

EVALUATION OF THE EFFECTS OF *SALVIA OFFICINALIS* ESSENTIAL OIL ON PLASMA BIOCHEMISTRY, GUT MUCUS AND QUANTITY OF ACIDIC AND NEUTRAL MUCINS IN THE CHICKEN GUT

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In this study the effects of *Salvia officinalis* L. essential oil on the thickness of the gut mucus layer and quantity of neutral and acidic mucins in chickens were observed. One-day-old chickens of Isa Brown breed were divided into five groups (n = 9) and fed 11 weeks as follows: control group (C): basal diet; experimental groups (E1 – E4) – same as C + sage essential oil (EO) in concentrations of 0.01%; 0.025%; 0.05% and 0.1%, respectively. The thickness of the mucus layer in the duodenum significantly increased in E3(0.05% sage EO) compared to E1 (0.01 % sage EO). In E3 (0.05% sage EO) the number of goblet cells containing acidic and neutral mucins was significantly decreased in the duodenum and jejunum and increased in the ileum compared to C. Feeding the diet supplemented with Se and 0.01% sage EO (E1) decreased plasma cholesterol level in comparison with E3(0.05 % sage EO). The addition of 0.05% (E3) and 0.1% sage EO (E4) to the diet caused a decrease in calcium plasma level compared to E2 (0.025 % sage EO). Plasma glucose level was significantly decreased in groups fed 0.05% (E3) and 0.025% sage EO (E2) compared with 0.01% sage (E1). The weight of internal organs was not affected by the diets. Our results suggest that effects of sage EO on the adherent mucus layer dynamics and mucin type distribution in the chicken intestine are dependent on sage EO dose and intestinal segment and there is still a need for further studies in order to obtain a plausible explanation.

Key words: intestine, mucus, mucin, poultry, sage

INTRODUCTION

The epithelium of the intestinal tract is covered by a layer of mucus composed predominantly of mucin glycoproteins that are synthesized and secreted by goblet cells [1]. The mucus layer acts as a medium for protection, lubrication, transport, a physical barrier and a trap for microbes [2]. Histologically mucins can be separated into two broad categories: neutral and acidic. Mucin subtype and goblet cell distribution vary throughout the gastrointestinal tract.

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The thickness of the mucus adherent layer is affected by the rate of mucin secretion and by the rate of mucin degradation. Mucin secretion occurs *via* both constitutive and regulated pathways. The constitutive pathway continuously secretes sufficient mucin to maintain the mucus layer, whereas the regulated pathway results in a massive discharge as a response to various stimuli, including cholinergic stimuli, inflammatory cytokines, prostaglandins, lipopolysaccharides, bile salts, nucleotides, nitric oxide, vasoactive intestinal peptide, neutrophil elastase [3] and diet [1].

Degradation of mucin occurs by the mechanical shear forces of peristalsis and by degrading enzymes. The mucosal barrier is constantly renewed and can potentially be rapidly adjusted to changes in the (patho) physiological stimuli.

The use of antibiotics as growth promotors in animal feedstuffs is facing a reduced social acceptance and therefore the research for alternative feed supplements has been increased. Attention has been turned to essential oils of herbs and spices. There is evidence that essential oils have modulatory effects on the animal digestive system [4, 5]. Sage plants belong to the *Lamiaceae* family, and their EOs are known as inhibitors of inflammation, suppressors of blood glucose levels and strong antioxidants [6].

The aim of this study was to investigate the effect of dietary sage supplementation on gut mucus dynamics, blood biochemical indices and performance of chickens.

