The effect of essential plant oils on mineral composition of egg mass and blood parameters of laying hens

Vplyv esenciálnych rastlinných olejov na minerálne zloženie vaječnej hmoty a krvné parametre nosníc

Róbert HERKEĽ[1], Branislav GÁLIK[1*], Daniel BÍRO[1], Michal ROLINEC[1], Milan ŠIMKO[1], Miroslav JURÁČEK[1], Henrieta ARPÁŠOVÁ[2] and Ondrej HANUŠOVSKÝ[1]

[1]Department of Animal Nutrition, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, Nitra, Slovak Republic

[2]Department of Poultry Science and Small Animal Husbandry, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, Nitra, Slovak Republic *correspondence: Branislav.Galik@uniag.sk

Abstract

The aim of study was to analyze the dietary effect of pumpkin and flaxseed oils on mineral composition of egg mass and blood parameters of laying hens. At 38 weeks of age, Lohmann Brown Lite hens were housed in three-floor cages, divided into three dietary groups (C-control, E1-pumpkin oil (3%), E2-flaxseed oil (3%)). There were housed six hens in one cage. A total 18 hens were monitored. In the control group hens were fed with standard complete feed mixture for laying hens and in the experimental groups by feed mixtures with supplementation of pumpkin or flaxseed oils. Vitamin E was added into feed mixture in the experimental groups. The experiment lasted 52 days. Twelve eggs from each dietary treatment were randomly selected and analyzed. As regards the mineral composition of eggs, only concentrations of calcium after both oil supplementations and zinc after flaxseed oil supplementation in diet were significantly ($P<0.05$) higher in yolk. Contents of magnesium in yolk and albumen and phosphorus in albumen were markedly ($P<0.05$) lower in E1 and E2 compared to the control. Also lower ($P<0.05$) concentrations of potassium after flaxseed oil supplementation in yolk and zinc after both oil supplementation in albumen compared to the control were found. The amounts of other minerals were relatively balanced among groups. Significant ($P<0.05$) differences among groups in blood parameters of laying hens were observed in mineral and energy profile. Higher ($P<0.05$) concentrations of calcium, triglycerides and cholesterol in both experimental groups in comparison with control group were found in blood serum of laying hens. The supplementation of oils in diets of laying hens had not notable ($P>0.05$) influence on enzymatic and protein profile.

Keywords: blood, eggs, flaxseed, laying hens, minerals, pumpkin

Abstrakt

Cieľom práce bolo analyzovať vplyv tekvicového a ľanového oleja na úžitkovosť nosníc a nutričné zloženie konzumných vajec. Do pokusu boli zaradené nosnice hybridu Lohmann Brown Lite vo veku 38 týždňov, ktoré boli rozdelené do troch diétnych skupín (C-kontrola, E1-tekvicový olej (3%), E2-ľanový olej (3%)). Nosnice boli ustajnené v trojetážovej klietkovej technológii (6 zvierat v jednej klietke). Spolu bolo monitorovaných 18 nosníc. V kontrolnej skupine nosnice prijímali štandardnú kompletnú kŕmnu zmes a v experimentálnych skupinách bola táto kŕmna zmes obohatená o prídavok tekvicového alebo ľanového oleja. Do pokusných diét bol pridávaný aj vitamín E. Experiment trval 52 dní. Z každej skupiny bolo náhodne vybraných a analyzovaných 12 vajec. Čo sa týka minerálneho zloženia vajec, preukazne ($P<0.05$) vyšší obsah vápnika bol zaznamenaný po oboch olejových prídavkoch a preukazne vyšší obsah zinku v skupine s ľanovým olejom v porovnaní s kontrolnou skupinou vo vaječnom žĺtku. Obsahy horčíka v žĺtku a bielku a fosforu v bielku boli preukazne ($P<0.05$) nižšie v E1 a E2 v porovnaní s kontrolou. Pokles ($P<0.05$) v koncentrácii draslíka po prídavku ľanového oleja v žĺtku a zinku po pridaní oboch olejov v bielku bol pozorovaný v porovnaní so skupinou bez olejového prídavku. Množstvo ostatných minerálnych látok bolo relatívne vyrovnané medzi skupinami. Významné ($P<0.05$) rozdiely v krvných parametroch nosníc boli pozorované v minerálnom a energetickom profile. Vyššie ($P<0.05$) koncentrácie vápnika, triglyceridov a cholesterolu sme zaznamenali v oboch experimentálnych skupinách v krvnom sére nosníc v porovnaní s kontrolnou skupinou. Prídavky olejov v diétach nosníc nemali preukazný ($P>0.05$) vplyv na enzymatický a bielkovinový profil.

