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PERFORMANCE, BIOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF HENS SUPPLEMENTED WITH ADDITION OF MILK THISTLE (*SILYBUM MARIANUM*) SEED CAKES IN DIET

Ondřej Šťastník[1], Eva Mrkvicová[1], Leoš Pavlata[1], Andrea Roztočilová[1], Barbora Umlášková[1], Eva Anzenbacherová[2]

[1]Department of Animal Nutrition and Forage Production, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

[2]Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic

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Abstract

The aim of this study was to evaluate the effect of milk thistle seed cakes addition in laying hens diet to performance, blood biochemical parameters and antioxidant activity. A total of 30 Bovans Brown hens were included to the experiment. The trial was performed from the age of 69 weeks to 80 weeks of hens age. The experimental group received feed mixture containing 7% milk thistle seed cakes. Control group received feed mixture without milk thistle seed cakes. After the 69th week of age, the laying hens in the experimental group reached a higher number of eggs and produced more egg mass compared to the control group. In the evaluation of the egg quality parameters, higher Haugh units, a higher millimeter height of the egg and thinner eggshell in the group receiving 7% of the seed cakes were found. When evaluating health indicators, higher antioxidant activity was found in the experimental group. Blood biochemical parameters was without any differences.

Keywords: silybum marianum, poultry nutrition, antioxidant activity, silymarin

INTRODUCTION

Many scientists have been trying to prove the benefits of Milk thistle in animal studies. It is assumed that silymarin is effective mainly due to its anti-inflammatory and antioxidant effects, stimulating hepatocyte regeneration (Vargas-Mendoza *et al.*, 2014). For example, Dumari *et al.* (2014) supplemented broiler chicken's diet with 0.5% and 1% milk thistle seeds and found a marked decrease in serum aspartate aminotransferase (AST) activity in the blood

serum of the experimental groups compared to the control group. Serum alanine aminotransferase (ALT) activity was significantly lower in aflatoxin B_1 (AFB_1) affected chickens compared to the control group. After the addition of silymarin to the feed mixture with AFB_1, there was found a marked increase in ALT activity (Tedesco *et al.*, 2004). Dumari *et al.* (2014) fed an AFB_1 inoculated feed mixture contained milk thistle seeds addition to fattened chickens. Authors did not find any influence on the parameters of biochemical and fat profile of chickens blood in this experiment.

Buzzelli *et al.* (1993) found that silymarin (silybin) at the dose 240 mg/day reduced the activity of ALT, AST, gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) in blood serum in human patients. These enzymes are considered as indicators of liver damage. In rats affected *diabetes mellitus* was found that silymarin reduced serum triglycerides, cholesterol and ALT and AST enzyme activity in blood plasma (Tuorkey *et al.*, 2015). Malayeri *et al.* (2014) report that silymarin supplementation in broiler chickens can be effective against carbon tetrachloride (CCl4) hepatotoxicity.

Silymarin has also a positive effect on feed intake, weight gain (Schönfeld *et al.*, 1997; Tedesco *et al.*, 2004; Gažák *et al.*, 2007; Chand *et al.*, 2011; Omid *et al.*, 2013) and chickens liver tissue morphology (Fani *et al.*, 2013). Additionally, silymarin supplementation shows effect on meat quality and its shelf life by increasing *post-mortem* oxidative stability (Schiavone *et al.*, 2007). However, the supplementation of 5% and 15% milk thistle seed cakes do not worsened sensory characteristic of breast or leg meat of broilers chickens and reflects optimal sensory quality traits (Šťastník *et al.*, 2016).

The above-mentioned findings are also confirmed by Tedesco *et al.* (2004) who found that the addition of silymarin phytosome to the diet at 600 mg/kg led to an increase in body weight of chickens by 14.83% compared to the control group. Gawel *et al.* (2003) found increase in hatching and increasing body weight gain of chickens and turkeys after silymarin supplementation. The ducklings under the oxidative stress receiving 200 mg/kg of silymarin in the diet had a higher body weight than ducklings in control group (Yi *et al.*, 2012). On the other hand, Schönfeld *et al.* (1997) indicate a decrease in feed intake after the addition 40 and 80 mg/kg silymarin to broiler chickens and 400 mg/kg silymarin to laying hens diet.

