Evaluation of the Effects of *Urtica Dioica* L. Supplementation on Egg Quality and Blood Parameters in Laying Hens

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Abstract. *The purpose of the current research was to study the influence of dietary supplementation of nettle (Urtica dioica) on laying performance, egg quality and blood serum biochemical parameters of layers. A total of 60 laying hens (42 weeks old) from Lohman Klassik Brown breed were randomly allocated into three groups: a control and two experimental groups (n = 20 hens per group). All layers received compound feed with the following nutritional value: 2710 Kcal/kg metabolizable energy; 16.44% crude protein; 3.32% crude fats; 4.58% crude fibres; 3.73% Ca; 0.49% P. The hens from the experimental groups received 0.3% (experimental group 1) and 0.5% (experimental group 2) of dried nettle with the diet. Both experimental groups had significantly higher egg yolk pigmentation ($p < 0.001$) compared with the control group. A significantly lower egg yolk cholesterol content was found in hens from experimental group 1 ($p < 0.05$). Nettle addition reduced significantly blood serum glucose ($p < 0.01$ and $p < 0.05$ in experimental groups 1 and 2, respectively) as well as the total serum cholesterol content ($p < 0.001$).*

Introduction

The nettle *(Urtica dioica* L., family *Urticaceae***)** is an ordinary plant with extraordinary properties (Bisht et al., 2012; Kregiel et al., 2018), widely grown in different parts of the world and used to promote health. Nettles have a high ratio of nutritious substances (vitamin C, carotenoids, minerals), active compounds such as tannins, formic acid, salicylic acid, thymol and carvacrol and make a readily digestible food (Viegi et al., 2003; Gülçin et al., 2004). Nettles possesse antioxidant, antimicrobial, antifungal and antiviral properties (Rutto et al., 2013, Upton, 2013). Recently, there has been a trend in the animal nutrition to improve the digestibility, gut health, immune response, and quality of the animal products (eggs, meat, milk) by using herbs and their extracts.

Szewczyk et al. (2006) have reported a reduction of monounsaturated fatty acids (MSFAs) and an increase of polyunsaturated fatty acids (PUFAs) in pig's muscle fat by nettle addition to their diet. Khanal et al. (2017) have established a beneficial effect of stinging nettle supplementation on quantity and quality of milk yield as well as on the body condition score in dairy cattle. Influence of diet nettle supplementation has been investigated by Stojčić et al. (2016) with broilers in two alternative housing systems. There were no effects on carcass weight, dressing percentage, abdominal fat and percent of parts of broiler chicken carcass. The authors found out that dietary supplementation of fresh nettle can improve the quality of chicken breast meat better than pasture intake.

The inclusion of 6% nettle in a quail's diet has led to reduction of egg yolk cholesterol, serum total cholesterol and serum triglyceride levels and has not negatively influenced quail performance (Moula et al., 2019). Loetcher et al. (2013a) have used nettles as natural yellow colorant for egg yolk. There was no substantial influence of nettle supplementation on laying performance and general egg quality. Nettle supplementation of layer diets is therefore considered as an effective means to naturally achieve the desired yolk yellowness, without risking unfavourable side-effects.

However, in available literature, there is no sufficient information about the effects of dried and milled nettle in a laying hen's diet.

The objective of our study was to investigate the influence of dried nettle addition on egg production, egg quality, yolk lipid oxidation, and some blood parameters in Lohman Klassik Brown layers.

Materials and methods

The experiment complied with Directive 2010/63/EU on the protection of animals used for scientific purposes, and the experimental procedures were approved by the Ethical Commission of National Research and Development Institute for Biology and Animal Nutrition.