Kľúčové slová: krv, ľanové semeno, minerálne látky, nosnice, tekvica

Introduction

Eggs are made up of a variety of chemical components, including water, protein, fatty acids, minerals, vitamins, and pigments (Li-Chan and Kim, 2008). Eggs are very good source of vitamins A, D, E a K and whole complex of vitamins of group B also comprising B12 which are necessary for well-functioning nervous system. Also includes folic acid, iron, phosphorus, sulphur, potassium, magnesium and from trace elements it is zinc, copper, manganese, bromine and iodine (Nagy et al., 2009). Feed additives are a group of feed ingredients that can cause a desired animal response in a non-nutrient role such as pH shift, growth or metabolic modifier (Hashemi and Davoodi, 2010). They may have a beneficial effect on vitality and health condition (Gerzilov et al., 2015). Vegetable oils, also called essential oils are aromatic, liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). It was reported that essential oils have a stimulating effect on animal digestive system (Ramakrishna et al., 2003). In animals, in particular, promote the secretion of gastric juices, while operating on gut motility and improve the integrity of the intestinal lining (Panda et al., 2009). In addition to antibacterial properties of essential oils (Gopi et al., 2013; Krishan and Narang, 2014) were demonstrated their antiviral effects, antifungal effects, effect of anti-producing of poisons, antiparasitic and insecticidal properties. Essential oils may like antibiotics to have positively influence on weight gain, utilization of nutrients, egg production, body weight, and feed intake. However, the sensory quality of eggs isn´t often affects (Garcia-Rebollar et al., 2008). The addition plant oils have positive effect on contents of total protein, albumin, cholesterol, triglycerides (TG), bilirubin, K, Na, Ca and Mg in blood serum (Gulec et al. 2013). Flaxseed is emerging as one of the key sources of phytochemicals in the functional food area. In addition to being one of the richest sources of α-linolenic acid oil and lignans, flaxseed is an essential source of high-quality protein and soluble fibre and has considerable potential as a source of phenolic compounds (Oomah and Mazza, 1999; Oomah, 2001). The pumpkin seeds contain 39.25% of crude protein, 27.83% of crude oil, 4.59% of ash, and 16.84% of crude fibre (Alfawaz, 2004).Using phytogenic or herbal plants containing essential oils in poultry farms has developed with successful results (Hashemi and Davoodi, 2010). The subject of this work was to determine the effect of an essential oils on mineral composition of egg mass and blood parameters of laying hens.

Material and methods

Animals and housing

The experiment was carried out in accredited testing station of the Department of Poultry Science and Small Animal Husbandry (Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra). At 38 weeks of age, Lohmann Brown Lite hens were housed in three-floor cages, divided into three diets of groups (C-control, E1-pumpkin oil, E2-flaxseed oil). There were six hens in one cage housed. The useful area provided for one laying hen presented 943.2 cm^2. Total 18 hens were monitored. The laying hens were kept in the standard bioclimatic and welfare conditions. In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by local ethic committee. All efforts were made to minimize suffering of animals. During experiment, the light regime was 16 hours. All hens were housed in a windowless and environmentally controlled room, with room temperature kept at 20 to 22°C. The experiment lasted 52 days.

Feeding

In the control group (C), hens were fed with standard complete feed mixture for laying hens, in the experimental groups by feed mixtures supplemented with pumpkin (E1) or flaxseed (E2) oils. Concentration of oils was 3%. Oils were added into mixtures before the start of feeding. These oils were obtained from business network in Slovakia. Analyzed oils have been declared by the producer as pure and virgin oils which were pressed mechanically by cold technology. Vitamin E (0.1g per 1 kg of feed) was added into feed mixture in the experimental groups. Each cage was equipped with four nipple drinkers. Laying hens in all groups received drinking water and feed mixture *ad libitum*. Feeding mixture was composed of wheat, corn, soybean meal, rapeseed meal, sunflower meal, animal fat, soybean oil, calcium carbonate, feed additives, sodium bicarbonate, monocalcium phosphate, sodium chloride and enzyme complex of phytase. All kinds of feed supplements used in the experiment were homogenously incorporated into the feed mixture in the feed mill. Nutrient composition of feed mixtures is presented in Table 1.