MATERIALS AND METHODS

Animals and experimental groups

Forty-five one-day-old female chickens of the laying strain Isa Brown (Párovské Háje, Slovakia hatchery) were used. They were divided into five groups ($n = 9$) and fed as follows: control group (C): basal diet (BD) + sodium selenite (Se) (0.4 ppm) + sunflower oil (SO) (1 %); experimental groups (E1 – E4) – same as C + sage essential oil (EO) in concentrations of 0.01 %; 0.025 %; 0.05 % and 0.1 %, respectively, for 11 weeks. The sage EO was dissolved in SO and then gently added to BD to reach the concentrations mentioned above. The birds were kept in group wooden cages with wooden shavings. The temperature and lightning regimen were in accordance with the recommendation of the breeder. All groups received the feed and water *ad libitum*. The composition of the basal diet supplemented with selenium as sodium selenite is shown in Table 1. The composition of the EO from the leaves of *Salvia officinalis* L., reported by the producer Calendula a.s., Nová Ľubovňa, Slovakia, was as follows: camphor 14.9 %, α -thujone 14.8 %, eucalyptol 8.5 %, β -thujone 7.2 %, borneol 3.7 %.

At the end of the experiment the chickens were anaesthetised by an intraperitoneal injection of xylazine (Rometar 2 %, Spofa, Czech Republic) and ketamine (Narkamon 5 %, Spofa, Czech Republic) at 0.6 and 0.7 ml.kg⁻¹ of body weight, respectively. The blood for biochemical measurements was collected into heparinized test-tubes and parameters (phosphorus, calcium, magnesium, AST, cholesterol, triglycerides and glucose) were determined by the colorimetric method using spectrophotometric kits (Randox lab, Ardmore, UK) and measured with Genesys 10 UV spectrophotometer. After laparotomy, internal body organs (liver without gall bladder, spleen, pancreas and empty gizzard without cuticula) were weighed and intestinal sections were collected.

Table 1. Composition of the basal diet – nutritional analysis per 1 kg

Ingredient	Amount	Ingredient	Amount
N x 6.25	min. 190 g	Co	0.4 mg
Crude ME	min. 12 MJ/kg	Se	0.1 + 0.4 mg
Ash	max. 50 g	Vitamin A	min. 12 000 IU
Fiber	max. 35 g	Vitamin D3	min. 4 000 IU
Lysine	min. 905 g	Vit. E (α -tocopherol)	min. 100 IU
Methionine	min. 4 g	Vitamin K3	4 mg
Cystine	min. 3.5 g	Vitamin B1	3 mg
Linoleic acid	min. 10 g	Vitamin B2	min. 9 mg
Ca	min. 7 g	Vitamin B6	6 mg
P	min. 5 g	Vitamin B12	min. 40 μ g
Na	1.5 g	Vitamin C	200 mg
Mn	min. 110 mg	Nicotinic acid	60 mg
Fe	min. 120 mg	Calcium pantothenate	15 mg
Cu	min. 15 mg	Folic acid	2 mg
Zn	min. 100 mg	Biotine	0.2 mg
I	1 mg	Choline	500 mg

Determination of gut mucus adherent layer

The gut samples (duodenum, jejunum, ileum and caecum) were processed for mucus determination using a method by Smirnov *et al.* [1], modified by Thompson and Applegate [7] and Faixová *et al.* [8]. The optical density of samples and standards was measured by ELISA reader (Opsys MR™ Microplate Reader, Dynex Technologies INC., USA) at 630 nm wavelength. The amount of the adherent gut mucus stained by alcian blue (AB) (AppliChem GmbH, Germany) was calculated by a standard curve and expressed as μ g AB.cm² of gut.

Determination of mucins

One - cm long middle segments of the duodenum, jejunum, ileum and caecum were removed, gently flushed with 0.9 % (wt/vol) NaCl and fixed in 10 % (vol/vol) formaldehyde for further processing. Segments were dehydrated, cleared, embedded in paraffin and cut into 3 μ m serial cross sections. Sections were deparaffinized in xylene and rehydrated in a graded alcohol series. Finally, they were stained with periodic acid-Schiff (PAS) (Acros Organics, New Jersey, USA; Merck KGaA, Darmstadt, Germany), alcian blue (AB) (AppliChem GmbH, Germany) and combined AB/PAS staining [9]. Three sections for each staining were prepared to examine each individual intestinal segment.

