Effect of Essential Oils on Enzymatic Activities in the Intestinal Apparatus and Growth Ability of Laboratory Mice

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Summary

The influence of essential oils (EOs) from medicinal and aromatic plants from sage (SA), cinnamon (CN), thyme (TH) and oregano (OR) on the amylolytic, proteolytic and cellulolytic activities in chyme of the duodenum (DU), the small (SI) and large intestine (LI), the caecum (CE) and the rectum (RE) as well as on the growth ability of laboratory ICR outbred mice were compared in four feeding trials. The negative control was present in the each trial. EOs were mixed into a feed mixture (crude protein (CP) 170.0, fibre 115.0, fat 27.0, lysine 7.0, methionine and cystine 6.7, Ca 9.0, P 6.0 $g.kg^{-1}$ dry matter (DM), metabolic energy (ME) 10 $MJ.kg^{-1}$ DM) of experimental group as follows: 1) 6 groups (n=36, age 63 days, period 14 days) SA, CN, TH, OR, the blend of SA with OR, the dosages of EOs 0.42 except OR 0.21 $ml.100\ g^{-1}$ feed, 2) 2 groups (n=12, age 28 days, period 30 days) blend of SA 0.42 with OR 0.21 $ml.100\ g^{-1}$ feed, 3) 3 groups (n=18, age 28 days, period 58 days) CN and TH, both 0.5 $ml.100\ g^{-1}$ feed, 4) 2 groups (n=12, age 28 days, period 8 days) the blend of CN with TH 0.42 $ml.100\ g^{-1}$ feed. The peroral intake of blend of EOs from OR with SA increased the weight gains by 25 %. Additionally, it stimulated the activities of digestive enzymes in the chyme of intestinal apparatus of laboratory mice in the experimental group compared to control as follows: amylolytic by 4,138 $\mu mol.s^{-1}.g^{-1}$ and proteolytic by 282.2 mg $azoalbumin.min^{-1}.g^{-1}$ in SI ($p<0.01$), cellulolytic by 23.58 in LI and by 34.87 mmol $glucose.min^{-1}.g^{-1}$ in CE ($p<0.01$).

Key words

Aromatic oils • Sage • Cinnamon • Thyme • Oregano • Mice • Growth ability

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Introduction

Plant extracts are sources of many different bioactive molecules with the influence on animal physiology and metabolism. Some of them can be used in the form of whole plant extracts for food and medicinal applications (Wallace *et al.* 2002). Phytoadditives, such as herbs or herbal derivates, have attracted attention for the utilization in animal nutrition. These are potential alternatives to antibiotic growth promoters in the animal breeding. Their effects depends on the quantitative level of phytogenic preparations in feed, the chemical composition, the content of feed ingredients and the physiology of a gastrointestinal apparatus (Regulation 1831/2003 EC).

Phytogenic feed additives contain various chemical compounds: EOs, saponins, tannins and flavonoids. The active chemical substances are isolated from medicinal, aromatic and spicy plants, which possess potential ability for manipulation of animal digestion and subsequently the growth ability.

The most important are EOs. These are complex mixtures of volatile, lipophilic compounds, which originate from plants. Because of lipophility, they possess good intestinal and percutaneous absorption properties. They stimulate the secretion of digestive enzymes and increase gastric and intestinal motility after dietary intake (Windisch *et al.* 2007).

The effects of EOs on the digestive

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characteristics and the enzymatic activities in the intestinal apparatus of laboratory mammals are not entirely evident. Therefore, the objective of this study was to compare the influence of dietary intake of EOs from sage, cinnamon, thyme and oregano on some digestive enzyme activities in the chyme of intestinal apparatus and the effects on the growth ability.

Methods

Plant essential oils

The plant EOs (100 % v/v), isolated from leafs of sage (SA; *Salvia officinalis* L., *Labiateae*), leafs of cinnamon (CN; *Cinnamomum zeylanicum* Ness, *Lauraceae),* stems of thyme (TH; *Thymus vulgaris* L. and *T. zigis* L., *Labiateae*) and tops of oregano (OR; *Origanum vulgare* L., *Lamiaceae*) were delivered from Calendula joint-stock company (Nová Ľubovňa, Slovak Republic; www.calendula.sk). The percentage ranges of the main components of the particular essential oil (EO) were declared in the product certificate according to the gas chromatography analysis (% mean ± SD) for SA: cineole 14±1, thujone 25±1, borneol 17±1, CN: eugenol 77±3, TH: beta-myrcene 2±1, gamma-terpinene 7.5±2.5, p-cymene 21.5±6.5, linalool 5.2±1.3, terpinen-4-ol 1.3±0.5, thymol 45±10, carvacrol 2.4±0.6, OR: carvacrol 65±3. EOs were stored in closed bottles in refrigerator at 5 °C and were used in all feeding experiments. It secured the quality and the reproducibility of experiments.

