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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

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## 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

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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 recieved BD with 0.5 g/kg EO and the fifth recieved 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 cardial 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 $cm^2$) 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 phosporous 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 (μkat/g Hb) and tissue (μkat/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 ± 0.07$^{ac}$ | 1.27 ± 0.04$^{c}$ | 1.54 ± 0.03$^{b}$ | 1.46 ± 0.06$^{bc}$ | 1.46 ± 0.05$^{bc}$ | 0.001 |
| MDA | 0.51 ± 0.03$^{a}$ | 0.40 ± 0.02$^{b}$ | 0.44 ± 0.03$^{ab}$ | 0.49 ± 0.03$^{ab}$ | 0.48 ± 0.02$^{ab}$ | 0.02 |
| Duodenal Mucosa | | | | | | |
| GPx | 0.20 ± 0.02$^{a}$ | 0.35 ± 0.04$^{b}$ | 0.23 ± 0.02$^{ab}$ | 0.24 ± 0.04$^{ab}$ | 0.22 ± 0.04$^{ab}$ | 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 ± 0.01$^{ac}$ | 0.20 ± 0.01$^{b}$ | 0.19 ± 0.01$^{b}$ | 0.18 ± 0.01$^{ab}$ | 0.14 ± 0.01$^{c}$ | <0.0001 |
| MDA | 169.2 ± 14.34$^{a}$ | 104.9 ± 10.12$^{b}$ | 125.5 ± 14.07$^{ab}$ | 111.7 ± 16.72$^{ab}$ | 123.0 ± 14.93$^{ab}$ | 0.0290 |
| Kidney | | | | | | |
| GPx | 0.26 ± 0.02$^{a}$ | 0.60 ± 0.08$^{b}$ | 0.36 ± 0.04$^{ac}$ | 0.41 ± 0.05$^{abc}$ | 0.54 ± 0.04$^{bc}$ | 0.0003 |
| MDA | 77.36 ± 4.14$^{ab}$ | 99.78 ± 5.48$^{a}$ | 68.56 ± 4.89$^{b}$ | 82.67 ± 6.60$^{ab}$ | 148.80 ± 7.97$^{c}$ | <0.0001 |

Notes: a,b,c Means in the same row with different superscripts are significantly different ($p<0.05$), values are means ± 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±0.22$^{a}$ | 42.50±0.43$^{b}$ | 42.17±0.40$^{b}$ | 42.00±0.37$^{b}$ | 41.50±0.34$^{ab}$ | 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 ± 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 concentration 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 ± 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.

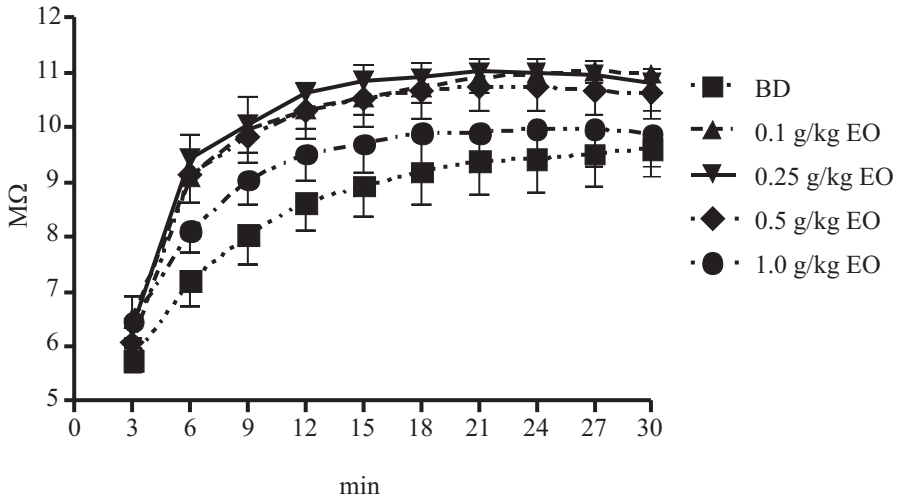


Fig. 1. Transepithelial electrical resistance values in duodenal tissue of laying strain growers.
Notes: BD – basal diet, EO – BD with *S. officinalis* essential oil supplementation. Values are means ± SEM (n=8).