Image analysis

For the PAS-, AB- and AB/PAS-stained sections, the color images were taken with a light microscope Nikon ECLIPSE E600 with Nikon digital camera DXM1200 and transferred to a TV monitor. From each section and each staining, 10 photos were taken and analyzed with Adobe Photoshop CS2 9.0. The surface area recorded on each photo was 0.09045 mm². The goblet cells in the villi were counted on the mentioned area

within each section and each staining. The number of goblet cells stained AB and PAS was converted to the area of 1 mm².

Statistical analysis

The results are expressed as means ± S.E.M. The statistical analysis was done by a one-way analysis of variance (ANOVA) with Tukey *post-hoc* multiple comparison test (GraphPad Software, USA).

The experiments were carried out in accordance with the established standards for the use of experimental animals. The protocol was approved by the local ethics and scientific authorities.

RESULTS

Measurement of the mucus adherent layer thickness

Feeding diet supplemented with Se and 0.05% sage EO (E3) significantly increased the thickness of the mucus layer in the duodenum compared with the group fed Se and 0.01% sage EO diet (E1) (Table 2).

Table 2. Effect of feeding the diet supplemented with selenium and *Salvia officinalis* L. essential oil on the thickness of gut mucus layer of chickens (µg AB.cm⁻² of gut)

Segment of gut	C	E1	E 2	E3	E4
	0.4 ppm Se+ SO	0.4 ppm Se+ SO+ 0.01% sage	0.4 ppm Se+ SO+ 0.025% sage	0.4 ppm Se+ SO+ 0.05% sage	0.4 ppm Se+ SO+0.1% sage
Duodenum	5.93 ± 0.22	4.81 ± 0.22 ^a	6.39 ± 0.34	7.27 ± 0.44 ^a	6.13 ± 0.37
Jejunum	5.15 ± 0.21	5.1 ± 0.25	6.47 ± 0.46	5.96 ± 0.33	6.37 ± 0.45
Ileum	5.33 ± 0.31	4.84 ± 0.21	6.29 ± 0.29	6.09 ± 0.34	6.28 ± 0.4
Caecum	5.48 ± 0.33	4.83 ± 0.29	5.23 ± 0.24	5.32 ± 0.33	6.22 ± 0.35

Significant differences within a row are indicated by the same superscript (a) at P < 0.05 level; mean ± S.E.M., n = 9; AB = alcian blue; SO = sunflower oil

Quantification of cells containing acidic and neutral mucins

In E3 (0.05% sage EO) the duodenal and jejunal goblet cells exhibited a marked decrease in the number of cells containing both acidic and neutral mucins (Figure 1), while in the ileal goblet cells the increase of both types of mucins was observed when compared to the control group (P < 0.05). The increase of number of cells containing acidic mucins in the duodenum and ileum was observed in E1 (0.01% sage EO) and E2 (0.025% sage EO), respectively (P < 0.05) when compared to the control. On the other hand, the number of cells containing acidic mucins in the ileum of E4 (0.1% sage EO) and neutral mucins in the duodenum and jejunum of E4 (0.1% sage EO) and E2 (0.025% sage EO), respectively, significantly decreased compared to control (P < 0.05). The number of goblet cells containing acidic and neutral mucins in the caecum did not differ significantly between groups (Table 3).

Table 3. Comparison between the mean goblet cell numbers containing acidic and neutral mucin in the chickens gut¹

