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Original article

Effects of dietary supplementation with sage (*Salvia officinalis* L.) essential oil on antioxidant status and duodenal wall integrity of laying strain growers

I. Placha¹, M. Ryzner¹, K. Cobanova¹, Z. Faixova², S. Faix¹

¹ Institute of Animal Physiology, SAS, Soltesova 4, 040 01 Kosice, Slovak Republic

² University of Veterinary Medicine and Pharmacy,
Department of Pathological Anatomy and Pathological Physiology,
Komenskeho 73, 041 81 Kosice, Slovak Republic

Abstract

The objective of this study was to compare the influence of four different concentrations of *Salvia officinalis* essential oil (EO) on animal health. A total of 50 laying strain chicks were randomly divided at the day of hatching into five dietary-treatment groups. Control group was given the basal diet (BD), the other four experimental groups contained BD supplemented with 0.1, 0.25, 0.5, 1.0 g *S. officinalis* EO/kg diet, respectively. 0.1 g/kg EO increased glutathion peroxidase activity (GPx) in duodenal mucosa, liver and kidney, phagocytic activity in blood (PA), transepithelial electrical resistance (TEER) in duodenal tissue and decreased malondialdehyde (MDA) concentration in plasma and liver. 0.25 g/kg EO increased GPx in liver, total antioxidant status (TAS) in plasma, PA in blood and TEER in duodenal tissue. Our results demonstrate that lower concentrations of EO improve animals' health status, and that it is necessary keep in mind the selection of sufficient concentration of EO used as animal feed additive.

Key words: duodenal wall integrity, phagocytic activity, feed additives, health

Introduction

Feed additives are used with healthy animals as substances or preparations favourably influencing animal production, performance and welfare, in contrast to veterinary drugs used just to treat health problems and applied for a limited period only. Several investigations have shown their antioxidative effect, effects on digestive physiology and on the microbiology of

the gut, but only little information is given about their mode of action, metabolism or generally on their science-based functionality (Franz et al. 2010, Wencelová et al. 2014, Bubel et al. 2015).

In normal physiological conditions the production of free radicals is balanced by the antioxidant defence system, but in certain circumstances a significant imbalance between reactive species and antioxidant defence system can occur, a situation called oxidative

Correspondence to: I. Placha, e – mail: placha@saske.sk, tel.: +421 55 792 29 69

stress. Halliwell and Gutteridge (1999) give a broader definition of an antioxidant as „any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate”. Antioxidative compounds prepared from sage have been well established and its extracts are marketed in the U.S., France, Germany and Japan as natural antioxidants for food (Miura et al. 2002).

Among the many benefits of dietary herbs is the ability to modulate the innate immunological properties. Innate immune response involves the detection, uptake and destruction of altered or non-self threats to the organism via phagocytosis (Henneke and Golenbock 2004).

The intestinal epithelium is a single cell-thick interface between the antigen-rich gut lumen and the internal milieu, and it acts as a barrier. The physical barrier function of the epithelium is achieved by the tight junctions between cells, made up of specialized protein complexes (Mannon 2005). The tightness of the intercellular junctional complex can be checked by measuring the transepithelial electrical resistance (TEER), as it is represented by movement of ions across the cell monolayers (Ward et al. 2000).

Compounds of essential oils (EO) are rapidly absorbed from the gastrointestinal tract and mainly conjugated with sulfate and glucuronic acid. If the capacity for conjugation is overwhelmed at high doses, alternative metabolic routes are activated, leading to the production of reactive metabolites (EFSA 2012). These metabolites are normally detoxified but at large doses sulfhydryl groups of hepatic proteins may react with reactive metabolites, resulting in hepatic necrosis (Laskin and Pilaro 1986). To date little information is available on effective doses of EO that can strengthen animal health and can be used in animals without inducing toxic effects. More research is therefore needed in this area (Acamovic and Brooker 2005).