The exact mechanism of increasing weight gain is not fully elucidated. This effect may be due to an improvement in the immune function of the birds receiving the milk thistle (Chand *et al.*, 2011). As has been said, the milk thistle supports the immune system by its substances with a strong antioxidant effect, free radical capture, ability to preserve the body's supply of other important antioxidants (glutathione) and direct effects on immune cells (Basaga *et al.*, 1997). Silymarin, as an antioxidant, has a protective effect against oxidative damage to cells and organs of the immune system of birds (bursa Fabricii, spleen and thymus) causes immunosuppression (Chand *et al.*, 2011).

The aim of this study was to evaluate the effect of addition 70 g/kg milk thistle seed cakes in laying hens diet to performance, blood biochemical parameters and antioxidant activity.

MATERIALS AND METHODS

The animal procedures were reviewed and approved by the Animal Care Committee of Mendel University in Brno and by the Ministry of Education, Youth and Sports MSMT-6XBCMU.

Animals and diets

A total of 30 Bovans Brown hens were included to the experiment. The trial was performed from the age of 69 weeks to 80 weeks of hens age. The experimental period lasted 11 weeks. Hens were divided into 2 groups per 15 hens. The experimental group (MT7) received feed mixture containing 7% milk thistle seed cakes. The control group (C) received feed mixture without milk thistle seed cakes. The nutrient composition of diets corresponds to the recommended nutrient requirements of the relevant category according to the recommended nutrient content in feed mixtures for poultry (Zelenka *et al.*, 2007). Tab. I shows chemical composition of used milk thistle seed cakes. The composition and chemical analysis of the feed mixtures is shown in Tab. II. The chemical composition of nutrient content of diets were determined for dry matter, crude protein, ether extract, crude fibre, and ash according to Commission Regulation (EC; Commission Regulation, 152/2009).

The diets were fed in non-pelleted form. Hens had an *ad libitum* access to feed and water. The feed was weighted daily. The feed residue was collected and weighted daily as well to calculate total feed consumption. The animals were weighed every week in the same time.

I: *Milk Thistle seed cakes chemical composition (in dry matter)*

Nutrient		
Gross energy	MJ/kg	20.3
Ether extract	g/kg	100.7
Crude fibre	g/kg	292.4
ADF	g/kg	413.8
NDF	g/kg	455.4
Ash	g/kg	68.0
Crude protein	g/kg	217.0
Amino acid	g/kg	
Aspartic acid		18.7
Threonine		6.1
Serine		10.0
Glutamic acid		37.9
Proline		17.0
Glycine		11.3
Alanine		7.6
Valin		10.2
Isoleucine		8.3
Leucine		13.4
Tyrosine		8.7
Phenylalanine		8.4
Histidine		5.5
Lysine		11.6
Arginine		21.6
Silymarin complex	mg/kg	
Taxifolin		580
Silychristin		3638
Silydianin		2520
Silybin B		6673
Silybin A		1473
Isosylibin		565
Flavonolignan's	mg/kg	37.3
Mycotoxins	µg/kg	
Ochratoxin A		< 0.2
Deoxynivalenol		154
Aflatoxin B1		< 0.1

ADF–acid detergent fibre; NDF–neutral detergent fibre

The conventional deep litter system with wood shavings were used. The roots, laying nest, automatic nipple drinkers and the self-draining feeders were in the cubicles. The room temperature, relative humidity and light mode were controlled. The light regime was set to 18 hours of light and 6 hours of darkness with gradual dimming and flashing. The experimental animals were regularly monitored for health and deaths were recorded.

Performance

The number of laid eggs and its total weight were recorded every day for 62 days during the experiment. The average weight of eggs in each group and the feed consumption per hen and eggs were calculated. Qualitative evaluation of eggs was ranged from 69 weeks to 78 weeks of laying hens age. Twenty eggs were taken every 14 days for two consecutive days from each group (n = 100). During experiment egg weight, egg length, egg width, albumen weight, albumen height, yolk weight and color, strength, weight and shell thickness were determined.