Table 1. Nutrient composition of feed mixtures

Tabuľka 1. Živinové zloženie kŕmnych zmesí

Nutritive	Unit	C	E1	E2
Dry matter	%	91.12	90.88	91.4
Crude protein	% of DM	17.68	17.81	17.45
Crude fat	% of DM	6.53	7.61	7.59
Crude fibre	% of DM	4.09	3.71	3.78
Ash	% of DM	12.01	11.49	11.26
NFE	% of DM	50.81	50.26	51.32
Organic matter	% of DM	79.11	79.39	80.14
Starch	% of DM	35.3	35.62	35.45
Sugar	% of DM	3.9	3.84	3.94
Ca	$mg*kg^{-1}$ DM	41468	38567	39059
P	$mg*kg^{-1}$ DM	8855	8436	8060
Mg	$mg*kg^{-1}$ DM	3501	2954	3053
Na	$mg*kg^{-1}$ DM	2442	2366	2500
K	$mg*kg^{-1}$ DM	8861	7934	7232
Cu	$mg*kg^{-1}$ DM	16.89	16.23	15.54
Fe	$mg*kg^{-1}$ DM	293	305	280
Mn	$mg*kg^{-1}$ DM	179	164	177.2
Zn	$mg*kg^{-1}$ DM	182	164	132.4

NFE: nitrogen-free extract, DM: dry matter.

NFE: bezdusíkaté látky výťažkové, DM: sušina

Sampling and laboratory analysis

At the last week the eggs were collected and processed for chemical analysis. Twelve eggs from each dietary treatment were randomly selected and analyzed. Blood samples from *vena basilica* from hens were taken from all animals by macromethod in the morning at the beginning and end of the experiment. Nutrients composition of diets, mineral composition of eggs and blood parameters were determined by standard laboratory methods and procedures (AOAC, 2000). Content of ash was determined by complete combustion of the sample in a muffle furnace at 550°C (4 to 6 hours). The contents of mineral nutrients were determined by High Resolution Continuum Source Atomic Absorption Spectrometer ANALYTIK JENA contrAA 700 (Ca, Mg, Na, K, Zn, Cu, Fe, Mn) and 6400 Spectrophotometer (P). The determination of individual elements' content was based on the absorptions measured at the following wavelengths: Ca content was detected at 422.7 nm, P at 666 nm, Mg at 285.2 nm, Na at 589 nm, K at 766.5 nm, Zn at 213.9 nm, Cu at 324.7 nm, Fe at 248.3 nm and Mn at 279.5 nm. Laboratory analysis of nutritional composition of diets and eggs was carried out in the Laboratory of quality and nutritional value of feeds at the Department of Animal Nutrition in Slovak University of Agriculture in Nitra, Slovakia. Samples of blood were centrifuged for 30 min at 3,000 xg. The following metabolites, electrolytes and enzymes in serum (calcium, phosphorus, magnesium, sodium, potassium, chlorides, total proteins, glucose, cholesterol, triglycerides, asparate aminotransferase AST, alanine aminotransferase ALT, gamma glutamyl transferase GGT, alkaline phosphatise ALP, bilirubin) were determined using Ecoline kits and automatic analyzer Microlab 300, spectrophotometer Genesys 10 and microprocessor-controlled analyzer EasyLite according to manufacturer conditions. Laboratory analysis of blood parameters was carried out at the Department of Animal Physiology in Slovak University of Agriculture.

Statistical analysis

The data used for statistical analysis of nutrients represents means of values obtained from 12 eggs from each group. The results of descriptive statistics have been expressed as mean with standard deviations or coefficient of variance. Differences between groups were analyzed with one-way analysis of variance (ANOVA) by using the statistical programme IBM SPSS 20.0. Results were analysing using Tukey test. Values with different superscripts within a column are significant at 0.05 level. Relationships between nutrients were assessed on the basis Pearson correlation coefficient.