For this reason the objective of this study was to compare the effect of four different concentrations of *S. officinalis* EO, and to find a sufficient concentration which can positively influence antioxidant status and immunity and strengthen the duodenal wall integrity in laying strain growers, and in this way improve their health. Moreover, this study evaluated the plasma biochemical profile of birds.

Materials and Methods

Animal care and use

The experiment was carried out in accordance with the established standards for use of animals. The

protocol was approved by the Ethical Commission of the Institute of Animal Physiology, Slovak Academy of Sciences in Košice, Slovakia and by Slovak governmental authority (Č.k. RO-820/10-221). Experimental design and housing: A total of 50 non sexed ISA BROWN laying strain chicks were randomly divided at the day of hatching into five dietary-treatment groups. All cages were placed in the same room, in which the temperature was controlled during the experiment. The birds were placed in cages with wood shavings. The light regimen from age of 5 weeks was 9 h of continuous light per day. The initial room temperature 32-33°C was reduced weekly by 2°C to a final temperature of 20-22°C. The relative humidity was within the range of 60 to 70%. All birds had free access to water and feed. The experiment finished at 11 weeks of age with sample collections. Feed intake was recorded daily, body weights were recorded once a week.

Diets

The birds were fed with the 5 experimental diets. The first group (control) was given the basal diet (BD), the second was fed with the same BD with 0.1 g *S. officinalis* EO/kg diet, the third received BD supplemented with 0.25 g/kg EO, the fourth received BD with 0.5 g/kg EO and the fifth received BD with 1.0 g/kg EO. Appropriate diets for growth and healthy development of laying strain chicks were used during the whole experiment (starter feed for the period 0-6 weeks and grower feed for the period 7-11 weeks, Table 1). Sage EO was dissolved in sunflower oil and mixed to the basal diet in appropriate concentration. The final concentration of sunflower oil in all diets was 1.0 %.

Sage oil used in the model experiment

Sage oil was obtained by steam distillation from selected fresh leaves of *Salvia officinalis* L., growing wild in the Balkan area. The EO was provided by HANUS s.r.o. (Slovakia). The major constituents identified in sage oil 0.1 g/kg diet were α -thujone 0.04 g/kg, limonene 0.02 g/kg, camphor 0.02 g/kg and α -humulene 0.01 g/kg; in 0.25 g/kg diet (0.11, 0.04, 0.06, 0.03 g/kg, respectively), in 0.5 g/kg diet (0.22, 0.08, 0.12, 0.06 g/kg, respectively), in 1.0 g/kg diet (0.43, 0.16, 0.25, 0.12 g/kg, respectively). These compounds in the EO were quantified using the high performance liquid chromatography (HPLC) method.

Table 1. Ingredients and composition of starter and grower diets (g/kg).

Ingredients	Starter (0-6 w)	Grower (7-11 w)
Wheat, ground	542.4	600
Maize, ground	100	106
Soybean meal, extracted	250	190
Barley, ground	30	30
Rapeseed	42	40
Limestone	16	16
Monocalcium phosphate	6	6
Feed salt	3	3
Premix	5*	5**
DL-methionin	3.2	4
DL-lysine	2.4	
Composition Dry matter	889	881
Crude protein	195	175
Ash	80	80
Crude fibre	50	50
Calcium	8.0	8.0
Phosphorus	5.0	5.0
Lysine	8.0	8.0
Methionine	4.0	3.5
Methionine + cysteine	7.5	7.5
Linoleic acid	10.0	10.0
Calculated MEn (N-adjusted metabolisable energy, MJ/kg)	11.9	11.5

Notes: Crude protein, dry matter and selenium are analysed data.

* The vitamin/mineral premix provided per kilogramme of complete diet: retinyl acetate 0.3 mg, cholecalciferol 0.05 mg, tocopherol 15.0 mg, riboflavin 4.0 mg, cobalamin 0.01 mg, choline 500 mg, natrium 2 500 mg, manganese 70 mg, iron 60 mg, copper 6 mg, zinc 50 mg, selenium 0.30 mg.