Feeding experiments

Four feeding experiments were performed with the laboratory ICR outbred mice (males/females) using a feed mixture (Table 1). One negative control group was present in each experiments which was fed with an identical ration without additives. All procedures were performed with the animals approved by the Animal Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice according to Directive 2010/63/EU.

Table 1. Analyzed chemical composition and ingredients of the experimental diet.

Parameter	Unit		Parameter	Unit	
Dry mater (DM)	$g.kg^{-1}$	890.07	*Cu*	$mg.kg^{-1}$	16.0
Crude protein (CP)	$g.kg^{-1}$	170.0	*Mn*	$mg.kg^{-1}$	74.0
Crude fat	$g.kg^{-1}$	27.0	*Zn*	$mg.kg^{-1}$	95.0
Crude fiber	$g.kg^{-1}$	115.0	*Retinol (A)*	I.U. kg^{-1}	10,000.0
Metabolizable energy (ME)	$MJ.kg^{-1}$	10.0	*Cholecalciferol (D3)*	I.U. kg^{-1}	2,000.0
Lysine	$g.kg^{-1}$	7.0	*Tocopherol (E)*	$mg.kg^{-1}$	50.0
Methionine + cysteine	$g.kg^{-1}$	6.7	*Thiamine (B_1)*	$mg.kg^{-1}$	6.0
Ca	$g.kg^{-1}$	9.0	*Riboflavin (B_2)*	$mg.kg^{-1}$	9.0
P	$g.kg^{-1}$	6.0	*Pyridoxine (B_6)*	$mg.kg^{-1}$	9.0
Na	$g.kg^{-1}$	3.4	*Cobalamin (B_{12})*	$mg.kg^{-1}$	10.0
Fe	$mg.kg^{-1}$	200.0			
Ingredients	barley, wheat bran, barley malt powder, dry alfalfa, dried sugar beet pulp, rapeseed meal 00, minerals, vitamin-mineral premix, NaCl				

a) The Experiment 1 was performed with 6 groups of animals (nex=30, nco=6 age 63 days) for 14 days. EOs isolated from SA, CN, TH, OR and the blend of SA with OR were added into diets of 5 experimental groups. The dosages of additives were set according to results of the chemical, microbiological and palatability tests. These were 0.42 for all EOs except OR 0.21 $ml.100\ g^{-1}$ feed. EOs were mixed with rapeseed oil (20 times diluted) and sprayed on the surface of feed pellets for experimental groups. This way prepared feed was used for animal feeding only for one day. The feed of control groups was sprayed with the same quantity of plant oil without EOs.

The quantitative analyses of amylolytic (Spofa test for alpha-amylase, Praha, Czech Republic), cellulolytic (Lever 1977) and proteolytic activities

(Broderick 1987) were performed in chyme of the duodenum, the small and large intestine, the caecum and the rectum after 14-day feeding trial as follows. The analyzed assay samples (in triplicate) contained strained chymus from an intestine diluted 10 times with 50 mmol.l^{-1} potassium phosphate buffer (PPB), pH 7.0.

The amylolytic activity was measured by the rate of hydrolysis of starch substrate bound with blue dye. As for amylase, α-amylase (α-1,4-glucan-4-glucan-hydrolase, EC 3.2.1.1) hydrolyze α-1-4 glycoside linkage with the pH optimum between 7.0 – 7.2. The assay sample 100 µl was incubated at 37 °C for 5 min. After addition of one starch tablet, the mixture was incubated at the same temperature for 30 min. The reaction was stopped by the addition of 4,000 µl 10 % acetone. Samples were centrifuged after 5 min at 1,500 x g for 5 min. The absorbance of supernatant with blue dye was measured spectrophotometrically at 620 nm against distilled water. The enzyme activity was read from diagram enclosed to test and after calculation expressed as µmol.s^{-1}.g^{-1} chyme.