Experimental design

The experiment was conducted in the Poultry Experimental Base of the Institute of Animal Science-Kostinbrod, Bulgaria, with a total of 60

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laying hens at the initial age of 42 weeks from Lohman Klassic Brown breed. The laying hens were randomly divided in three groups: control (n = 20) and two experimental (n = 20 per group), kept in separate pens. The poultry was raised on a deep litter pen on a 16-hour lighting schedule, 70–85% relative air humidity, and 21–24°C air temperature. Water was supplied via nipple drinkers. The trial lasted 50 days: the preparatory period was 10 days, and the experimental period lasted 40 days. During the preparatory period, all the groups received compound feed for layers in the amount of 130 g/day/hen in order to eliminate the influence of the previous diet. During the experimental period, the hens received 130 g/day/hen of this compound feed, whereas the diet of experimental hens was supplemented with 0.3% (experimental group 1) and 0.5% (experimental group 2) dried and milled nettle. In our research, a dry mass of the above ground part of *Urtica dioica* was used as a diet supplement. The chemical composition and antioxidant properties of dried nettle are presented in Table 1.

The ingredients and chemical composition of the diets are presented in Table 2.

The feed nutritive value was determined by the conventional Weende analysis: crude protein, crude fat, and crude fibres (by Weende analysis); the contents of both Ca (BSS 11 374-86, 1990) and P (BSS 4336-73, 1990); the pH value, determined, using a pH meter Stirrer, type OP-951. The metabolizable energy was calculated according to WPSA (1989).

At the beginning and at the end of the trial, the live body weight of the hens from the control and experimental groups was measured.

Table 1. Chemical composition, total antioxidant capacity and pH of nettle

Nettle	
Moisture, %	9.75
Crude proteins, %	24.52
Crude fat, %	2.86
Crude fibre	11.39
Ca, %	3.95
P, %	0.556
Total phenolic content, mg GAE/100 g	357.00
Total antioxidant capacity, mmolTE/100 g	1230.20
pH	8.33

Table 2. Composition and nutritive value of the feed for laying hens

Ingredients, %	Control	Experimental group 1	Experimental group 2
Wheat	63.34	63.04	62.84
Soybean meal	9.0	9.0	9.0
Sunflower meal	14.0	14.0	14.0
Sunflower oil	2.5	2.5	2.5
Nettle	0.0	0.3	0.5
Limestone	9.0	9.0	9.0
Mono calcium phosphate	0.4	0.4	0.4
Complex premix 6015*	1.25	1.25	1.25
Nutritive value			
Metabolizable energy, kcal kg^{-1}	2710	2710	2710
Crude proteins, %	16.44	16.44	16.44
Crude fat, %	3.32	3.32	3.32
Crude fibre, %	4.58	4.58	4.58
Ca, %	3.73	3.73	3.73
P, %	0.49	0.49	0.49

* Complex premix contains: Mn (MnO): 120 mg/kg; Zn (ZnO): 110 mg/kg; Fe ($FeSO_4$): 140 mg/kg; Cu ($CuSO_4$): 18 mg/kg; I ($Ca(IO_3)_2$: 1.80 mg/kg; Se (Na_2SeO_3): 0.35 mg/kg; vitamin A (retinyl acetate): 9900 UI; vitamin D_3 (cholecalciferol): 3000 UI; vitamin E (DL-alpha-tocopherol): 30 mg/kg. It does not contain nutritive antibiotics, synthetic dyes and carotenoids or other stimulants.

The egg production (in percent) for each group was controlled every day. There was no mortality noticed in the experimental groups during the trial.

Thirty eggs from each group, laid within two consecutive days, were taken at the beginning and at the end of the experiment and the following measurements were made:

– The weight of the egg, egg shell with a shell membrane, egg yolk, and albumen were measured with an electronic scale BOECO within 0.001 g;

– The shape index was measured by an index meter;

– The height of albumen and egg yolk as well as the egg yolk width were measured with a calliper (in mm);

– The haugh unit was calculated by the formula:

$$HU = 100 \log (h + 7.17 - 1.7\ W0.37)$$

Where: H is height of the thick albumen (in mm) and W is egg weight;

– The shell thickness (mm) without a shell membrane was measured by a micrometer Amer 25EE with the precision of 0.0001 mm;

– The egg yolk colour was determined visually by the 15 Roche colour fan having a 15 degrees scale.