Results and discussion

Mineral composition of egg mass

The main role of minerals is their structural function. They are very important as regulators of physical-chemical processes in the organism, are involved in the maintenance of acid-base balance, perform an important function in the regulation of the osmotic pressure and in formation of buffer system of biological fluid (Pajtáš et al., 2009). Egg yolk also contains a trace amount of minerals. The mineral content in egg yolk is about 1%. Phosphorus is the most abundant mineral owing to the high content of phospholipid (Li-Chan and Kim, 2008). Macroelements and microelements composition of eggs components are summarized in Tables 2, 3. Significant ($P<0.05$) differences in contents of calcium and magnesium among control and experimental groups were found in eggs yolk and albumen. After oils addition in to diets of laying hens content of magnesium was evidently lower in E1 (pumpkin oil) and E2 (flaxseed oil) in both components of egg. Negative effect of oils was detected also in sodium concentration. However, higher amount of phosphorus was observed in both experimental groups compared to the control in all components of egg but significant ($P<0.05$) differences were observed only in eggs albumen. The potassium concentration was lower after pumpkin and flaxseed oil supplementation in comparison with control group in shell ($P<0.05$) and albumen ($P>0.05$). Significantly ($P<0.05$) lowest content of potassium was recorded in E2 against control and E1. The contents of iron and manganese in table eggs were not markedly affected of oils addition in feed mixture. Values of manganese were balanced in all groups. The concentration of zinc was significantly ($P<0.05$) lower after pumpkin and flaxseed oil addition compared to the control in eggs albumen. Significant ($P<0.05$) differences in content of copper were observed only in eggs yolk and egg shell. Tendency ($P>0.05$) of a higher copper content in albumen was found in the control group in comparison with experimental groups. Relative to mineral composition, yolks from egg produced by hens fed the control treatment (no oil) were significantly different only from those derived from hens fed the diet supplemented with 2.5% canola oil + 2.5% soybean oil, which presented lower mineral content (Faitarone et al., 2013). The effect of dietary plant extracts added into feed mixture of layers on the content of phosphorus, calcium and magnesium in eggshell were not significant (Lokaewmanee et al., 2014). Herkeľ et al. (2016) also reported that phytogenic additive including essential oil may significantly affect mineral composition in poultry products. In the study of Aydin et al. (2001) who researched effect of conjugated linoleic acid and olive oil on nutritional composition of eggs yolk and albumen contents of Mg, Na and Cl of eggs yolks stored at 4°C for 10 week was significantly higher in eggs from conjugated linoleic acid fed laying hens. The lower concentrations of Ca, Zn and Fe in the yolk were found in the treatment with conjugated linoleic acid compared to the control. In contrast, eggs from laying hens fed the conjugated linoleic acid diet had greater concentrations of Fe, Ca and Zn and lower concentrations of Mg, Na and Cl in albumen relative to eggs from hens fed control diet. Total Fe level in the albumen of the eggs from conjugated linoleic acid fed laying hens was 22-fold greater than those from hens fed control diet. Feeding olive oil along with conjugated linoleic acid completely prevented changes in the mineral content of egg yolk and albumen. Average contents of Ca 344 $g*kg^{-1}$, P 1.17 $g*kg^{-1}$, Mg 3.32 $g*kg^{-1}$ and Zn 4.82 $g*kg^{-1}$ were observed in egg shell of Lohmann Brown laying hens (Tůmová et al., 2014).

Table 2. Macroelements composition of eggs mass

Tabuľka 2. Obsah makroprvkov vo vaječnej hmote

Component of egg	Group		Ca	P	Mg	Na	K
					$mg*kg^{-1}$		
Yolk	C	Mean	2167.18[a]	11060	356.93[a]	1655	3407.29[a]
		S.D.	486.04	836.53	13.31	197.31	396.55
	E1	Mean	2691.86[b]	11708	321.48[b]	1622.4	3506.93[a]
		S.D.	138.82	572.28	16.86	154.49	159.4
	E2	Mean	2663.36[b]	11463	312.93[b]	1569.8	2941.88[b]
		S.D.	129.94	412.62	12.42	107.48	201
Albumen	C	Mean	1065.81[a]	1610.85[a]	1138.58[a]	15166	11447.1
		S.D.	186.12	132.46	73.39	2067.9	1247.4
	E1	Mean	652.16[b]	1342.58[b]	846.73[b]	14067	10983.6
		S.D.	120.14	119.64	39.12	516.55	895.45
	E2	Mean	612.56[b]	1387.42[b]	898.4[b]	13955	9725.02
		S.D.	133.93	86.74	77.59	744.73	1698.61

S.D.: standard deviation, C: control group, E1: pumpkin oil addition, E2: flaxseed oil addition. Values with different superscripts in a column are significant at the 0.05 level.

S.D.: smerodajná odchýlka, C: kontrolná skupina, E1: prídavok tekvicového oleja, E2: prídavok ľanového oleja. Hodnoty s rozdielnym indexom v stĺpci sú preukazne rozdielne na hladine 0.05.