** The vitamin/mineral premix provided per kilogramme of complete diet: retinyl acetate 2.4 mg, cholecalciferol 0.04 mg, tocopherol 12.0 mg, riboflavin 4.0 mg, cobalamin 0.01 mg, choline 300 mg, natrium 2 500 mg, manganese 50 mg, iron 60 mg, copper 6 mg, zinc 50, selenium 0.32 mg.

Sample collections

At the age of 11 weeks, eight randomly chosen chickens from each treatment group were anaesthetized with an intraperitoneal injection of xylazine (Rometar 2%, SPOFA, Czech Republic) and ketamine (Narkamon 5%, SPOFA, Czech Republic) at doses 0.6 and 0.7 ml/kg body weight, respectively. After laparotomy, blood for analysis was collected using cardiac puncture and placed in heparinised tubes. The tubes with blood for determination of malondialdehyde (MDA) concentration and total antioxidant status (TAS) were centrifuged at 3000 RPM for 10 minutes. Plasma and blood samples were stored at -70°C until analysis. Following euthanasia samples of their liver, kidney and duodenal mucosa tissues were collected for biochemical analysis and stored at -70°C

until analysis. Their duodenum was separated to measure TEER *in vitro*.

Duodenal wall integrity

Intestinal wall integrity was tested by measuring the trans-epithelial electrical resistance (TEER) value. Tissues of duodenal mucosa (0.71 cm²) were incubated at 37°C in chambers with Tyrode's solution. TEER values were recorded every 3 min over a period of 30 min. The chambers used were constructed similarly to those described by Ussing and Zerahn (1951), with some modifications. The chambers were composed of two symmetrical half-cells each with volume 10.5 ml. A sheet of chicken's duodenum tissue was mounted between these half cells. Transepithelial electrical resistance was measured with electrodes using a Volt Ohm Meter (MXD-5040RS232 Digital Multimeter with True RMS, METEX Instruments, Korea).

Analysis

Haemoglobin (Hb) content of blood and TAS in plasma were analysed using commercial kits from Randox, UK. To analyse the activities of glutathione peroxidase (GPx, EC 1.11.1.9) in the liver, duodenal mucosa and kidney, pre-weighed pieces of tissue were homogenized in phosphate-buffered saline. Homogenates were centrifuged at 13 680 x g at 4°C for 20 min. The enzyme activity in the supernatant as well as in the blood was measured by monitoring oxidation of NADPH at 340 nm in accordance with Paglia and Valentine (1967), using a commercial kit for the blood (Ransel, Randox, UK).

The tissue samples of duodenal mucosa, liver and kidney for MDA measurement were homogenized with de-ionized distilled water and 50 µl of 7.2% butylated hydroxytoluene. MDA concentrations in these tissues were measured using the modified fluorimetric method of Jo and Ahn (1998).

The protein concentrations in the examined tissues were measured using the spectrophotometric method published by Bradford (1976).

Alkaline phosphatase (ALP, EC 3.1.3.1.), aspartate transaminase (AST, EC 2.6.1.1.), cholesterol, triglycerides, glucose, calcium, potassium and total protein in blood plasma were measured using commercial kits (Randox, U.K.) and phosphorous and magnesium in plasma (BIOLA-test, PLIVA-Lachema, Czech Republic) with the colorimetric method using a Genesys 10 UV spectrophotometer analyser (Thermo Spectronic, Rochester, NY, USA).

Table 2. Activity of GPx in blood ($\mu\text{kat/g Hb}$) and tissue ($\mu\text{kat/g protein}$), TAS (mmol/l) in plasma, concentration of MDA in plasma (nmol/ml), duodenal mucosa, liver and kidney (nmol/g protein) and IgA (mg/g) in duodenal mucosa of laying strain growers.

