daily.

#### *Eimeria* sp. oocysts detection

*Eimeria* sp. oocysts were enumerated in the faecal samples microscopically at the start of the experiment (day 0–1), at days 7, 21 and 42 of the experiment and expressed in counts of oocysts per 1 g of faeces (OPG). The samples were evaluated by the quantitative flotation technique - modified McMaster method (Ministry of Agriculture, Fisheries and Food, Manual of veterinary parasitological laboratory techniques, 1986).

#### Statistical analysis

The results are quoted as mean  $\pm$  SD (standard deviation), statistical evaluation of the results was performed by the one-way ANOVA post-hoc Tukey test (*P* < 0.05, 0.01).

## Results

The counts of CoNS in E1 were slightly decreased compared to E2 at days 21 and 42. The counts of CoPS were also lower in E1 compared to E2 and control at day 42. Moreover, in experimental groups, the reduction of *Clostridium*-like bacteria were found at day 21

Table 3. Biochemical indicators (mean  $\pm$  standard deviation) comparing oregano and Xtract™ administration in experimental groups and control group of rabbits

	Total proteins (g/l)			Cholesterol (mmol/l)			Glucose (mmol/l)			Calcium (mmol/l)			Total lipids (g/l)			ALT ( $\mu$ kat/l)		
	S	A	B	S	A	B	S	A	B	S	A	B	S	A	B	S	A	B
E1	52.8 $\pm$ 6.6	64.9 $\pm$ 5.5	65.5 $\pm$ 8.8	3.7 $\pm$ 2.7	1.8 $\pm$ 0.2	0.7 $\pm$ 0.3	6.8 $\pm$ 1.1*	5.1 $\pm$ 0.6	6.2 $\pm$ 0.8	3.0 $\pm$ 0.7	3.3 $\pm$ 0.4	3.1 $\pm$ 0.6	6.7 $\pm$ 2.1*	3.6 $\pm$ 1.2	2.9 $\pm$ 0.4	0.4 $\pm$ 0.1*	0.12 $\pm$ 0.05	0.17 $\pm$ 0.06
E2	52.8 $\pm$ 6.6	66.4 $\pm$ 6.2	65.8 $\pm$ 6.1	3.7 $\pm$ 2.7	2.2 $\pm$ 0.5	1.21 $\pm$ 0.4	6.8 $\pm$ 1.1*	6.2 $\pm$ 1.2	7.2 $\pm$ 0.5	3.0 $\pm$ 0.7	3.2 $\pm$ 0.1	3.2 $\pm$ 0.1	6.7 $\pm$ 2.1*	5.5 $\pm$ 2.9	3.6 $\pm$ 1.3	0.4 $\pm$ 0.1*	0.09 $\pm$ 0.03	0.09 $\pm$ 0.06
C	52.8 $\pm$ 6.6	58.2 $\pm$ 9.8	66.1 $\pm$ 4.6	3.7 $\pm$ 2.7	1.9 $\pm$ 0.3	1.1 $\pm$ 0.4	6.8 $\pm$ 1.1*	6.3 $\pm$ 0.9	5.9 $\pm$ 0.4	3.0 $\pm$ 0.7	3.2 $\pm$ 0.1	2.8 $\pm$ 0.3	6.7 $\pm$ 2.1*	3.3 $\pm$ 1.2	3.3 $\pm$ 0.9	0.4 $\pm$ 0.1*	0.14 $\pm$ 0.12	0.15 $\pm$ 0.05

E1- experimental group of rabbits with oregano administration; E2 - Xtract™ experimental group 2; C - control group; S - the start of the experiment; A - after 21 days of additive; B - after 21 days of additive withdrawal; \*mixed sample; ALT - alaninaminotransferase

(in E1 and E2) as well as at day 42 (in E1) compared to control. Coliforms in E1 were significantly lower than in E2 group ( $P < 0.01$ , Table 2) at day 7. The bacterial counts in caecum were lower (from 2 to 3 log cycle) compared to those in faeces and no significant changes in bacteria were noted.