Glisson and Walker (2010) reported that sodium, potassium and chlorides occurs for the most part as free minerals and other minerals are usually bound to proteins and phospholipids. Main factor which affect amount of minerals in eggs is feed. Eggs are important source of phosphorus, iron, zinc, copper and other elements.

Table 3. Microelements composition of eggs mass

Tabuľka 3. Obsah mikroprvkov vo vaječnej hmote

Component of egg	Group		Cu	Zn	Fe	Mn
			mg*kg^{-1}			
Yolk	C	Mean	7.47^{ab}	46.8^{a}	150.14	1.48
		S.D.	1.58	1.3	16.01	0.14
	E1	Mean	8.72^{a}	45.53^{a}	161.18	1.68
		S.D.	1.44	2.73	18.31	0.19
	E2	Mean	6.09^{b}	59.88^{b}	138.23	1.51
		S.D.	1.64	15.99	15.79	0.08
Albumen	C	Mean	5.09	3.47^{a}	86.68	0.12
		S.D.	2.22	2.04	12.76	0.03
	E1	Mean	3.56	1.01^{b}	56.14	0.11
		S.D.	0.71	1.2	24.56	0.01
	E2	Mean	4.13	1.03^{b}	59.53	0.09
		S.D.	1.45	1.37	27.93	0.05

S.D.: standard deviation, C: control group, E1: pumpkin oil addition, E2: flaxseed oil addition. Values with different superscripts in a column are significant at the 0.05 level.

S.D.: smerodajná odchýlka, C: kontrolná skupina, E1: prídavok tekvicového oleja, E2: prídavok ľanového oleja. Hodnoty s rozdielnym indexom v stĺpci sú preukazne rozdielne na hladine 0.05.

Relationships among content of fat and macroelements are expressed in Figure 1. Positive correlation was found in Ca and P contents to fat. Very strong negative correlation was detected in Mg and Na concentrations to fat content in egg yolk.