Indices	BD	0.1 g/kg EO	0.25 g/kg EO	0.5 g/kg EO	1.0 g/kg EO	P-value
Blood (plasma)						
GPx	2.59 ± 0.19	2.74 ± 0.90	2.88 ± 0.06	2.78 ± 0.26	2.72 ± 0.15	0.92
TAS	$1.23 \pm 0.07^{\text{ac}}$	$1.27 \pm 0.04^{\text{c}}$	$1.54 \pm 0.03^{\text{b}}$	$1.46 \pm 0.06^{\text{bc}}$	$1.46 \pm 0.05^{\text{bc}}$	0.001
MDA	$0.51 \pm 0.03^{\text{a}}$	$0.40 \pm 0.02^{\text{b}}$	$0.44 \pm 0.03^{\text{ab}}$	$0.49 \pm 0.03^{\text{ab}}$	$0.48 \pm 0.02^{\text{ab}}$	0.02
Duodenal Mucosa						
GPx	$0.20 \pm 0.02^{\text{a}}$	$0.35 \pm 0.04^{\text{b}}$	$0.23 \pm 0.02^{\text{ab}}$	$0.24 \pm 0.04^{\text{ab}}$	$0.22 \pm 0.04^{\text{ab}}$	0.03
MDA	47.12 ± 4.55	55.24 ± 4.89	41.68 ± 2.54	43.44 ± 4.23	42.27 ± 5.04	0.23
IgA	0.46 ± 0.03	0.55 ± 0.01	0.54 ± 0.02	0.59 ± 0.04	0.45 ± 0.05	0.09
Liver						
GPx	$0.16 \pm 0.01^{\text{ac}}$	$0.20 \pm 0.01^{\text{b}}$	$0.19 \pm 0.01^{\text{b}}$	$0.18 \pm 0.01^{\text{ab}}$	$0.14 \pm 0.01^{\text{c}}$	<0.0001
MDA	$169.2 \pm 14.34^{\text{a}}$	$104.9 \pm 10.12^{\text{b}}$	$125.5 \pm 14.07^{\text{ab}}$	$111.7 \pm 16.72^{\text{ab}}$	$123.0 \pm 14.93^{\text{ab}}$	0.0290
Kidney						
GPx	$0.26 \pm 0.02^{\text{a}}$	$0.60 \pm 0.08^{\text{b}}$	$0.36 \pm 0.04^{\text{ac}}$	$0.41 \pm 0.05^{\text{abc}}$	$0.54 \pm 0.04^{\text{bc}}$	0.0003
MDA	$77.36 \pm 4.14^{\text{ab}}$	$99.78 \pm 5.48^{\text{a}}$	$68.56 \pm 4.89^{\text{b}}$	$82.67 \pm 6.60^{\text{ab}}$	$148.80 \pm 7.97^{\text{c}}$	<0.0001

Notes: a,b,c Means in the same row with different superscripts are significantly different ($p < 0.05$), values are means \pm SEM ($n=8$). BD – basal diet, EO – BD with *S. officinalis* essential oil, GPx – glutathione peroxidase, TAS – total antioxidant status, MDA – malondialdehyde, IgA – immunoglobulin A.

Table 3. The effect of sage essential oil on blood phagocytic activity and its index in laying strain growers.

Indices	BD	0.1 g/kg EO	0.25 g/kg EO	0.5 g/kg EO	1.0 g/kg EO	P-value
PA(%)	$40.50 \pm 0.22^{\text{a}}$	$42.50 \pm 0.43^{\text{b}}$	$42.17 \pm 0.40^{\text{b}}$	$42.00 \pm 0.37^{\text{b}}$	$41.50 \pm 0.34^{\text{ab}}$	0.01
IPA	2.03 ± 0.04	2.14 ± 0.04	2.14 ± 0.03	2.07 ± 0.06	2.09 ± 0.05	0.39

Notes: a,b Means in the same row with different superscripts are significantly different ($p < 0.05$), values are means \pm SEM ($n=8$). PA – phagocytic activity, IPA – index of phagocytic activity, BD – basal diet, EO – BD with *S. officinalis* essential oil.