Biochemical indicators were not influenced by administration of additives (Table 3). In caecum of rabbits in both groups E1 and E2, higher values of acetic, propionic and butyric acids were noted at days 21 and 42 (Table 4); moreover, a significant increase ( $P < 0.01$ ) of the butyric acid value was observed in E2 compared to control.

The value of phagocytic activity (PA) was higher in E1 ( $21.6 \pm 0.51$  %) compared to group E2 ( $13.4 \pm 0.51$ %) at day 21 of oregano and Xtract™ additions. At the end of experiment PA in E1 reached  $18.8 \pm 0.37$  % compared to E2 group ( $16.6 \pm 0.51$  %).

Concerning the nutritional variables (Table 5), in E1 as well as E2 were observed higher ( $P < 0.05$ ) feed conversion ratio at day 21, higher final weight in E1 at day 42, higher daily weight gain in E1 and E2 at day 42 and lower mortality in E1 and E2 at day 42 compared to control.

The anti-coccidial effect was recorded in both experimental groups at days 21 and 42 compared to control. In E1 250 OPG were counted, whereas in E2 33.4 OPG were found, and in the control group 1306.7 OPG were counted at day 21. The oocysts were also reduced in both experimental groups at day 42 (183.4 OPG were counted in E1, 133.3 OPG in E2) compared to control (1435.7 OPG). Comparison between E1 and E2 showed at both days of sampling, a slight decrease of *Eimeria* sp. oocysts in E1 (from 250 OPG at day 21 to 183.4 OPG at day 42), whereas an increase of oocysts was noted in E2 (from 33.4 OPG at day 21 to 133.3 OPG at day 42).

The lowest values of GPx were measured in E1 ( $207.1 \pm 67.3$  U/gHb) compared to E2 ( $275.5 \pm 114.8$  U/gHb) and control ( $250.2 \pm 109.8$  U/gHb) at day 21. Also the lowest values of GPx were observed in E1 ( $216.1 \pm 67.24$ ) compared to E2 ( $267.1 \pm 101.9$  U/gHb) as well as control ( $265.4 \pm 135.7$  U/gHb) at day 42.

## Discussion

A decrease of CoNS and CoPS was observed in oregano group E1 compared to E2 and control. The administration of oregano plant extract lead to significant anti-coliforms ( $P < 0.01$ ) and anti-clostridial effect. There are many studies showing the antibacterial potential of the oregano essential oil (its components such as carvacrol or its isomer thymol) in food and feed (Burt 2004). Marcin et al. (2006a) reported the inhibitory activity of oregano aromatic oils against pig isolates *E. coli* S143 and *Salmonella enterica* var. Enteritidis. In addition, Bozin et al. (2006) showed the *in vitro* antibacterial activity expressed by oregano essential oil on multiresistant strains of *E. coli*.

Table 4. Values (mean  $\pm$  standard deviation) of volatile fatty acids in the caecum content after administration of oregano extract and Xtract™ (at day 21) and at the end of the experiment (3 weeks after discontinuing administration of oregano and Xtract™ at day 42)

Group	Volatile fatty acid	Day 21	Day 42
E1	Lactic acid (g/100g)	0.049 $\pm$ 0.001*	0.024 $\pm$ 0.003
	Acetic acid (mmol/l)	4.966 $\pm$ 0.721	6.063 $\pm$ 0.726
	Propionic acid (mmol/l)	0.372 $\pm$ 0.066	0.527 $\pm$ 0.077
	Butyric acid (mmol/l)	1.374 $\pm$ 0.044	1.679 $\pm$ 0.142
E2	Lactic acid (g/100 g)	0.042 $\pm$ 0.009	0.042 $\pm$ 0.003
	Acetic acid (mmol/l)	5.910 $\pm$ 0.411	5.222 $\pm$ 0.557
	Propionic acid (mmol/l)	0.380 $\pm$ 0.027	0.455 $\pm$ 0.052
C	Butyric acid (mmol/l)	1.808 $\pm$ 0.189 <sup>a</sup>	1.585 $\pm$ 0.034
	Lactic acid (g/100 g)	0.041 $\pm$ 0.005	0.044 $\pm$ 0.005
	Acetic acid (mmol/l)	4.188 $\pm$ 1.115	4.949 $\pm$ 1.490
	Propionic acid (mmol/l)	0.275 $\pm$ 0.027	0.451 $\pm$ 0.244
	Butyric acid (mmol/l)	1.095 $\pm$ 0.113 <sup>b</sup>	1.238 $\pm$ 0.507