At the end of the treatment, 10 hens from each group were chosen randomly and blood samples were taken from *Vena cutanea ulnaris*. The serum levels of total cholesterol, glucose, and triglycerides were measured by commercial kits using biochemical analyser BioSystems (S.A. Costa Brava, Spain).

At the end of the experimental period, some lipid fractions of egg yolks of 10 eggs from each group were determined. The total lipids were determined by the method of Bligh and Dyer (1959). The total cholesterol content in the yolk was determined by the method of Schoenheimer-Sperry modified by Sperry and Webb (1950).

At the end of the trial, the lipid oxidation of egg yolk of 6 eggs from each group was evaluated as TBARS according to the method of Castellini et al. (2006). Oxidation products were quantified as malondialdehyde equivalents (mg MDA 100 g^{-1}).

The results were expressed as means with their standard errors. Statistical examination of the data obtained was determined by SPSS, single factor, ANOVA program. A t-test was used to compare the results between control and experimental groups. Statistical significance was set at $p < 0.05$.

Results and discussion

Effect of nettle powder on laying performance

The values of live body weight egg intensity and mortality of the hens from the control and experimental groups are shown in Table 3. The live body weight of laying hens at the beginning of the experimental period varied within narrow range: 1846 g; 1782 g, 1868 g for control, and experimental groups 1 and 2, respectively ($p > 0.05$). This parameter increased by 90 g, 35 g, 78 g for control, and experimental groups 1 and 2, respectively, at the end of the trial ($p > 0.05$). No significant differences ($p > 0.05$) between the groups about these parameters were found.

The hens' laying intensity at the beginning of the experimental period was as follows: control – 86.67%, experimental group 1 – 85%; experimental group 2 – 86.00%. At the end of the treatment, this indicator was 92%, 89% and 89.5% for control and experimental groups 1 and 2, respectively. At the end of the trial, within the groups, there was an increase in laying intensity by 5.33% in the control and by 4% and 3.5% for experimental groups 1 and 2. The differences between the groups were insignificant ($p > 0.05$).

Effect of nettle supplementation on egg quality

The effects of nettle powder supplementation on egg morphological parameters of laying hens are presented in Table 4. According to Song et al. (2000), Toussant and Latshaw (1999), and Wolanski et al. (2007), the egg morphological parameters can be classified into 2 main groups: external (egg weight, shell thickness, shape index) and internal traits (albumin index, yolk index, Haugh unit, egg yolk color). As can be seen from Table 4, the addition of 0.3% and 0.5% dried and milled nettle to the layers diet had no significant effect on egg-, albumen-, yolk- and shell weights; shell thickness; Haugh units; and shape index, albumen index and yolk index. The present finding is in agreement with nettle supplementation of layer diets that showed no considerable effect on internal and external traits (Loetscher et al., 2013).

Yolk color is one of the important factors for egg marketing. In general, consumers *prefer eggs with an orange yolk colour*. To achieve desirable yolk

Table 3. Live body weight, feed intake, egg intensity and mortality of laying hens (X ± SE)

Dietary treatments	Control	Dietary nettle powder (%)	
		0.3	0.5
Initial body weight (g)	1846 ± 27.65	1782 ± 22.63	1868 ± 30.29
Final body weight (g)	1936 ± 28.34	1817 ± 48.92	1946 ± 32.98
Egg intensity (%) Start	86.67 ± 1.44	85.00 ± 2.67	86.00 ± 1.25
Egg intensity (%), End of experiment	92.00 ± 0.82	89.00 ± 1.80	89.50 ± 1.17

Table 4. Effect of dietary nettle powder on egg morphological parameters (X ± SE)