The width and length of the egg were measured using a sliding scale. The egg shape index characterizes the shape properties of the egg and it is calculated as the ratio of the width to length of the egg (width/length). The yolk weight was measured after separation of the egg yolk including the chalases. The weight of the albumen was calculated. After weights of the main parts of the egg was determined, percentages were calculated. Subsequently, Haugh units (HU)

II: *Composition and nutrients content of the experimental diets for laying hens*

Components (g/kg)	C	MT7
Wheat	600	600
Soybean meal	200	168
Limestone	74	74
Milk Thistle seed cakes	0	70
Maize	54.1	0.8
Rapeseed oil	31.7	39
Vitamin-mineral premix[1]	30	30
Monocalcium phosphate	5	5.4
Wheat gluten	4.7	10.8
DL-Methionine	0.5	1
L-Lysine	0	1
Analysed composition (per kg)		
AME_N (MJ)[2]	11.46	11.41
Dry matter (g)	880	880
Crude protein (g)	159.49	162.29
Ether extract (g)	50.58	54.39
Crude fiber (g)	43.92	53.23
Ash (g)	130.50	118.80
Calcium (g)	35.6	33.6
Total phosphorus (g)	62.2	65.4

C: control group; MT7: 7% milk thistle seed cakes in diet

[1]Vitamin-mineral premix per kg diet: 0.39 g lysine; 1.35 g methionine; 8.85 g Ca; 2.01 g P; 1.38 g Na; 9.00 mg Cu; 54.00 mg Zn; 60 mg Fe; 72.00 mg Mn; 0.9 mg I; 0.24 mg Se; 9,900 IU vitamin A; 3,000 IU vitamin D_3; 15.00 mg vitamin E; 1.2 mg B_1; 3.6 mg B_2; 1.62 mg B_6; 12.00 mg B_{12}; 0.09 mg biotin; 0.9 mg folic acid; 12.6 mg niacinamide; 7.5 mg calcium pantothenate; 180 mg choline chloride; 0.3 mg butylhydroxyanisole; 1.5 mg butylhydroxytoluene; 3 mg etoxyquin

[2]AME_N–apparent metabolizable energy (calculated value)

were calculated: HU = 100 × log (solid albumen height + 7.6 – 1.7 × egg weight $^{0.37}$).

The height of the solid albumen was measured from the height gauge TSS (Great Britain) at several millimeters from the yolk and was expressed in millimeters. The color of the yolk was subjectively determined by the DSM color fan. The shell was dried at room temperature. The weight of the shell was determined by weighing on a laboratory scale to the nearest 0,01 g, including eggshell membranes. The eggshell strength was expressed by the force required to deform/rupture the shell. It was measured using the Egg Force Reader (US) in Newtons (N). The thickness of the shell was measured at the blunt end, the sharp end and middle of the egg, and the arithmetic mean was calculated from the measured values.

Determination of amino acids, flavonolignans and mycotoxin content in seed cakes

The sample was hydrolyzed by acid hydrolysis of 6 N HCl for 24 hours at 110 °C. The resulting hydrolysate was filtered, transferred to a volumetric flask of the appropriate amount and made up to distilled water. From the volumetric flask, pipette a possible amount of sample and evaporate in a vacuum rotary evaporator. Transfer the residue with sodium citrate buffer to a 25 ml flask volume and make up with the appropriate buffer. The determination of amino acids is performed on an automatic amino acid analyzer AAA 400 from INGOS a.s. Prague based on color-forming reactions with the oxidizing agent ninhydrin.

Flavonolignans determination was performed according to Kosina *et al.* (2017). 1 g of homogenized sample was combined with 10 mL of the extraction mixture (H_2O:ACN:MeOH in the ratio 10:50:40, v/v/v); the sample in the presence of the extraction mixture was vortexed for 1 min, sonicated in an ultrasonic bath for 30 min and then macerated for 12 h at room temperature in the dark. After 1 min of vortexing, 1 mL of the sample in the extraction mixture was collected, centrifuged for 5 min at 12,000 × g at room temperature. 200 µL of supernatant was mixed with 400 µL of mobile phase A (MeOH:H2O:CH3COOH in the ratio 37:63:0.5, v/v/v), vortexed and centrifuged again. 10 µL of final supernatant was injected into an HPLC column (Shimadzu Class VP HPLC system (Kyoto, Japan) with UV detection. LiChrospher RP-18 column (5 µm) 250 × 4 mm equipped with a 4 × 4 mm guard column). All analyses were carried out in triplicate.