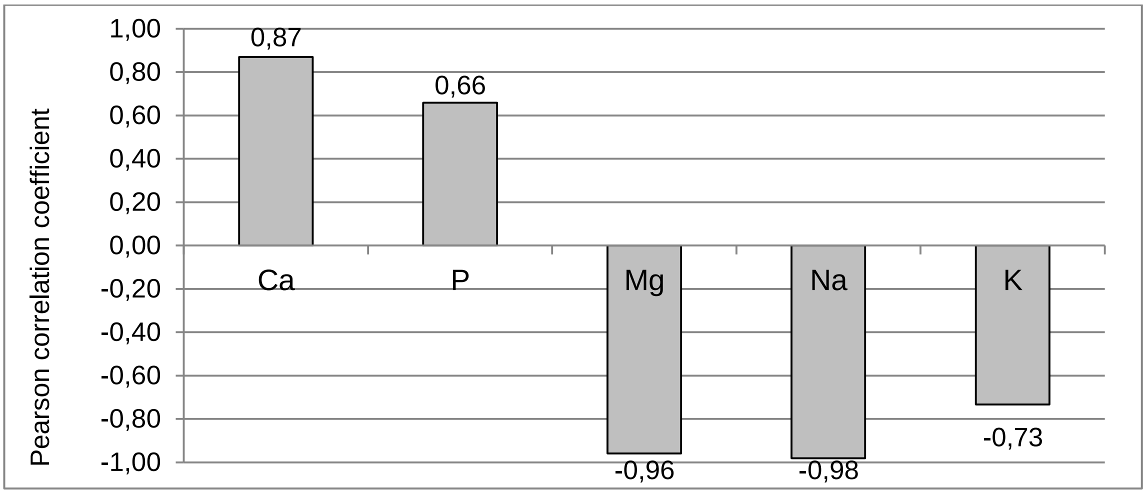

Figure 1. Correlation analysis among fat content and macroelements in egg yolk

Obrázok 1. Korelačná analýza medzi obsahom tuku a makroprvkami vo vaječnom žĺtku

Blood parameters of laying hens

In the experiment, there was analyzed the effect of essential oils on selected blood parameters in laying hens. The majority of performance traits of livestock species is determined by maintaining a dynamic equilibrium between the extent of anabolic and catabolic changes in the body and by effective metabolism (Kapelański et al., 2004). Biochemistry of the blood gives us an indication of what is happening within the body. Internal milieu of the organism includes also parameters of blood and its status. Basically it is homeostasis of the cells or stable environment within the cells. Changes in internal milieu can be caused by various factors. One of them is also diets and feed (Capcarová and Kolesárová, 2010). The blood serum of laying hens at the beginning of the experiment contained 3.38 mmol*l^{-1} Ca, 3.68 mmol*l^{-1} P, 1.59 mmol*l^{-1} Mg, 147 mmol*l^{-1} Na, 4.21 mmol*l^{-1} K, 116.8 mmol*l^{-1} Cl, 75.61 g*l^{-1} crude protein, 11.24 mmol*l^{-1} glucose, 2.72 mmol*l^{-1} cholesterol, 8.8 mmol*l^{-1} triglycerides, 3.11 µkat*l^{-1} AST and 0.36 µkat*l^{-1} ALT. The effect of essential oils on mineral profile of blood serum at the end of experiment is shown in Table 4. Significant differences ($P<0.05$) were found in content of calcium (Ca), sodium (Na) and potassium (K). The current experiment demonstrated that the experimental treatments did not markedly ($P>0.05$) affect the concentration of phosphorus (P), magnesium (Mg) and chlorine (Cl). The content of Ca was significantly ($P<0.05$) higher in both experimental groups compared to the control. The addition of flaxseed oil into diets of layers affected ($P<0.05$) increase of Na and K contents. The reduction ($P>0.05$) of plasma Mg was found in the experimental groups in comparison with control group. The decrease of plasma magnesium was detected in the experiment of Gálik et al. (2015). The contents of chlorides and phosphorus were also lower in dietary treatment (blend of essential oils) compared to the control. Phytogenic feed additives containing essential oils of thyme and star anise as main active components were studied by Cho et al. (2014). They found reduced blood total cholesterol. Gulec et al. (2013) noted significantly higher ($P<0.05$) contents of total protein, albumin, cholesterol, triglycerides, bilirubin, K, Na, Ca and Mg after addition of plant oils (*Thymus vulgaris* and *Foeniculum vulgare*) compared to the control. Present findings are not in agreement with those presented by Levkut et al. (2011) who observed that chickens fed oregano oil supplemented diet had significantly higher serum magnesium concentration and slightly lower serum phosphorus level in comparison with birds from control group. Tůmová et al. (2014) found average contents of serum Ca 4.18 mmol*l^{-1}, serum P 1.8 mmol*l^{-1}, serum Mg 1.45 mmol*l^{-1} and serum Zn 81 µmol*l^{-1} in blood of Lohmann Brown laying hens.

Table 4. Mineral profile of blood of laying hens at the end of experiment

Tabuľka 4. Minerálny profil krvi nosníc na konci experimentu

Group		Ca	P	Mg	Na	K	Cl
				mmol*l^{-1}			
C	Mean	3.05^{a}	1.91	1.2	150.55^{a}	3.5^{a}	120.2
	S.D.	0.43	0.71	0.3	0.93	0.14	2.14
	C.V.	0.18	0.5	0.09	0.87	0.02	4.57
E1	Mean	4.23^{b}	2.35	0.85	153.2^{ab}	4.02^{ab}	122.9
	S.D.	0.32	0.4	0.35	3.45	0.53	3.19
	C.V.	0.1	0.16	0.12	11.89	0.28	10.18
E2	Mean	4.42^{b}	2.11	0.85	156.73^{b}	4.33^{b}	126.38
	S.D.	0.17	0.11	0.35	2.85	0.47	3.94
	C.V.	0.03	0.01	0.12	8.14	0.22	15.51

S.D.: standard deviation, C.V.: coefficient of variance, C: control group, E1: pumpkin oil addition, E2: flaxseed oil addition. Values with different superscripts in a column are significant at the 0.05 level.

S.D.: smerodajná odchýlka, C.V.: variačný koeficient, C: kontrolná skupina, E1: prídavok tekvicového oleja, E2: prídavok ľanového oleja. Hodnoty s rozdielnym indexom v stĺpci sú preukazne rozdielne na hladine 0.05.