Immunoglobulin A (IgA) in the intestinal mucosa was measured with a Chicken IgA enzyme-linked immunosorbent assay (ELISA Quantitation Set, Bethyl Laboratories, Inc., USA). Intestinal mucosa was prepared using the method described by Nikawa et al. (1999).

Phagocytic activity was measured by direct counting procedure using yeast cells according to the method of Steruska (1981). Blood smears were prepared and stained with May-Grunwald and Giemsa-Romanowski painting. Phagocytic activity was calculated as the number of white cells containing at least three engulfed particles per 100 white cells (monocytes/granulocytes) and the index of phagocytic activity was calculated as the number of engulfed particles per total number of phagocytes observed. The percentage of phagocytic cells was evaluated using an optical microscope, by counting PMN up to 100.

Dry matter content of diet and tissues was determined by the standard method of drying samples at 105°C . Crude protein in complete diet was analysed using the Kjeldhal method. The selenium concentra-

tion in diet was measured using the fluorimetric method of Rodriguez et al. (1994).

Statistical analysis

Statistical analysis of the results was performed using one-way analysis of variance (ANOVA) with the post hoc Tukey multiple comparison test using GraphPadSoftware (USA). The data presented are the mean values \pm SEM. Probability values of less than 0.05 were considered significant.

Results

Growth performance

Animals were in good health, average weight gain was 976.2 ± 7.63 g at 11 weeks of age and the average feed intake was 61.63 ± 0.4 g/day.

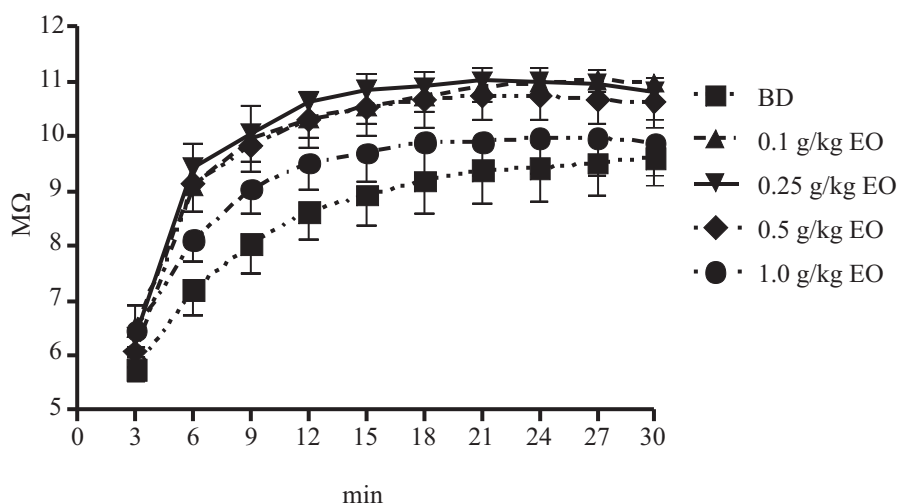


Fig. 1. Transepithelial electrical resistance values in duodenal tissue of laying strain growers.

Notes: BD – basal diet, EO – BD with *S. officinalis* essential oil supplementation. Values are means \pm SEM (n=8).

Table 4. The effect of sage essential oil on biochemical indices in blood plasma of laying strain growers.