The deoxynivalenol content was analyzed immunochemically by ELISA using RIDASCREEN DON kits, R-Biopharm (Darmstadt, Germany). Ochratoxin and aflatoxin B1 content was analyzed by HPLC method, Shimadzu liquid chromatograph (Kyoto, Japan), with fluorescence detector.

Sample collection and chemical analysis

Hens blood was collected into heparinized tubes and centrifuged for 15 minutes at 3,000 rpm during 2 hours after collection. The separated blood plasma was frozen (–20 °C) until biochemical examination. The following parameters were determined using standardized biochemical methods using Erba Lachema (Czech Republic) commercial sets on the Ellipse automatic biochemical analyzer (AMS Spa, Italy) in blood plasma samples (n = 7): enzymes activity AST–aspartate aminotransferase (AST/GOT 500); GMT–gamma-glutamyltransferase (GGT 250); ALT–alanine aminotransferases (ALT/GPT 500); ALP–alkaline phosphatase (ALP AMP 500) and LD–lactate dehydrogenase (LDH-L 100). As other markers of hepatic metabolism, fat and nitrogen metabolism, as well as kidney activity, was determined concentrations of the total bilirubin–Bili (BIL T JG 350), cholesterol (CHOL 250); TG–triglycerides (TG 250), uric acid (UA–UA 500, no. 10010225 Erba Lachema, Czech Republic), TP–total protein (TP 500) and albumin (Alb 500). The globulin content (TP minus albumin) was calculated.

The antioxidant capacity of plasma (n = 6) was measured by the FRAP (ferric reducing antioxidant power) method described by Benzie and Strain (1996). Subsequently, absorbance at 593 nm was measured spectrophotometrically using a Tecan Infinite M200 Pro instrument (Tecan, Switzerland). All samples were measured in 3 replicates. The results correspond to the concentration of ascorbic acid using a standard curve. All chemicals were purchased from Sigma-Aldrich (Czech Republic).

Statistical analysis

Data were processed by Microsoft Excel (USA) and StatSoft Statistica version 12.0 (USA). The basic statistical characteristics of the set of values (means and standard errors) were calculated from the results of the individual groups. One-way analysis of variance (ANOVA) was used. To ensure evidential differences Scheffe's test was applied and $P < 0.05$ was regarded as statistically significant

difference and the $P < 0.01$ was considered as statistically highly significant.

RESULTS

The mean laying hens live weight during the trial is shown in Tab. III. During the experiment was not found significant differences in hens live weights ($P > 0.05$) and two deaths were recorded in both groups of hens.

For laying hen's performance evaluation, it was performed 100 egg analyses, ranging from week 69 to week 78. Results are shown in Tab. IV.

A group of animals with 7% milk thistle seed cakes in the diet showed a statistically significant higher ($P < 0.01$) number of eggs per group and per day (11.48 pcs) compared to the control group.

The average egg weight was 64.8 g in both groups. The average egg mass production was statistically significantly higher ($P < 0.05$) in MT7 compared to control group (51.38 vs. 48.29 g/pcs/day). Similarly, a significantly higher ($P < 0.05$) laying intensity was found in experimental group MT7 compared to the control group (79.32% versus 74.72%).

The mean daily feed consumption (Tab. IV) was higher in MT7 by 6.35 g compared to C. The difference was statistically significant ($P < 0.05$). Feed consumption and feed conversion ratio (FCR) per one egg was without significant differences ($P > 0.05$).

Tab. V shows the qualitative evaluation of the eggs. Experimental monitoring revealed a stronger ($P < 0.05$) eggshell in the control group. The HU were significantly higher ($P < 0.01$) in the experimental group as well as the millimeter height of the albumen.

Results of the blood biochemical examination of laying hens are given in Tab. VI. According to the table, no significant differences were found in the blood biochemical parameters analysis ($P > 0.05$). The experimental group with the inclusion of 7% seed cakes in diet showed a significantly higher ($P < 0.05$) antioxidant activity of the blood plasma (Tab. VII).