Segment of gut	C						E1		E2		E3		E4	
	0.4 ppm Se+SO		0.4 ppm Se + SO +0.01% sage		0.4 ppm Se + SO +0.025% sage		0.4 ppm Se + SO +0.05% sage		0.4 ppm Se + SO +0.1% sage		0.4 ppm Se + SO +0.05% sage		0.4 ppm Se + SO +0.1% sage	
	Acidic	Neutral	Acidic	Neutral	Acidic	Neutral	Acidic	Neutral	Acidic	Neutral	Acidic	Neutral	Acidic	Neutral
Duodenum	1.187±0.066 ^{ab}	1.148±0.156 ^{cd}	1.702±0.098 ^a	1.424±0.106	1.202±0.059	0.910±0.049	0.390±0.050 ^b	0.363±0.041 ^c	0.811±0.033	0.691±0.038 ^d	0.363±0.041 ^c	0.363±0.041 ^c	0.811±0.033	0.691±0.038 ^d
Jejunum	1.171±0.059 ^a	1.200±0.053 ^{bc}	1.269±0.100	1.291±0.043	0.805±0.036	0.713±0.03 ^b	0.645±0.054 ^c	0.602±0.054 ^c	1.133±0.028	1.097±0.054	0.602±0.054 ^c	0.602±0.054 ^c	1.133±0.028	1.097±0.054
Ileum	1.933±0.168 ^{abc}	1.525±0.130 ^d	1.717±0.162	1.726±0.069	2.520±0.085 ^a	1.411±0.097	2.458±0.103 ^b	2.200±0.072 ^d	1.205±0.057 ^c	1.385±0.065	2.200±0.072 ^d	2.200±0.072 ^d	1.205±0.057 ^c	1.385±0.065
Caecum	0.394±0.022	0.214±0.024	0.295±0.049	0.265±0.025	0.348±0.012	0.297±0.024	0.241±0.031	0.127±0.001	0.174±0.001	0.135±0.004	0.127±0.001	0.127±0.001	0.174±0.001	0.135±0.004

¹Values are number of goblet cells (x 10³)/mm² expressed as mean ± S.E.M., n = 9; Se = selenium; SO = sunflower oil. Significant differences within a row are indicated by the same superscripts (a,b,c, d) at P < 0.05 level

Blood analysis

The addition of sage extract (0.05 and 0.1%) to the Se –supplemented diet led to a decreased level of plasma calcium compared to E2 (0.025% sage EO) (Table 4) (Ca reference values: 2.09 – 2.52 mmol.l⁻¹) [10]. Feeding diet supplemented with 0.01% sage EO and Se caused a significant decrease in plasma cholesterol level compared to E3 (0.05% sage EO) (cholesterol reference values: 1.90 – 2.40 mmol.l⁻¹) [10].

Table 4. Effect of feeding the diet supplemented with selenium and *Salvia officinalis* L. essential oil on biochemical parameters in the plasma of chicks.

	C 0.4 ppm Se+ SO	E1 0.4 ppm Se+ SO+ 0.01% sage	E2 0.4 ppm Se+ SO+ 0.025% sage	E3 0.4 ppm Se+ SO+ 0.05% sage	E4 0.4 ppm Se+ SO+ 0.1% sage
Glucose (mmol.l ⁻¹)	19.85±1.51	24.13±2.28 ^{ab}	15.53±1.45 ^b	16.9±1.1 ^a	18.6±0.9
Ca (mmol.l ⁻¹)	2.47±0.16 ^{cd}	1.82±0.07	2.19±0.13 ^{ab}	1.39±0.03 ^{bc}	1.5±0.1 ^{ad}
P (mmol.l ⁻¹)	2.01±0.01	1.98±0.21	2.1±0.07	2.08±0.15	2.60±0.11
AST (μkat.l ⁻¹)	0.96±0.03	1.04±0.05	0.94±0.05	0.93±0.02	0.9±0.03
Mg (mmol.l ⁻¹)	0.68±0.05	0.73±0.04	0.85±0.05	0.69±0.07	0.84±0.04
Triglycerides (mmol.l ⁻¹)	0.28±0.05	0.23±0.03	0.20±0.02	0.38±0.06	0.35±0.05
Cholesterol (mmol.l ⁻¹)	1.78±0.12	1.23±0.08 ^a	1.52±0.12	2.28±0.3 ^a	1.98±0.15

Significant differences within a row are indicated by the same superscripts (a,b,c,d) at P < 0.01 level; mean ± S.E.M., n = 9; SO = sunflower oil