The proteolytic activity was measured by the rate of hydrolysis of the protease substrate azoalbumin (Merck Ltd., Germany) (Broderick, 1987). There were added the assay sample 250 µl, 250 µl PPB and solution of 2.5 mg substrate in 500 µl PPB. After 90 min incubation at 37 °C, the reaction was stopped by the addition of 1,000 µl 5 % trichloroacetic acid. Samples were centrifuged at 3,000 x g for 10 min. In the case of the blank sample, the substrate solution was added immediately after the incubation. Acid-soluble azodye was dissolved in the supernatant. It was measured spectrophotometrically at 450 nm after addition of 1 mol.l^{-1} NaOH (1:1). The proteolytic values were expressed as mg azoalbumin.min^{-1}.g^{-1} chyme.

The cellulolytic activity was measured by the rate of hydrolysis of the substrate methylhydroxyethylcellulose (Merck Ltd., Germany) (Lever 1977). The assay sample 250 µl, 250 µl PPB and solution of 5.0 mg substrate in 500 µl PPB. After 12 h incubation at 37 °C, samples were immediately chilled at 4 °C and then centrifuged at 3,000 x g for 10 min. The substrate was added into the blank sample after the incubation. 20 µl of experimental or blank samples were taken from supernatant and were added to 2,000 µl of 4-hydroxybenzoic acid hydrazide (PAHBAH) reagent in alkali. The reagent contained 0.05 mol.l^{-1} PAHBAH and 0.5 mol.l^{-1} NaOH. The mixture was subsequently heated at 70 °C for 10 min. After immediate cooling, the absorbance was measured at 410 nm against a blank sample. The values of cellulolytic activities were expressed as mmol glucose.min^{-1}.g^{-1} chyme.

b) In the feeding trials 2, 3 and 4, the growth abilities of mice were determined after peroral intake of EOs as follows.

Two groups of mice were used in the experiment 2 (nex=6, live weight (LW) 18.83 ± 2.409 g; nco=6, LW 22.33±1.972 g, age 28 days) for 30-day trial. The blend of EOs from SA 0.42 ml.100 g^{-1} and OR 0.21 ml.100 g^{-1} feed was mixed into the diet of experimental group.

EOs from CN and TH were applied into diet at the level 0.5 ml.100 g^{-1} feed in the experiment 3. Two experimental (nex_1=12, LW 12.5±0.530 g; nex_2=12, LW 12.5±0.510 g) and one control group (nco=6, LW 12.0±1.200 g) at the age 28 days were used in the trial for 58-day trial.

In the experiment 4, two groups of mice (nex=6, LW 10.86±1.374 g; nco=6, LW 10.33±1.599 g, age 28 days) were used. The blend of EOs isolated from CN and TH was applied into feed mixture of experimental group at the dosage 0.42 ml.100 g^{-1} feed for 8-day trial.

All mice from each group were anesthetized with the intraperitoneal injections of xylazine 0.6 ml.kg^{-1} (Rometar 2 %, Spofa, Czech Republic) and ketamine 0.7 ml.kg^{-1} (Narkamon 5 %, Spofa, Czech Republic) after finishing of feeding trials.

Data in the presented paper are expressed as means ± standard deviation (SD) of a single values (SAS, Version 8.2, 1999). Means of results from treatment were compared by one-way analysis of variance. Treatment means were statistically compared by Tukey-Kramer multiple comparison test. Significance was declared at $p<0.05$ (*) and $p<0.01$ (**).

Results

The results of the feeding trial 1 are demonstrated in Tables 2 and 3. There were observed amylolytic, proteolytic and cellulolytic activities in the gastrointestinal apparatus of laboratory mice with the following results.

The amylolytic activities (µmol.s^{-1}.g^{-1} chyme) were increased ($p<0.01$) in the case of addition of the blend of EOs from SA with OR in the small intestine by 4,138.08 and by 2,557.51 in the large intestine as well as after intake of EO from OR in the large intestine ($p<0.05$) by 967.01 compared to control group (Table 2).