Enzymatic profile of blood of laying hens at the end of experiment is recorded in Table 5. AST, ALT, GGT, ALP and bilirubin of enzyme complex were observed in this trial. Significant differences among groups in enzyme profile were not found (P>0.05). Values of AST were balanced in all treatments. Tendency (P>0.05) of highest activity of AST and GGT was observed in E1 (pumpkin oil addition). A slight increase in plasma ALT was found in the both experimental groups compared to control. The lower activities for plasma ALP and bilirubin were recorded in the experimental groups in comparison with control group. Those marker enzymes are normally localised within the cells of the liver, heart, gill, kidney, muscles and other organs (Yakubu et al., 2005). Moreover, mentioned “adaptive enzymes” are of importance for diagnosis of diseases (Beňová et al., 2003). Abd El-Ghang and Ismail (2013) who used oregano essential oil in broiler feed, observed increase in the activity of ALT and AST. The above mentioned finding agree with the results of Traesel et al. (2010), who reported that the serum AST levels in the group supplemented with essential oils from oregano, sage, rosemary, and pepper crude extract at 150 mg*kg^{-1} were significantly higher than in the control group. Gálik et al. (2015) added phytogenic additive including essential oils into feed mixture for poultry. In contrast to the present study, significantly higher concentrations of ALT as well as AST were found in the control group, while lower concentration was detected in birds from experimental group. The lowest activities for plasma ALT and AST recorded for individuals fed diets supplemented with phytoadditive indicate that the used treatment did not negatively alter liver enzyme activity but also had a non-toxic effect on the kidney and liver (Saleh, 2014). Furthermore, no increase in serum concentration of ALT and AST may provide evidence to protect of liver against hepatocellular degeneration (Al-Jaff, 2011). Presented experiment did not confirm results of other authors (Habibi et al., 2014; Saleh, 2014; Zhu et al., 2014). Shi et al. (2014) who added phytosterols into feed mixture of layers reported some minor changes in clinical chemistry parameters, but these changes were small and considered to be of no toxicological significance. Total bilirubin was relatively balanced between control and experimental groups with different doses of phytosterol what is in agreement with current findings. Similar results with present work were found in enzyme GGT. Differences in ALP were not significant. Lokaewmanee et al. (2014) researched effect of plant extract addition in diets on blood components of laying hens. Among the dietary treatments were not detected significant differences in content of AST, ALT, ALP and bilirubin.

Table 5. Enzymatic profile of blood of laying hens at the end of experiment

Tabuľka 5. Enzymatický profil krvi nosníc na konci experimentu

Group		AST	ALT	GGT	ALP	Bilirubin
			$\mu kat*l^{-1}$			$\mu mol*l^{-1}$
C	Mean	2.83	0.14	14.57	46.09	26.05
	S.D.	0.14	0.06	4.26	29.91	8.08
	C.V.	0.02	0	18.17	894.89	65.25
E1	Mean	2.9	0.17	19.96	24.72	21.23
	S.D.	0.16	0.06	3.32	19.91	1.6
	C.V.	0.03	0	10.99	396.51	2.56
E2	Mean	2.76	0.17	15.57	11.88	26.02
	S.D.	0.18	0.03	5.74	3.4	10.86
	C.V.	0.03	0	32.96	11.55	117.93

S.D.: standard deviation, C.V.: coefficient of variance, C: control group, E1: pumpkin oil addition, E2: flaxseed oil addition. Values with different superscripts in a column are significant at the 0.05 level.

S.D.: smerodajná odchýlka, C.V.: variačný koeficient, C: kontrolná skupina, E1: prídavok tekvicového oleja, E2: prídavok ľanového oleja. Hodnoty s rozdielnym indexom v stĺpci sú preukazne rozdielne na hladine 0.05.

Energy and protein profile of blood of laying hens at the end of experiment are shown in Table 6. The glucose content was statistically ($P<0.05$) the highest in control group compared to E1 (pumpkin oil supplementation). Both cholesterol and triglycerides concentrations was markedly ($P<0.05$) higher in the E1 and E2 (flaxseed oil supplementation) in comparison with control group. Significant differences between experimental groups in the content of cholesterol were not recorded ($P>0.05$). The greatest ($P<0.05$) impact on increasing of triglycerides concentration had pumpkin oil addition in laying hens diet. The comparison of biochemical blood parameters of birds received diet with blend of essential oils from origanum, anise and citrus fruits and from control group demonstrated that the latter had only slightly lower concentration of glucose (-4.11%), which is substance that in animals directly oxidises to provide energy (Gálik et al., 2015). Mansoub (2011) reported that using oregano oil in chicken´s diet had not significant effects on plasma glucose level. Insignificant differences between control and experimental groups (addition of *Nigella sativa*) were found in concentration of glucose in the experiment of Ghasemi et al.