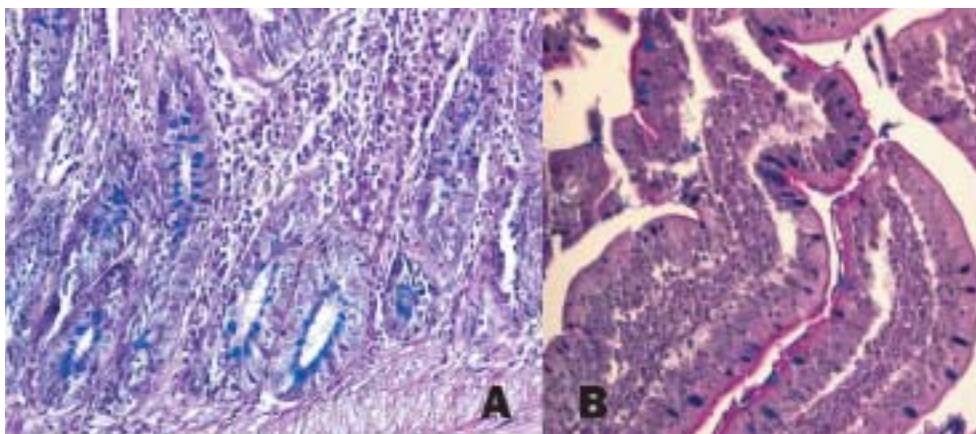


Figure 1. The effect of dietary sage on the number of cells containing acid and neutral mucins in jejunum, PAS (B). Decrease of the number of goblet cells containing acid and neutral mucins in E3 (0.05% sage) compared to control, AB (A)

Plasma glucose level was significantly decreased in groups of chicks fed the diet supplemented with Se and 0.05 % and 0.025% sage in comparison with group fed

diet with Se and 0.01 % sage EO (glucose reference values: 11.5 – 18.0 mmol.l⁻¹ [10]. Plasma phosphorus, magnesium and triglycerides levels and AST enzyme activity did not differ significantly between treatments (phosphorus, magnesium, triglycerides and AST reference values: 1.60 – 2.10 mmol.l⁻¹, 0.75 – 1.3 mmol.l⁻¹, 0.30 – 0.50 mmol.l⁻¹ and 1.09 – 1.20 μ kat.l⁻¹, respectively) [10].

There were no statistically significant differences in the weights of internal organs.

DISCUSSION

The gastrointestinal mucosa is constantly exposed to luminal oxidants from the ingested food. The small intestine is covered with a thinner or discontinuous mucus layer compared to the colon. In our study, the feeding diet supplemented with 0.05% sage EO significantly increased the thickness of the mucus layer in the duodenum compared with the group fed 0.01% sage EO diet (Table 2). This effect could be explained by initiating a higher rate of mucin production in the intestinal mucosa by sage treatment. The observed increase in the mucus layer thickness could be considered as beneficial and could indicate a dietary sage protective role. Our results are similar to the work of Tsirtsikos *et al.* [11] who observed a linear increase of the duodenal mucus layer thickness with increasing phytogetic feed additive dietary levels (carvacrol, anethol and limonene at concentrations of 115 g.kg⁻¹ feed additive) in broiler chickens. According to Jamroz *et al.* [12], in groups of chickens fed with plant extract mixture (containing carvacrol, cinnamaldehyde and capsaicin) showed an increased release of large amounts of mucus and the creation of a thick layer of mucus on the wall of the jejunum.

The acidic mucins are reported to dominate in the large intestine and play a considerable role in innate immunity as they predominate in fetal and early life stages. Later, they play an important role in the local defense against pathogens. Some bacteria possess mucin-specific glycosidases and proteases which are able to degrade mucus and facilitate colonization of the epithelial surface [13]. The hydroxyl groups of sialic acid are highly substituted by acetyl esters which serve as added protection as they block against further glycosidic degradation, with reports that 2 or more acetyl groups inhibit enteric bacterial sialidases [13].

Feeding the diet supplemented with 0.05 % sage EO caused an increase of numbers of cells containing both acidic and neutral mucins in the ileum. However, in the duodenum and jejunum of E3 (0.05% sage EO) they were decreased. In the study of Forder *et al.* [14], a higher number of goblet cells containing acidic mucin in the ileum was observed compared to the jejunum. Similarly, in the current study, a greater amount of acidic mucins was observed in ileal goblet cells within the groups compared to the duodenum and jejunum.