The increases of proteolytic activities (mg azoalbumin.min^{-1}.g^{-1} chyme) were observed a) in the duodenum ($p<0.05$) after intake of EOs from SA by 240.54 and the blend of SA with OR by 218.14, b) in the small intestine ($p<0.01$) after addition of EO from SA by 434.44, blend of EOs from SA with OR by 282.80 and EO from TH by 312.74, c) in the large intestine ($p<0.01$) in the case of EOs from SA by 102.99 and TH by 291.56, d) in the caecum ($p<0.01$) after addition of EO from TH by 139.01, e) in the rectum ($p<0.01$) after intake of EOs from SA by 91.65 and TH by 166.27 (Table 3).

Table 2. Amylolytic activity in the intestinal apparatus of laboratory mice (age 63 days) after 14-day feeding trial.

Essential oils	Amylolytic activity (μmol.s^{-1}.g^{-1} chyme)				
	Duodenum (DU)	Small intestine (SI)	Large intestine (LI)	Caecum (CE)	Rectum (RE)
Sage	535.38 ± 82.366	1,114.10 ± 64.402	352.44 ± 48.937	28.18 ± 0.478	50.11 ± 1.984
Cinnamon	163.62 ± 10.100	676.20 ± 77.265	1,588.73 ± 97.475	448.95 ± 55.247	209.47 ± 20.099
Thyme	1,691.65 ± 59.025	2,383.81 ± 74.060	2,225.11 ± 20.826	208.26 ±21.307	178.66 ± 31.66
Oregano	3,804.364 ± 94.626	3,205.5 ± 309.804	3,087.54 ± 318.877*	782.81 ±176.29	125.05 ± 47.443
Sage with oregano	4,220.33 ± 150.365	6,919.12 ± 349.987**	4,678.04 ± 162.03**	443.19 ±85.553	480.78 ± 27.221
Control	5,494.65 ± 77.214	2,781.04 ± 36.942	2,120.53 ± 28.99	781.51 ± 63.251	717.65 ± 29.832

Data are means ± SD from 6 animals in each group, SD – standard deviation, ** – $p<0.01$, * – $p<0.05$. The amylolytic activities (μmol.s^{-1}.g^{-1} chyme) were increased ($p<0.01$) in the case of addition of the blend of EOs from SA with OR in the small intestine by 4,138.08 and by 2,557.51 in the large intestine as well as after intake of EO from OR in the large intestine ($p<0.05$) by 967.01 in comparison to control group.

Table 3. Proteolytic activity in the intestinal apparatus of laboratory mice (age 63 days) after 14-day feeding trial.

Essential oils	Proteolytic activity (mg azoalbumin.min^{-1}.g^{-1} chyme)				
	Duodenum (DU)	Small intestine (SI)	Large intestine (LI)	Caecum (CE)	Rectum (RE)
Sage	454.78 ± 4.017*	655.61 ± 18.396**	285.08 ± 0.597**	63.76 ± 12.037	150.47 ± 4.475**
Oregano	109.38 ± 4.972	328.64 ± 7.458	185.95 ± 5.966	12.19 ± 0.994	31.08 ± 2.983
Sage with oregano	432.38 ± 43.752*	503.97 ± 19.887**	224.23 ± 10.441	26.35 ± 2.138	72.09 ± 3.033
Thyme	242.41 ± 12.052	533.91 ± 6.026**	473.65 ± 33.142**	149.39** ± 8.662	225.09 ± 8.286**
Cinnamon	57.49 ± 10.169	228.10 ± 43.688	263.12 ± 4.896	19.65 ± 6.299	20.28 ± 1.260
Control	214.24 ± 31.636	221.17 ± 0.242	182.09 ± 17.968	10.38 ± 1.967	58.82 ± 1.505

Data are means ± SD from 6 animals in each group, SD – standard deviation, ** – $p<0,01$, * – $p<0,05$. The increases of proteolytic activities (mg azoalbumin .min^{-1}.g^{-1} chyme) were observed a) in the duodenum ($p<0.05$) after intake of EOs from SA by 240.54 and the blend of SA with OR by 218.14, b) in the small intestine ($p<0.01$) after addition of EOs from SA by 434.44, blend of SA with OR by 282.80 and TH by 312.74, c) in the large intestine ($p<0.01$) in the case of EOs from SA by 102.99 and TH by 291.56, d) in the caecum ($p<0.01$) after addition of TH by 139.01, e) in the rectum ($p<0.01$) after intake of EOs from SA by 91.65 and TH by 166.27.