III: *Live weight of laying hens during experiment (kg)*

Age	C		MT7	
	n	mean ± SE	n	mean ± SE
69th week	15	1.81 ± 0.073	15	1.79 ± 0.074
74th week	13	1.80 ± 0.067	13	1.74 ± 0.095
77th week	13	1.75 ± 0.070	13	1.81 ± 0.084
80th week	13	1.74 ± 0.075	13	1.74 ± 0.070

C: control group; MT7: 7% milk thistle seed cakes in diet
n–number of observations
SE–standard error
Differences were not statistically significant $P > 0.05$

IV: *Evaluation of the daily laying of eggs and average feed consumption during the experiment*

n	C	MT7
	62 days	62 days
	mean ± SE	
Number of eggs per group and day (pcs)	10.58 ± 0.201[a]	11.48 ± 0.212[a]
Laying intensity (%)	74.72 ± 1.437[a]	79.32 ± 1.539[a]
Mean egg weight (g)	64.81 ± 0.133	64.79 ± 0.139
Egg mass production (g/pcs/day)	48.29 ± 0.948[a]	51.38 ± 1.049[a]
Feed consumption (g/hen/day)	110.56 ± 1.867[a]	116.91 ± 2.263[a]
Feed consumption per egg (g)	150.83 ± 4.011	150.15 ± 4.716
Feed conversion ratio per egg	2.33 ± 0.06	2.31 ± 0.07

C: control group; MT7: 7% milk thistle seed cakes in diet
n–number of observations
SE–standard error
[a:a] the same characters in the one line show a statistically significant difference $P < 0.05$

V: *Egg quality parameters of laying hens*

n	C	MT7
	100 eggs	100 eggs
	mean ± SE	
Egg weight (g)	64.00 ± 0.562	64.81 ± 0.324
Eggshell strenght (N)	34.34 ± 0.664[a]	32.05 ± 0.831[a]
Egg lenght (cm)	5.72 ± 0.024	5.74 ± 0.022
Egg width (cm)	4.49 ± 0.029	4.46 ± 0.014
Shape index	1.28 ± 0.008	1.28 ± 0.007
Haugh units	83.86 ± 1.335[a]	88.60 ± 0.954[a]
Albumen height (mm)	7.49 ± 0.180[a]	8.23 ± 0.181[a]
Yolk weight (g)	16.06 ± 0.162	16.15 ± 0.196
Albumen weight (g)	42.05 ± 0.470	42.85 ± 0.363
Albumen (%)	65.55 ± 0.267	66.06 ± 0.360
Yolk (%)	25.16 ± 0.211	24.97 ± 0.317
Eggshell (%)	9.29 ± 0.135	8.96 ± 0.117
Egg yolk colour score	4.31 ± 0.101	4.18 ± 0.088
Eggshell weight (g)	5.89 ± 0.061	5.80 ± 0.078
Eggshell thickness (mm)	38.78 ± 0.438	38.14 ± 0.563

C: control group; MT7: 7% milk thistle seed cakes in diet
n–number of observations
SE–standard error
[a:a] the same characters in the one line show a statistically significant difference $P < 0.05$

VI: *Blood biochemical parameters of laying hens*

n	C	MT7
	7	7
	mean ± SE	
AST (µkat/l)	2.68 ± 0.227	2.48 ± 0.023
GGT (µkat/l)	0.53 ± 0.170	0.62 ± 0.266
ALP (µkat/l)	4.62 ± 0.608	6.82 ± 0.968
ALT (µkat/l)	0.06 ± 0.013	0.09 ± 0.013
LD (µkat/l)	36.89 ± 5.599	25.10 ± 5.407
Bili (µmol/l)	11.17 ± 1.544	11.64 ± 0.579
UA (µmol/l)	341 ± 54.18	480 ± 71.72
Chol (mmol/l)	3.23 ± 0.750	3.56 ± 0.365
TG (mmol/l)	14.37 ± 2.915	18.70 ± 3.180
TP (g/l)	53.36 ± 3.053	54.63 ± 1.731
Alb (g/l)	18.71 ± 0.702	19.13 ± 0.949
Glob (g/l)	34.64 ± 2.803	35.50 ± 1.870