The neutral mucins are reported to dominate in the gastric mucosa, however, in mammals, Sakata and Engelhardt [15] reported that neutral mucin formed the main constituents of luminal mucin in the caecum and proximal colon of mice, rats and guinea pigs. Forder *et al.* [14] observed an increase in goblet cells containing neutral mucin in the ileum from day 4 to day 7 posthatch in chicks compared to jejunum. Another report has demonstrated a distal increase in the density of goblet cells along the duodenal-ileal axis [16]. Similarly, in our study, the number of neutral mucins was

much higher in the ileum within the groups in comparison with the duodenum and jejunum. On the other hand, the mean values of intestinal goblet cells containing the neutral mucin were lower in the experimental groups compared to the control.

Blood analysis showed that addition of sage extract (0.025% and 0.05%) to the basal diet had a lowering effect on the level of glucose compared to the control. The hypoglycaemic effect of sage extract has been reported in chickens [17], rats [18] and mice [19]. There are reports that sage increases hepatocyte sensitivity to insulin and inhibits gluconeogenesis [19].

Our results showed that feeding diet supplemented with sage EO had a reducing effect on plasma calcium levels. This is consistent with our previous finding on chicks fed a diet supplemented with graded levels of borneol (constituent of many herbal essential oils including sage), which has demonstrated a reducing effect of borneol on plasma calcium levels [20]. The effects of some herbs (sage, rosemary and thyme) and their constituent EOs and monoterpenes on bone resorption were tested in ovariectomized rats [21]. Bone resorption was inhibited by the addition of 1 g of powdered leaves of each herb, and the EOs extracted from sage and rosemary also inhibited resorption. Pure components from the EOs were also studied and a mixture of the four major monoterpenes which occur in sage oil (thujone, eucalyptol, camphor and borneol in proportion similar to that found in the natural oil) and were shown to inhibit bone resorption in a similar manner to the unmodified sage extract. Experiments were also carried out *in vitro* on osteoclasts and it was found that borneol, thymol and camphor all inhibited bone resorption at a concentration of 1.0 mmol.l⁻¹. It is thought that these monoterpenes act directly on osteoclasts, not *via* the stimulation of calciotropic hormones, and the mode of action is thought to be *via* inhibition of the mevalonate pathway and prenylation of small G- proteins such as Ras, Rho and Rac [21]. 3- carene, bicyclic monoterpene in the essential oil extracted from pine trees, was shown to effect osteoblastic differentiation of mouse osteoblastic MC3T3-E1 subclone 4 cells [22]. At low concentrations of 3- carene was shown to stimulate significantly the activity and expression of alkaline phosphatase, an early phase marker of osteoblastic differentiation on differentiation day 9. On day 15, it dramatically promoted the induction of calcium in a dose – dependent manner. Although these studies report apparently contradictory data, they should not be considered to be mutually exclusive, since bone metabolism is a complex multifactorial process and it is known that stimuli can give rise to anabolic or catabolic effects depending on dosage, timing, and site of application [23]. In addition, calcium level also depends on albumin level and pH. It is known that a minor drop of calcium exists because of a decrease in albumin level. Surprisingly, there were no differences between groups in plasma phosphorus levels. In addition to the role of the intestinal mucus layer as a defensive barrier, there is evidence suggesting that mucin has a major effect on the absorption of cations. Metal ions traverse the mucosally adherent mucus layer with an efficiency of $M^+ > M^{2+} > M^{3+}$ before transport by membrane proteins into the enterocytes. Thus Ca^{2+} binds directly to anions of goblet cell mucin before uptake by the enterocytes [24]. We might suggest that sage essential oils should have a beneficial effect on bone mineralization.