The enhancements of cellulolytic activities (mmol.l^{-1} glucose .min^{-1}.g^{-1} chyme) were observed in a) the large intestine ($p<0.01$) after intake of EO from OR (20.70±1.661), the blend of EOs from SA with OR (25.02±0.096) and TH ($p<0.05$; 7.74±0.839) compared to control (1.44±0.241), b) the caecum ($p<0.01$) after addition of EOs from OR (39.94±0.895), the blend of SA with OR (35.91±0.831) and CN (94.22±4.098) compared to control (1.039±0.053), c) the rectum ($p<0.01$) in the case of intake of blend of EOs from SA with OR (51.05±0.728) and TH (41.04±0.516) in comparison to control (8.50±0.411).

The achieved results of the growth of laboratory mice were the following:

In the second feeding trial with the blend of EOs from SA with OR (Fig. 1), the differences of weights

(g.mouse^{-1}) decreased from the start to the end of experiment from 3.5 to 2.0. The finishing weights of mice from experimental vs. control groups were 26.33±4.346 vs. 28.33±3.399. The individual weight gains (g.mouse^{-1}.day^{-1}) were higher by 25 % in the experimental group compared to control.

In the third feeding trial (Fig 2), the differences of starting live weights (g.mouse^{-1}) between two experimental and control groups were 0.50. Whereas, the finishing live weights (g.mouse^{-1}) were higher in the experimental groups by 2.0 (TH) and 9.5 (CN). Similarly, the individual weight gains (g.mouse^{-1}day^{-1}) were enhanced in TH or CN group by 0.026 and 0.155, respectively.

In the fourth feeding trial the finishing live weights (g.mouse^{-1}) of experimental group were 8.17±1.067 (the blend of EOs from TH with CN) and of control group 16.83±2.115. The individual weight gains (g.mouse^{-1}.day^{-1}) of experimental group were negative and subsequently lower -0.311±0.174 in comparison to control 0.813±0.265. The mortality of 2 mice was observed in the experimental group during 8-day trial.

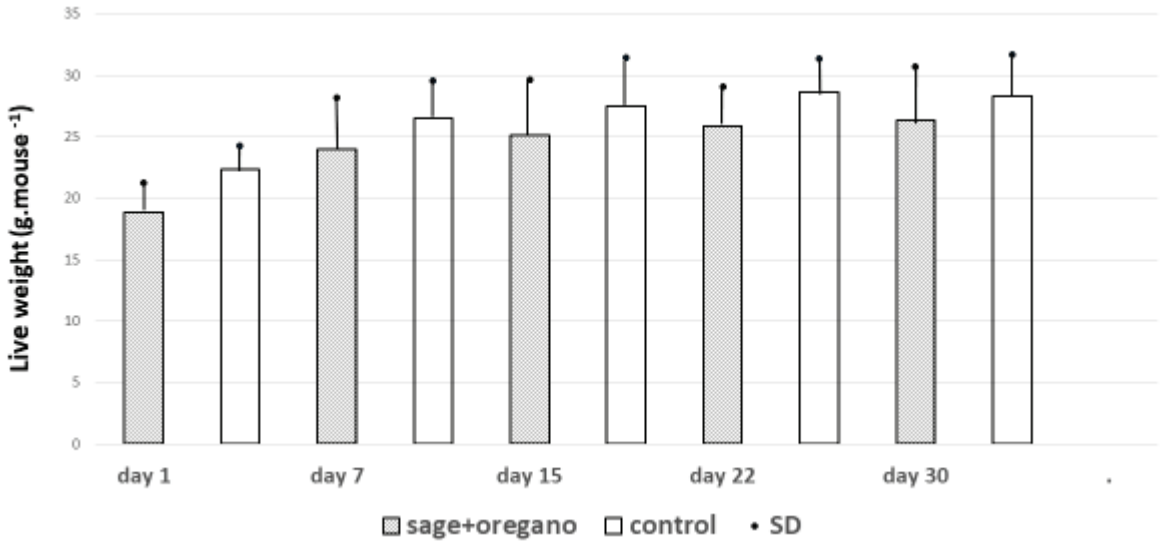

Fig. 1. Live weights of mice (n=12, age 28 days) during 30-day feeding trial (mean ± SD). In the second feeding trial with the blend of EOs from SA with OR the differences of live weights (g.mouse^{-1}) between groups decreased from the start to the end of experiment from 3.5 to 2.0. The individual weight gains (g.mouse^{-1}.day^{-1}) were higher by 25 % in the experimental group compared to control.