E1 - experimental group (oregano; n = 3); E2 - experimental group (Xtract™; n = 3); C - control group (n = 3);  
<sup>a,b</sup>P < 0.01

Table 5. Nutrition indicators (mean  $\pm$  standard deviation) of rabbits in response to dietary supplementation of oregano (E1) and Xtract™ (E2) compared to control group

Nutrition indicators	E1 (oregano)	E2 (Xtract™)	C (control group)
Initial weight (g)	950 $\pm$ 100	968 $\pm$ 120	964 $\pm$ 109
Final weight (g)	2 521 $\pm$ 263	2 460 $\pm$ 225	2 465 $\pm$ 180
Daily weight gain (g/day)	38.9	40.48	38.29
Feed conversion ratio at day 21 (g/g)	3.01 $\pm$ 0.03 <sup>a</sup>	2.99 $\pm$ 0.02	2.81 $\pm$ 0.07 <sup>b</sup>
Feed conversion ratio at day 42 (g/g)	3.49 $\pm$ 0.02	3.56 $\pm$ 0.04	3.63 $\pm$ 0.11
Daily feed intake (g/day)	130.5 $\pm$ 10.2	126.5 $\pm$ 13.6	129.4 $\pm$ 8.5
Mortality (n)	2	3	5

<sup>a,b</sup>P < 0.05

The feeding of either oregano or Xtract™ in rabbits in our study did not influence the biochemical indicators; neither did it have a negative effect on the health status and growth performance of rabbits. Marcin et al. (2006b) also reported positive influence on growth performances and the incidence of diarrhoeal diseases in the experimental weaned pigs administrating aromatic oils from oregano, clove and cinnamon.

It can be stated that oregano administration has an immuno-stimulatory effect. Moreover, the higher PA was also demonstrated in blood samples of rabbits after sage administration (Szabóová et al. 2008) and after sage administration with bacteriocin-producing strain *Enterococcus faecium* CCM 4231 and its bacteriocin enterocin 4231 (Szabóová et al. 2011).

Eimeriosis in rabbit breeds presents a serious health and economic problem. Our study showed that both the oregano plant extract and Xtract™ administration lead to reduction of *Eimeria* sp. oocysts. The mechanisms of reduction of *Eimeria* sp. oocysts in the intestinal tract after administration of natural substances have not been rigorously studied up to now. However, we hypothesise that plant extracts/essential oils inhibit target cells in the membrane, depleting the transmembrane potential and/or the pH gradient, which results in the leakage of cellular materials and destruction of oocysts (Cleveland et al. 2001). Giannenas et al. (2003) reported reduction of *Eimeria tenella* oocysts after dietary supplementation with oregano essential oil in

broiler chicken. The anti-coccidial effect ( $P < 0.05$ ) of another natural substance, green tea-based diets were also evaluated in chickens (Seung et al. 2007). Administration of chamomile essential oil (Simonová et al. 2006) as well as a plant extract of sage (member the *Labiatae* family; Szabóová et al. 2008) resulted in the reduction of *Eimeria* sp. oocysts in faeces of rabbits.

The beneficial effect of oregano plant extract administration was manifested by antimicrobial activity, by the increase of phagocytic activity as well as by a new phenomenon, the anti-coccidial effect in rabbits.

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