Indices	BD	0.1 g/kg EO	0.25 g/kg EO	0.5 g/kg EO	1.0 g/kg EO	P-value
ALP (μ kat/l)	21.98 \pm 1.76	14.33 \pm 0.87	20.73 \pm 2.40	19.81 \pm 1.75	22.85 \pm 2.76	0.13
AST (μ kat/l)	1.01 \pm 0.04	1.09 \pm 0.06	1.03 \pm 0.05	0.97 \pm 0.03	0.91 \pm 0.03	0.11
Cholesterol (mmol/l)	1.62 \pm 0.05	1.23 \pm 0.08	1.41 \pm 0.15	1.84 \pm 0.28	1.80 \pm 0.12	0.05
Triglyceridy (mmol/l)	0.31 \pm 0.05	0.24 \pm 0.04	0.23 \pm 0.03	0.38 \pm 0.06	0.35 \pm 0.05	0.06
Glukóza (mmol/l)	17.10 \pm 1.04	21.65 \pm 2.37	16.25 \pm 1.49	15.53 \pm 0.87	16.36 \pm 1.11	0.05
Ca (mmol/l)	2.26 \pm 0.18 ^a	1.79 \pm 0.06 ^{ab}	2.13 \pm 0.14 ^a	1.47 \pm 0.05 ^b	1.56 \pm 0.10 ^b	<0.0001
P (mmol/l)	2.46 \pm 0.35	2.21 \pm 0.30	2.18 \pm 0.10	2.18 \pm 0.16	2.77 \pm 0.14	0.32
K (mmol/l)	6.33 \pm 0.28 ^a	7.56 \pm 0.33 ^{ac}	6.69 \pm 0.34 ^a	7.73 \pm 0.26 ^{bc}	8.50 \pm 0.41 ^{bc}	0.0004
Mg (mmol/l)	0.76 \pm 0.08	0.85 \pm 0.08	0.99 \pm 0.15	0.74 \pm 0.08	0.94 \pm 0.07	0.28
Total protein (g/l)	33.67 \pm 1.17	32.82 \pm 3.84	33.77 \pm 2.31	39.63 \pm 1.30	35.81 \pm 0.94	0.21

Notes: a,b,c Means in the same row with different superscripts are significantly different ($p < 0.05$), values are means \pm SEM (n=8). BD – basal diet, EO – BD with *S. officinalis* essential oil. ALP- alkaline phosphatase, AST- aspartate transaminase.

The effect of sage oil on antioxidant status

Glutathion peroxidase activity in duodenal mucosa, liver and kidney was significantly higher when 0.1 g/kg EO was added to the diet, as well as in the liver with 0.25 g/kg EO in the diet and in the kidney with 1.0 g/kg EO addition ($p < 0.05$). Concentration of MDA was significantly lower in plasma and liver with 0.1 g/kg EO addition and significantly higher in kidney with 1.0 g/kg EO addition in the chickens' diet ($p < 0.05$). Total antioxidant status in plasma significantly increased in the group with the diet supplemented with 0.25 g/kg EO ($p < 0.05$, Table 2).

The effect of sage oil on phagocytic activity

Blood phagocytic activity was significantly higher in chickens obtaining 0.1, 0.25 and 0.5 g/kg EO in the diet in comparison with the group fed the BD (Table 3).

The effect of sage oil on duodenal wall integrity

When TEER values were measured in the course of time, they increased over the first 12 min of intestine incubation. After this time the TEER values were stable up to 30 min. The significantly highest values were reached in the groups where 0.1 and 0.25 g/kg sage oil was added in comparison with the control group (9.10 \pm 0.48, 9.43 \pm 0.44 vs 7.20 \pm 0.49 – 6 min, 9.96 \pm 0.41, 10.06 \pm 0.48 vs 8.04 \pm 0.56 – 9 min, 10.71 \pm 0.30, 10.62 \pm 0.19 vs 8.63 \pm 0.52 M Ω – 12 min respectively; $p < 0.05$, Fig. 1).

The effect of sage oil on biochemical indices in blood plasma

Calcium significantly decreased and potassium significantly increased in the groups of animals with 0.5 and 1.0 g/kg EO in their diet ($p < 0.05$, Table 4).