(2014). Some reports have indicated that cholesterol from eggs does not have a negative effect on serum cholesterol levels (Kritchevsky and Kritchevsky 2000; Hu et al. 2001). On the contrary, findings of a research works conducted by Soltan et al. (2008) and Gálik et al. (2015) who noted that dietary anise seeds respectively blend of essential oils supplementations in poultry diet not significantly affected serum cholesterol level. No differences on serum cholesterol levels observed also Bampidis et al. (2005) and Sarica et al. (2005) who evaluated the effect of dietary supplementation with oregano oil. In contrast to present study, decreasing effect of phytoadditives on serum triglycerides level recorded Puvača et al. (2015) and Rahimi et al. (2011). Abou-Elkhair et al. (2014) researched bioactive effect of dietary supplementation with essential oils blends of oregano, thyme and garlic on blood parameters of broilers. The content of triglycerides in dietary treatments was lower than in the group without essential oils addition in diet. Have been suggested that the lower serum concentrations of triglycerides and cholesterol in birds who received phytogenic feed additive could be related to the complex antistress, antioxidant and antimicrobial effect of herbs included in the feed supplement (Gálik et al., 2015). Saki et al. (2014) added raw herbal powders including garlic, marigold, fennel seeds and thyme into feed mixture of Leghorn laying hens. The serum triglyceride and cholesterol were not affected significantly by inclusion of different levels of phytogenic feed additives in layer diets. However, serum HDL and LDL were increased by inclusion of phytogenic additives. Zhang and Kim (2014) researched effect of dietary olive oil on serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides in blood of laying hens. At the end of experiment, serum total cholesterol and HDL-cholesterol concentration in group with 5% olive oil addition was lower than in control group. Triglycerides level in blood of layers was unaffected by dietary treatments. Park et al. (2015) who added dietary *Marine microalgae* into diets of layers found that blood triglyceride concentrations increased quadratically in experimental group compared to control treatment. Similarly, total cholesterol concentrations of blood also increased markedly. In presented study, significant differences in plasma total protein concentration between control and experimental groups were not observed ($P>0.05$). Tendency ($P>0.05$) of the highest crude protein content was found after flaxseed oil addition in diet. Similar results were reported by Gálik et al. (2015). Corduk et al. (2013) showed that supplementation of phytoadditives (oil of oregano or red pepper) did not markedly affect the serum total protein. However, in some another studies carried out in poultry, the increase in the plasma total protein resulted from the phytoadditves supplementation has been observed (Elagib et al., 2012; Amad et al., 2013).

Table 6. Energy and protein profile of blood of laying hens at the end of experiment

Tabuľka 6. Energetický a bielkovinový profil krvi nosníc na konci experimentu

Group		Glucose	Cholesterol	Triglycerides	Crude protein
			mmol*l^{-1}		g*l^{-1}
C	Mean	13.22^{a}	1.38^{a}	11.83^{a}	42.32
	S.D.	1.24	0.22	2.28	7.42
	C.V.	1.55	0.05	5.2	55.04
E1	Mean	11.14^{b}	3.76^{b}	25.13^{b}	44.53
	S.D.	0.94	0.97	2.65	8.93
	C.V.	0.89	0.95	6.99	79.68
E2	Mean	11.54^{ab}	4.19^{b}	17.73^{c}	54.19
	S.D.	0.76	17.73	1.84	6.72
	C.V.	0.58	54.19	3.37	45.17

S.D.: standard deviation, C.V.: coefficient of variance, C: control group, E1: pumpkin oil addition, E2: flaxseed oil addition. Values with different superscripts in a column are significant at the 0.05 level.

S.D.: smerodajná odchýlka, C.V.: variačný koeficient, C: kontrolná skupina, E1: prídavok tekvicového oleja, E2: prídavok ľanového oleja. Hodnoty s rozdielnym indexom v stĺpci sú preukazne rozdielne na hladine 0.05.

Conclusions

The goal of this study was to analyse the impact of essential oils on mineral composition of egg mass and blood parameters of laying hens. Evidently (P<0.05) lower contents of magnesium in egg mass, phosphorus, calcium and zinc in albumen, in E1 (pumpkin oil addition) and E2 (flaxseed oil addition) against control group were found. The supplementation of oils had positive effect only on the concentration of calcium in yolk (P<0.05). The amounts of other minerals were relatively balanced between groups and were not considerably affected of oil addition in diet. In blood serum of layers, pumpkin and flaxseed oil had significant (P<0.05) impact on mineral and energy profile. The content of calcium was markedly (P<0.05) higher in experimental groups in comparison with control in blood serum. Cholesterol and triglycerides concentration of blood of hens increased (P<0.05) after oil inclusion compared to the control. The supplementation of oils in diets of laying hens had not notable (P>0.05) influence on enzymatic and protein profile.

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