Our previous experiments showed that feeding a diet supplemented with graded levels

(0; 0.1; 0.05 and 0.025%) of essential oil from rosemary leaves (*Rosmarini aetheroleum* of *Rosmarinus officinalis* L.) of the Spanish type (main constituents are as follows: α -pinene 18 – 26%, camphene 8 – 12%, cineole 16 - 25%, camphor 13 – 21%, borneol 2 – 4.5%) caused a significantly lower plasma calcium level in broiler chickens and the effect of rosemary essential oil on plasma calcium level tended to be dose- dependent [25].

Our results showed that feeding a diet supplemented with sage had a modulatory effect on plasma cholesterol level. There are several reports on *in vivo* and *in vitro* metabolic studies on herbal essential oils rich in small aromatic terpenoids that have been shown to reduce plasma cholesterol level by downregulating 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) expression [26].

In the present study, no significant difference in the relative weight of the internal organs was observed, which is in agreement with Hernández *et al.* [27]. Similar results were reported by Çabuk *et al.* [28]. They found out that dietary herbal EO mixture had no effect on body weight of broiler chicks.

In conclusion, the results of our study showed that sage EO in chickens increased the mucus layer thickness in duodenum, increased the ileal number of goblet cells containing acidic and neutral mucins, modulated plasma cholesterol, glucose and calcium levels and did not influence the relative weights of internal organs. This study contributes to the knowledge on the impact of sage on the gut, plasma indices and performance of chickens. The interactions between sage essential oil, mucin dynamics and biochemistry changes in chickens need to be further examined and better explained.

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EVALUACIJA UTICAJA ESENCIJALNOG ULJA *SALVIA OFFICINALIS* NA BIOHEMIJSKE VREDNOSTI U PLAZMI, SLUZNICU CREVA I KVANTITET KISELIH I NEUTRALNIH MUCINA U CREVIMA PILIĆA

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Studija se odnosi na posmatranje efekata *Salvia officinalis* L. esencijalnih ulja na debljinu sluznice creva i kvantitet neutralnih i kiselih mucina kod pilića. Formirano je pet grupa sa po 9 jednodnevni pilića rase Isa Brown koji su u narednih 11 nedelja hranjeni na sledeći način: kontrolna (C) grupa je dobijala osnovni obrok sa dodatkom Na selenita (Se) u koncentraciji 0,04 ppm + suncokretovo ulje (1%); eksperimentalne grupe E1 do E4: isto kao C grupa uz dodatak esencijalnog ulja žalfije (EO) u koncentraciji 0,01%, 0,025; 0,05% odnosno 0,1%. Debljina sloja mukusa u duodenumu je značajno porasla kod pilića E3 grupe (Se i 0,05% EO ulja) u poređenju sa E1 grupom (Se i 0,01% EO ulja). U E3 grupi (Se i 0,05% EO ulja) broj peharastih ćelija koje sadrže kisele i neutralne mucine je značajno bio smanjen u duodenumu i jejunumu, a povećan u ileumu u poređenju sa C grupom. Ishrana sa dodatkom Se i 0,01% EO ulja žalfije (E1) izazivala je smanjenje koncentracije holesterola u plazmi u poređenju sa E3 (Se i 0,05% EO žalfije). Dodavanje Se i 0,05% EO žalfije (E3) kao i 0.1% EO ulja (E4) izazvalo je smanjenje koncentracije kalcijuma u plazmi u poređenju sa E2 (Se i 0,025% EO ulja). Koncentracije glukoze u plazmi su značajno bile smanjene u grupama koje su dobijale Se i 0,05% (E3) i 0,025% EO (E2) ulja žalfije, u poređenju sa E1 grupom (0,01% EO ulja). Različiti sastavi obroka po grupama nisu imali uticaja na mase unutrašnjih organa pilića u ogledu. Rezultati ukazuju da efekat EO žalfije na dinamiku adherencije sloja mukusa kao i na tip i distribuciju mucina u crevima pilića zavisi od doze EO kao i od segmenta creva uz istovremeno postojanje potrebe da se obave dalja ispitivanja u cilju detaljnog objašnjenja ovog uticaja.