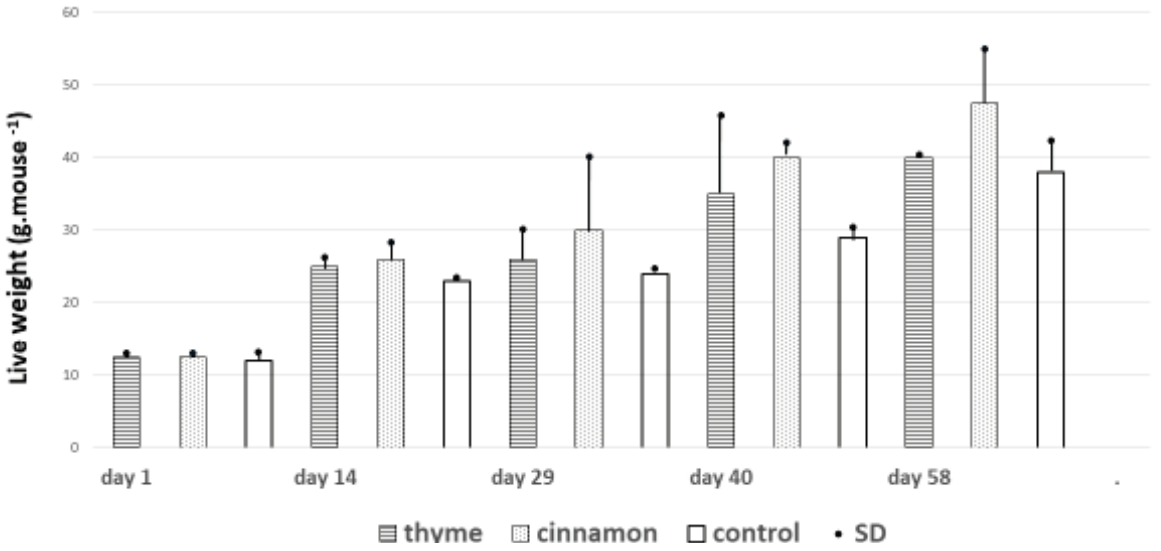

Fig. 2. Live weights of mice (n=18, age 28 days) during 58-day feeding trial (mean ± SD). In the third feeding trial, the differences of starting live weights (g.mouse^{-1}) between two experimental and control groups were 0.50. Whereas, the finishing live weights (g.mouse^{-1}) were higher in the experimental groups by 2.0 (TH) and 9.5 (CN). Similarly, the individual weight gains (g.mouse^{-1} day^{-1}) were enhanced in TH or CN group by 0.026 and 0.155, respectively.

Discussion

Our study demonstrated that the dietary addition of EOs from SA, CN, TH and OR can be substantial for laboratory mice in various aspects.

As for enzymatic activities in an intestine, the significant increase of amylolytic activity was observed in the small or large intestine after intake of EOs from OR and the blend of SA with OR.

The increase of proteolytic activity was observed after intake of EOs from SA and the blend of SA with OR in the duodenum and the small intestine. The intake of EOs from TH caused the increase of this enzymatic activity in the small and large intestine.

The cellulolytic activity was enhanced in the large intestine as a result of dietary intake of EOs from TH, OR and the blend of SA with OR. The increase was observed in the caecum after addition of EOs from CN, OR and the blend SA with OR.

On one hand, Hernández *et al.* (2004) added the blend of EOs – OR, CN, pepper (200 ppm) or SA, TH, rosemary (5,000 ppm) into feed of broiler chickens and it resulted in significantly increased digestibility of DM and starch but not CP. On the other hand, Basmacioglu *et al.* (2010) determined the effect of dietary supplementation of oregano EO at two levels 250 and 500 $mg.kg^{-1}$ on the digestive enzymes and nutrient digestibility of broiler chickens fed wheat-soybean meal based diets. It significantly increased chymotrypsin activity in the digestive system and improved CP digestibility.

The weight gains were increased after dietary intake of the blend of EOs from SA with OR and single application of CN into feed mixture.