C: control group; MT7: 7% milk thistle seed cakes in diet
n–number of observations
SE–standard error
Differences were not statistically significant $P > 0.05$
AST–aspartate aminotransferase; GGT–gamaglutamyltransferase; ALP–alkaline phosphatase; ALT–alanine aminotransferase; LD–lactate dehydrogenase; Bili–bilirubin; UA–uric acid; Chol–cholesterol; TG–triacylglycerols; TP–total protein; Alb–albumin; Glob–globulins

VII: *Hens blood plasma antioxidant activity (FRAP, µmol/l)*

	n	Mean ± SE
C	6	106.65 ± 8.708[a]
MT7	6	170.76 ± 25.229[a]

C: control group; MT7: 7% milk thistle seed cakes in diet
n–number of observations
SE–standard error
[a:a] the same characters in the column show a statistically significant difference P < 0.05

DISCUSSION

This experiment was carried out on laying hens after 69 weeks of age to investigate the possible influence of milk thistle (*Silybum marianum*) addition on hens liver and performance (including egg quality parameters). In older laying hens, summation of hepatic tissue occurs during the laying cycle due to its own high performance. In our experiment, 7% of milk thistle seed cakes was added to the mixture, which was 5.3% crude fiber content in the diet. Albiker *et al.* (2015) recommends up to 7% of crude fiber in diet for laying hens. The Lohmann Tierzucht Chicken Hedge Technological Guidelines provide the recommended content of crude fiber in feed mixtures for laying hens 5–6% (Lohmann Tierczucht, 2016).

Feed consumption, laying and egg quality parameters evaluation

In the experimental group MT7, was found a statistically significant higher (P < 0.01) number of eggs per group and day. The egg mass production in the experimental group was also significantly higher (P < 0.05). The higher laying intensity (P < 0.05) was accompanied by a statistically higher (P < 0.05) total feed consumption in the MT7 group. The results of our experiment correspond to the findings of Erisir *et al.* (2016) who found an increase in Japanese quail laying when feeding a 10 g/kg milk thistle seeds in feed mixture. Hashemi Jabali *et al.* (2017) performed an experiment (for 70 days) with leghorn type hybrids (from 40 weeks of age). In this experiment were fed diets with addition 0, 15, 30 and 60 g/kg milk thistle flour. The authors found that a dose of 30 g per kilogram of milk thistle flour led to a significantly lower intake of feed over the duration of the experiment, and this group had the best feed conversion (1.86) compared to other groups. In the MT7 group was found higher (P < 0.05) feed intake without effect to FCR per egg in our experiment. Hashemi Jabali *et al.* (2017) noted that the addition of 30 g of milk thistle flour led to a significant increase in laying intensity (86.27% vs. 80.89%) in the second phase of the experiment (35–70 days) and a significant increase in egg mass production (96 vs. 50.27 g/hen/day). This trend was confirmed by our trial, although the absolute values compared with Hashemi Jabali *et al.* (2017) were lower but it could be influenced by selected hen hybrid. Additionally, the addition of milk thistle flour in the experiment (Hashemi Jabali *et al.*, 2017) did not influenced the weight of the eggs, whereas in our experiment the total egg mass production was statistically significantly higher (P < 0.05), and average weight of one egg was not significantly higher (P > 0.05). On the other hand, Quarantelli *et al.* (2009) examined the addition of 0.2 and 0.4 g/kg silymarin in feed mixture of laying hens did not detect improvement in egg laying or FCR in the experimental group containing 0.2 g/kg of silymarin. The authors report that no other differences were found among the experimental and control groups in other monitored parameters, such as average egg weight, feed consumption per egg and shell thickness.