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 (μkat/l) | 21.98 ± 1.76 | 14.33 ± 0.87 | 20.73 ± 2.40 | 19,81 ± 1.75 | 22.85 ± 2.76 | 0.13 |
| AST (μkat/l) | 1.01 ± 0.04 | 1.09 ± 0.06 | 1.03 ± 0.05 | 0.97 ± 0.03 | 0.91 ± 0.03 | 0.11 |
| Cholesterol (mmol/l) | 1.62 ± 0.05 | 1.23 ± 0.08 | 1.41 ± 0.15 | 1.84 ± 0.28 | 1.80 ± 0.12 | 0.05 |
| Triglyceridy (mmol/l) | 0.31 ± 0.05 | 0.24 ± 0.04 | 0.23 ± 0.03 | 0.38 ± 0.06 | 0.35 ± 0.05 | 0.06 |
| Glukóza (mmol/l) | 17.10 ± 1.04 | 21.65 ± 2.37 | 16.25 ± 1.49 | 15.53 ± 0.87 | 16.36 ± 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 ± 0.35 | 2.21 ± 0.30 | 2.18 ± 0.10 | 2.18 ± 0.16 | 2.77 ± 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 ± 0.08 | 0.85 ± 0.08 | 0.99 ± 0.15 | 0.74 ± 0.08 | 0.94 ± 0.07 | 0.28 |
| Total protein (g/l) | 33.67 ± 1.17 | 32.82 ± 3.84 | 33.77 ± 2.31 | 39.63 ± 1.30 | 35.81 ± 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 ± 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±0.48, 9.43±0.44 vs 7.20±0.49 – 6 min, 9.96±0.41, 10.06±0.48 vs 8.04±0.56 – 9 min, 10.71±0.30, 10.62±0.19 vs 8.63±0.52 MΩ – 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, Pessayer 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, Raoof 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 effectivness 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 glutathion 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. Aproximately 80% of absorptive enterocytes are mainly intestinal epithelial cells which are tightly bound together by intercellular junctional complexes that regulate the paracellulal 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 demage 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 demage 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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## References

Acamovic T, Brooker JD (**2005**) Biochemistry of plant secondary metabolites and their effects in animals. Proc Nutr Soc 64: 403-412.

Al-Tawaha A, Al-Karaki G, Massadeh A (**2013**) Antioxidant activity, total phenols and variation of chemical composition from essential oil in sage (*Salvia officinalis* L.) grown under protected soilless condition and open field conditions. Adv Environ Biol 7: 894-901.

Amensour M, Sendra E, Abrini J, Bouhdid S, Perez-Alvarez JA, Fernandez-Lopez J (**2009**) Total phenolic content and antioxidant activity of myrtle (Myrtus communis) extracts. Nat Prod Commun 4: 819-824.

Bors W, Heller W, Michel C, Saran M (**1990**) Flavonoids as antioxidants – determination of radical – scavenging efficiencies. Methods Enzymol 186: 343-355.

Bradford MM (**1976**) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing

the principle of protein-dye binding. Anal Biochem 72: 248-254.

Brigelius-Flohé R (**1999**) Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 27: 951-965.

Bubel F, Dobrzański Z, Gaweł A, Pogoda-Sewerniak K, Grela ER (**2015**) Effect of humic-plant feed preparations on biochemical blood parameters of laying hens in deep litter housing system. Pol J Vet Sci 18: 131-139.

Castell JV, Gomez-Lechon MJ, Ponsoda X, Bort R (**1997**) *In vitro* investigation of the molecular mechanisms of hepatotoxicity. In: Castell JV, Gomez-Lechon MJ (eds) In vitro Methods in Pharmaceutical Research. Academic Press, London, pp 375-410.

Cooper MR, Johnson AW (**1998**) Poisonous plants and fungi in Britain: Animal and Human Poisoning, 2 nd ed., Norwich, The Stationary Office, pp 202-205.

Cuvelier ME, Berset C, Richard H (**1994**) Antioxidant constituents in sage (*Salvia officinalis*). J Agric Food Chem 42: 665-669.

European Food Safety Authority (**2012**) Scientific Opinion on the safety and efficacy of phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group (chemical group 25) when used as flavourings for all species. EFSA Journal 10: 2573.

Ferguson LR (**2001**) Role of plant polyphenols in genomic stability. Mutat Res 475: 89-111.

Franz C, Baser KHC, Windisch W (**2010**) Essential oils and aromatic plants in animal feeding – a European perspective. A review. Flavour Frag J 25: 327-340.

Gutteridge JM, Halliwell B (**2000**) Free radicals and antioxidants in the year 2000. A historical look to the future. Ann NY Acad Sci 899: 136-147.

Halliwell B, Gutteridge JMC (**1999**) Free radicals in Biology and Medicine, 3rd ed., Oxford University Press, New York, pp 617-783.

Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, von Wright A (**1998**) Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem 46: 3590-3595.

Henneke P, Golenbock DT (**2004**) Phagocytosis, innate imunity, and host-pathogen specifity. J Exp Med 199: 1-4.

Jager W, Mayer M, Platzer P, Reznicek G, Dietrich H, Buchbauer G (**2000**) Stereoselective metabolism of the monoterpene carvone by rat and human liver microsomes. J Pharm Pharmacol 52: 191-197.