In like manner, Basmacioglu *et al.* (2010) observed higher weight gains after addition of 250 $mg.kg^{-1}$ oregano EO into feed of poultry. Koiyama *et al.* (2014) observed the increase of growth performance and better feed conversion rate of broiler chickens after addition of blend of EOs from SA, CN and TH too. Similarly, according to Garcia *et al.* (2007) 200 ppm of plant extracts (OR, CN, pepper) were beneficial for improving growth traits and nutrient apparent ileal digestibility of poultry.

EO from OR is characteristic with its potential to improve the health of animals and the intestine. The reasons are antimicrobial and anti-inflammatory effects of the basic components. The components with the substantial inhibitory activity against bacteria are carvacrol, eugenol and thymol. The highest concentration of carvacrol was analyzed present in EOs from OR and TH, eugenol in CN and thymol in TH.

As for antimicrobial activity, Marcin *et al.* (2006) quantitatively evaluated the inhibitory activity of EOs from SA and OR (100 % v/v) by the agar diffusion paper disc measured as the diameter of inhibitory zones on the Mueller-Hinton agar. EOs from SA or inhibited growth of the reference bacterial strains isolated from the pig intestine compared to physiological solution as follows: a) pathogenic *Escherichia coli* S143 (hemolytic, K antigen positive) 7.68±1.992 or 14.96±4.91 vs. 6.24±1.05, b) *E. coli* (hemolytic, K antigen negative) 6.33±0.943 or 10.75±1.953 vs. 5.33±0.471, c) *Enterococcus spp.* 31.17±6.793 vs. 5.83±0.898. The antibacterial activities of EOs from SA and TH were confirmed by Puškárová *et al.* (2017) against pathogenic *E. coli* and *Salmonella typhimurium* as well. Similarly, Aminzare *et al.* (2017) used extracts from SA, TH, OR and Santurio *et al.* (2011) from CN, TH, OR in the testing of the effectiveness against *E. coli* with the positive results.

The integrity of intestinal barrier is important in the processes of protection against bacteria and intake of nutrients from a feed as well. EOs can positively influence the health state of the intestinal apparatus.

Zou *et al.* (2016) showed significant decrease of endotoxin level in serum and significant increase of villus height as well as expression of occludin and zonula occludens-1 in the jejunum after diet supplementation with 25 $mg.kg^{-1}$ oregano EO for 4 weeks. They demonstrated that the integrity of intestinal barrier was improved by this treatment through modulating intestinal bacteria and immune status in pigs.

Whereas, Karimzadeh and Farahpour (2017) used wound healing properties of hydroethanolic leaf extract of SA because of highest total flavonoid and phenolic content as well as antioxidant capacity.

There was observed the toxic effect of blend of EOs from TH and CN containing eugenol (77.0 %) and carvacrol (2.4 %) after peroral intake by mice. The results were the decreased feed intake and the weight losses during short feeding period. These both active substances are phenolic compounds. The carvacrol and eugenol are alternatively occurring mainly in the fractions of EOs from oregano, thyme, calamint and cinnamon and clove. The additive effect of both EOs and their phenolic compounds were toxic to experimental mice.

The negative effect is observable in the case of bacterial cells. Ultee *et al.* (1999) studied the effect of

carvacrol on bioenergetic parameters of vegetative cells of the foodborne pathogen *Bacillus cereus*. These bacteria were used as model experimental cells. Only 2 mmol.l^{-1} carvacrol significantly depleted the intracellular ATP pool. The depletion of the internal ATP pool was associated with the change of membrane potential. An increase of the permeability of the cytoplasmic membrane was observed for protons and potassium ions. The dissipation of ion gradients leads to impairment of essential processes in the cell and finally to the cell death. On the other hand, Puškárová *et al.* (2017) observed that EOs from SA, TH and OR, applied individually, have not any genotoxic effect on HEL 12,469 human embryo cells and did not induced significant DNA damage *in vitro*.

The antioxidative characteristics of some EOs with the positive effects on cells of macroorganism could explain these observations. The anti-lipoperoxidative and antioxidant properties of phenol extracts from leaves of SA on SH-SY5Y human neuroblastoma cells at dose level below 0.125 mg.ml^{-1} were confirmed by Pacifico *et al.* (2017).

Conflict of Interest

There is no conflict of interest.

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