In the quality eggs evaluation, which has always tested ten randomly selected eggs, was found that the experimental treatment was not influenced average weight of the egg or the average weight of the egg yolk, probably due to the balanced content of nutrients (nitrogenous substances, fat) in diets. In the MT7 group, was found a significantly higher (P < 0.01) millimeter height of the albumen and associated statistically significant higher Haugh units (P < 0.01). On the other side, the experimental group (MT7) has significantly thinner eggshell compared to the control group without the addition of milk thistle seed cakes, although the calcium (3.6% C and 3.4% MT7) and phosphorus (6.2% C and 6.5% MT7) content in the diets was basically balanced (Tab. II). Jiang *et al.* (2013) argue that changes

in liver function can affect the metabolism of mineral nutrients including Ca and P, which are essential for skeletal integrity and eggshell quality. According to Ayerza and Coatese (1999), the egg quality parameters, including the weight of yolk, can be influenced by the amount of fat in the hens diet.

Haugh's units are important value for assessment the quality of eggs (LI *et al.*, 2017) and are also known as indicators of freshness related to egg shelf life (Özek *et al.*, 2011). In our experiment the egg was collected and evaluated at the same time, so the differences in HU were not due to the different freshness of the eggs.

The HU is calculated from the height of the internal dense albumen and the egg weight (reflecting the content of the dense egg albumen). The dense albumen viscosity is attributed to the presence of ovomucine (Omana *et al.*, 2010) and the value of HU is mainly influenced by ovomucine content in eggs (Kovacs-Nolan *et al.*, 2005). It has been found that low intake of nitrogenous substances by laying hens reduces the weight and strength of the albumen due to reduced albumin synthesis (Novak *et al.*, 2006).

Certain explanations of the differences in the quality of the albumen may be related to the fact that proteins taken from diets from non-traditional plant products may contain excess or deficiency of some digestible amino acids (such as arginine, phenylalanine, histidine and leucine) or antinutritional factors (phytochemicals, tannins and other substances), which affect the digestion of proteins and their deposition into egg albumen (especially ovomucine; Novak *et al.*, 2006; Phelps, 1966). At present days there is little more information about the effect of milk thistle active substances related to qualitative eggs evaluation.

Blood biochemical parameters and antioxidant activity

In our experiment, no statistically differences in blood biochemical parameters was found. It means that no effect of used seed cakes to liver function and other selected parameters of the hepatic metabolism was found. The same results found Blevins *et al.* (2010) who added 1,000 mg/kg of silymarin to the feed mixture. They not found differences in GGT and AST enzymes activity but also in cytochrome (CYP) 450 34A activity. On the other hand, the feeding of different levels of milk thistle flour to laying hens led to a marked decrease of triglyceride level in the blood of animals compared to the control group (Hashemi Jabali *et al.*, 2017). The authors of Hashemi Jabali *et al.* (2017) further found that 60 g/kg of milk thistle meal reduced the blood cholesterol concentration and raised the high-density lipoprotein (HDL) cholesterol concentration in animal blood ($P > 0.05$). The same conclusion achieved Suchý *et al.* (2008), who found a significantly lower blood cholesterol concentration in fattened chickens at the day 43 following the addition of 1% of milk thistle seed cakes to the feed mixture. Gyenis *et al.* (2006) published a range of plasma proteins in laying hens. The authors claim that the total protein content is 35–88 g/l and 13–28 g/l for albumin, which corresponds to our results.

In our experiment milk thistle seed cakes feeding lead to a statistically significant higher antioxidant activity of the laying hens blood, which could be caused by the content of silymarin, which has a polyphenolic structure that causes its antioxidant properties. The hydroxyl groups of the silymarin complex have the potential to absorb free radicals (Miranda *et al.*, 2000).

CONCLUSION

Perspective results are found in the addition of 7% milk thistle seed cakes in the diet of laying hens. After the 69th week of age, the laying hen in the experimental group reached a higher number of eggs and produced more egg mass compared to the control group. In the evaluation of the egg quality parameters, higher Haugh units were found, a higher millimeter height of the egg, but a thinner shell in the laying hens receiving 7% of the seed cakes. When evaluating health indicators, higher antioxidant activity was found in the experimental group.

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Contact information

Ondřej Šťastník: ondrej.stastnik@mendelu.cz
Eva Mrkvicová: eva.mrkvicova@mendelu.cz
Leoš Pavlata: leos.pavlata@mendelu.cz
Andrea Roztočilová: andrea.roztocilova@mendelu.cz
Barbora Umlášková: barbora.umlaskova@mendelu.cz
Eva Anzenbacherová: eva.anzenbacherova@upol.cz