Discussion

The antioxidant properties of EO are well known, but they are dependent on their concentrations. High concentrations of EO lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander et al. 1998). The antioxidant activity of EO has been the subject of many studies and their mode of action is still not clearly understood. Amensour et al. (2009) reported that it is mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. However, some reactive metabolites can initiate lipid peroxidation of polyunsaturated lipids in cells, particularly present in membranes. During lipid peroxidation, unsaturated lipids are oxidised and small fragments such as alkanes, alkenals and malondialdehyde are created. These lipid radicals are very reactive and may cause cellular damage (Castell et al. 1997, Pessayre et al. 1999).

Earlier studies on the antioxidative activity of sage were limited to its diterpenoid compounds (Cuvelier et al. 1994). According to Al-Tawaha et al. (2013) sage is very rich in phenolic compounds such as flavonoids, phenolic acids and phenolic diterpenes which are responsible for its high antioxidant activities. The term polyphenol mean phenolic compounds containing two or more phenol groups, although currently the term polyphenol is used to refer to phenolic compounds regardless of the number of phenol groups in the molecules (Jiang and Dusting 2003).

In general, metabolic biotransformation of EO compounds occurs in two phases and the final products are glucuronide and sulfate conjugates (Jager 2000). Although the liver is considered the most important organ for biotransformation, Raouf et al. (1996) and Shipkova et al. (2001) in their studies demonstrated more effective glucuronidation of phenolic compounds in kidney than in liver or intestinal microsomes. During metabolism reactive species are produced in cells, which may have a pro-oxidant action if their levels rise above the homeostatic point (Willcox et al. 2004). It is uncommon for phenolic compounds that both antioxidant and pro-oxidant activities are observed at different doses (Ferguson 2001). The highest dose of sage oil used in our experiment probably had pro-oxidant activity and significantly increased the MDA content in the kidney (Table 2).

The metabolic processes of plant compounds undergo enzymatic transformation which may generate toxic substances from an initially harmless compound. These metabolic processes differ with animal species, quantity, structure, specificity and activity of plant compounds (Cooper and Johnson 1998). We

can suppose from these studies that the highest sage oil concentration in our experiment could produce toxic substances during metabolic processing, in a quantity that could cause oxidative stress in the kidney as the predominant organ of phenolic compound glucuronidation.

Oxidative stress is characterized by abnormal quantity of reactive oxygen species in the body. Antioxidants are compounds or mechanisms that dampen or counteract oxidative stress, either by reducing the cause or the consequences of oxidative stress. The antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase serve as a primary line of defence in destroying free radicals (Gutteridge et al. 2000). It is important to keep in mind that hydroperoxides play a role in the proliferation and differentiation of cells, as well as contribute to maturation of red blood cells, and thus it is more advantageous to maintain a certain peroxide tone which is necessary for adequate functioning of cells. Regulation of the redox balance might therefore be one of the more important functions of peroxidases and of glutathione peroxidases in particular (Brigelius-Flohe 1999). We suppose that the GPx increasing in the kidney in birds with 1.0 g/kg EO addition was the answer to the lipid peroxidase enhancing. MDA increasing in the kidney in this group suggests the production of reactive oxygen species, and GPx increasing in this group probably regulates the redox balance (Table 2).

Polyphenols which contain two or more phenol groups are ubiquitous in plant foods. In general, their effectiveness in protecting against oxidative stress depends on their reactivity towards free radicals. Flavonoids, the largest group of polyphenols, contain strong antioxidants such as quercetin or catechin which can interact with intracellular antioxidative species such as glutathione peroxidase, and may enhance their antioxidative activities (Bors et al. 1990, Nagata et al. 1999). We found significant increases in TAS in plasma with 0.25 g/kg EO addition and GPx in duodenal mucosa, liver and kidney of birds receiving 0.1 and 0.25 g/kg EO in their diet (Table 2). We can suppose that components of sage oil used in our experiment could positively influence these antioxidant parameters in the way described above, when added to the diet in lower concentrations.