Jamroz D, Wertelecki T, Houszka M, Kamel C (**2006**) Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. J Anim Physiol Anim Nutr 90: 255-268.

Jiang F, Dusting GJ (**2003**) Natural phenolic compounds as cardiovascular therapeutics: potential role of their antiinflammatory effects. Curr Vasc Pharmacol 1: 135-156.

Jo C, Ahn DU (**1998**) Fluorometric analysis of 2-thiobarbituric acid reactive substances in turkey. Poult Sci 77: 475-480.

Laskin DL, Pilaro AM (**1986**) Potential role of activated macrophages in acetaminophen hepatotoxicity. 1. Isolation and characterization of activated macrophages from rat liver. Toxicol Appl Pharmacol 86: 204-215.

Ma DY, Shan AS, Chen ZH, Du J, Song K, Li JP, Xu QY (**2005**) Effect of *Ligustrum lucidum* and *Schisandra chinensis* on the egg production, antioxidant status and immunity of laying hens during heat stress. Arch Anim Nutr 59: 439-447.

Maffei M, Camusso W, Sacco S (**2001**) Effect of Mentha x Piperita essential oil and monoterpenes on cucumber root membrane potential. Phytochemistry 58: 703-707.

Mannon P (**2005**) Normal Gut Mucosal Immunity: A Dynamic Balance of Tolerance and Defence. Gastroenterol Hepatol 1: 50-56.

Miura K, Kikuzaki H, Nakatani N (**2002**) Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. J Agric Food Chem 50: 1845-1851.

Muhlbauer RC, Lozano A, Palacio S, Reinli A, Felix R (**2003**) Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. Bone 32: 372-380.

Nagata H, Takekoshi S, Takagi T, Honma T, Watanabe K (**1999**) Antioxidative action of flavonoids, quercetin and catechin, mediated by the activation of glutathione peroxidase. Tokai J Exp Clin Med 24: 1-11.

Nikawa T, Odahara K, Koizumi H, Kido Y, Teshima S, Rokutan K, Kishi K (**1999**) Vitamin A Prevents the Decline in Immunoglobulin A and Th2 Cytokine Levels in Small Intestinal Mucosa of Protein-Malnourished Mice. J Nutr 129: 934-941.

Paglia DE, Valentine WN (**1967**) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70: 158-169.

Pessayre D, Larrey D, Biour M (**1999**) Drug- induced liver injury. In: Bircher J, Benhamou JP, McIntyre N, Rizzeto M, Rodes J (eds) Oxford Textbook of Clinical Hepatology. Oxford University Press, Oxford, pp 1261-1315.

Raoof AA, van Obbergh LJ, de Goyet JD, Verbeeck RK (**1996**) Extrahepatic glucuronidation of propol in man: possible contribution of gut wall and kidney. Eur J Clin Pharmacol 50: 91-96.

Rodriguez EM, Sanz MT, Romero CD (**1994**) Critical study of fluorometric determination of selenium in urine. Talanta 41: 2025-2031.

Shipkova M, Srassburg CP, Braun F, Streit F, Gröne HJ, Armstrong VW, Tukey RH, Oellerich M, Wieland E (**2001**) Glucuronide and glucoside conjugation of mycophenolic acid by human liver, kidney and intestinal microsomes. Br J Pharmacol 132: 1027-1034.

Steruska M (**1981**) Tests for the investigation of leukocyte function. In: Hrubisko M, Steruska M (eds) Hematology and Transfusiology. Osveta Martin, Slovakia, pp 228-236.

Taylor EL, Megson IL, Haslett C, Rossi AG (**2003**) Nitric oxid: a key regulator of myeloid inflammatory cell apoptosis. Cell Death Differ 10: 418-430.

Ussing HH, Zerahn K (**1951**) Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol Scand 23: 110-127.

van der Flier LG, Clevers H (**2009**) Stem cells, self-renewal and differentiation in the intestinal epithelium. Annu Rev Physiol 71: 241-260.

Vattem DA, Lester Ce, Deleon Rc, Jamison By, Maitin V (**2013**) Dietary supplementation with two Lamiaceae herbs-(oregano and sage) modulates innate imunity par-

ameters in *Lumbricus terrestris*. Pharmacognosy Res 5: 1-9.

Ward PD, Tippin TK, Thakker DR (**2000**) Enhancing paracellular permeability by modulating epithelial tight junctions. Pharm Sci Technol To 3: 346-358.

Wencelová M, Váradyová Z, Mihaliková K, Jalč D, Kišidayová S (**2014**) Effects of selected medicinal plants on rumen fermentation in a high-concentrate diet in vitro. J Anim Plant Sci 24: 1388-1395.

Willcox JK, Ash SL, Catignani GL (**2004**) Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 44: 275-295.