Mühlbauer et al. (2003) found that essential oils and their monoterpene components could affected bone metabolism. The mechanism by which monoterpenes inhibit bone resorption is not known to date. Because of their high hydrophobicity, monoterpenes may be incorporated into the cell membrane, affect cell function, impair the membrane potential. Oxidative damage of membrane potential causes entrance of calcium into the cell and escape of potass-

ium from the cell which ends in cell death (Castell et al. 1997). Loss of homeostasis in our experiment should be a result of cells oxidative damage due to the higher concentrations of EO. Maffei et al. (2001) found that after removal of the monoterpene, it would also be released from the membrane and the function of the cell would return to normal. According to Mühlbauer et al. (2003), who fed rats with sage oil, bone resorption resumed control value 52 h after the end of sage oil feeding, which suggests that the inhibition of bone resorption was not due to any toxic effect of monoterpenes. We can explain the observed significant increasing of potassium and decreasing of calcium (0.5 and 1.0 g/kg EO) in plasma in our experiment in the way described by the authors above. Moreover we also suppose that impaired cell homeostasis was not due to sage oil toxic effect, which is also confirmed by other investigated biochemical parameters in plasma which were not negatively affected by the EO concentrations used in our experiment (Table 4).

According to Taylor et al. (2003) mobilization of undifferentiated cell progenitor in circulation, immunopoietic reconstitution, and increasing neutrophil distribution were accompanied with controlling the rate of oxidant formation. Increased cell viability, cell count and distribution of neutrophils caused increases in phagocytic activity. According to Vattem et al. (2013) regulation of the redox balance is very important and more controlled respiratory burst may result from a robust antioxidant defense as a response to sage intake. Ma et al. (2005) suggested that the antioxidant properties of some plant bioactives may play a role in the development of immune response in birds by protecting cells from oxidative damage and enhancing the function and proliferation of these cells. Phagocytic activity in our experiment was significantly higher in the groups with lower concentrations of sage EO (0.1, 0.25 and 0.5 g/kg), which we can explain by the results of authors above, suggesting that lower EO concentrations in our experiment had antioxidant influence on phagocytic cells and protected them against free radicals (Table 3).

We obtained similar results in our small intestine integrity study, where TEER values showed that lower EO concentrations (0.1 and 0.25 g/kg) positively influenced the intestine epithelial barrier. The intestinal epithelium is a single layer of epithelial cells that separates the intestinal lumen from the underlying lamina propria. Approximately 80% of absorptive enterocytes are mainly intestinal epithelial cells which are tightly bound together by intercellular junctional complexes that regulate the paracellular permeability (van der Flier and Clevers 2009). According to Ma et al. (2005) the antioxidant properties of some plant

bioactive compounds are able to protect these cells from oxidative damage and enhance their function and proliferation. Jamroz et al. (2006) suggested that due to their strong antioxidant properties oregano leaf extract and EO could accelerate the renewal rate of mature enterocytes at the surface of the intestinal villi. We suppose that in our study sage oil was also able to protect intestinal epithelial cells from oxidative damage and in this way improve the paracellular permeability, which is crucial for the integrity of the epithelial barrier.

Our results indicate that lower concentrations of sage EO improve antioxidant defence through induction of antioxidant enzymes, as well as the innate immune system function by increasing phagocytic activity. Lower concentrations of EO also positively influenced gut barrier function, which might be the mechanism underlying the protective effects of sage EO against pathogen penetration, possibly meaning that animals are less exposed to microbial toxins or other undesired metabolites, so that their health may be improved this way. Our results demonstrate that it is necessary to keep in mind the selection of sufficient concentration of EO used as feed additive for animals. More studies are needed to define available levels of EO in laying strain growers for improving their health status.

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