Groups / Indices	Control	Dietary nettle powder supplementation (%)		Control	Dietary nettle powder supplementation (%)	
		0.3	0.5		0.3	0.5
	Start of the experiment			End of the experiment		
Egg weight, g	63.84 ± 0.64	61.36 ± 0.78	62.30 ± 0.76	64.10 ± 0.72	62.06 ± 0.89	62.13 ± 0.88
Albumen, g	41.50 ± 0.45	40.00 ± 0.61	40.17 ± 0.64	41.44 ± 0.49	40.08 ± 0.69	40.05 ± 0.72
Yolk, g	15.56 ± 0.21	14.74 ± 0.21	15.53 ± 0.20	15.96 ± 0.24	15.64 ± 0.22	15.50 ± 0.18
Shell, g	6.93 ± 0.12	6.61 ± 0.09	6.77 ± 0.08	6.54 ± 0.11	6.34 ± 0.15	6.27 ± 0.11
Shell thickness, mm	0.39 ± 0.005	0.38 ± 0.003	0.40 ± 0.003	0.39 ± 0.004	0.39 ± 0.005	0.39 ± 0.005
Haugh units	81.50 ± 1.30	76.20 ± 1.41	77.45 ± 1.33	73.43 ± 1.51	71.38 ± 1.30	71.13 ± 1.42
Shape index %	78.55 ± 0.36	79.50 ± 0.47	80.23 ± 0.40	78.60 ± 0.37	79.40 ± 0.36	79.25 ± 0.44
Albumen index %	9.74 ± 0.29	8.10 ± 0.34	8.69 ± 0.38	7.73 ± 0.30	6.49 ± 0.32	6.74 ± 0.32
Yolk index %	38.30 ± 0.64	37.76 ± 0.70	41.56 ± 0.85	40.52 ± 0.61	38.46 ± 0.51	38.82 ± 0.57
Yolk color	2.89 ± 0.18	2.81 ± 0.19	2.78 ± 0.18	2.93 ± 0.16	4.73 ± 0.27 A***	5.20 ± 0.24 B***

Significance by: * – $p \leqslant 0.05$; ** – $p \leqslant 0.01$; *** – $p \leqslant 0.001$
A – control group / experimental group 1
B – control group / experimental group 2

colour, intensity hens' feed is often supplemented with synthetic carotenoids because they are cheaper. The increased demand of safety animal products during the recent years requires further studies on the possibilities to use various natural sources of carotenoids as layers' diet ingredients (Grigorova and Petkova, 2014). At the beginning of our study, yolk colour intensity in the groups varied within close range – from 2.78 to 2.89 points on the Roche Colour Fan. At the end of the trial, this parameter increased significantly ($p < 0.001$) in both experimental groups compared with the control group (Table 4). Based on the fact supported by scientific investigations of Kang et al. (2003) and Karadas (2006), the carotenoids from feed compound passed unchanged in egg yolk, so the nettle powder used in different amount (0.3% and 0.5%) in our study was a suitable applicant for egg yolk pigmentation.

Egg is one of the major sources of dietary cholesterol, which may lead to lower consumption of eggs of the consumers instead of the scientific thesis that the cholesterol improves lipid profile (Fernandez-Robredo et al., 2008). There are a lot of investigations for reducing the cholesterol in egg yolk and enhancing the nutritional value of egg with supplementation of the hen's diet with plants and herbs (Grigorova et al., 2021; Chowdhury et al., 2002; Chen et al., 2005). In the current research, a significant decrease of total yolk cholesterol in hens receiving 0.3% dried nettle with the diet was established (Table 5).

These results are similar with the findings of Mansoub (2011) in which 2% nettle powder supplementation to the diet of laying hens reduced the total cholesterol and triglycerides in eggs, but are slightly different from the limited effects reported by Keshavarz et al. (2014). The present study is consistent with the findings of Moula et al. (2019) who have reported that nettle at the level of 6% reduced egg yolk cholesterol in quails.

In general, herbs have antioxidant properties, so as a feed ingredient they deserve a special attention for decreasing the oxidation level that may cause the changes in organoleptic properties of eggs. Nettle leaves are rich in polyphenols, mainly flavonoids (i.e., kaempferol, isorhamnetin, quercetin, and their rutinoside or glycoside derivatives) and phenolic acids (i.e., caffeic acid and its ester derivatives, like chlorogenic acid and caffeoylmalic acid) (Upton, 2013). The phenolic compounds of nettle stabilize the lipid peroxidation (Akbay et al., 2003). In recent years, some researchers have presented results according to which supplementation of feed with some plant products indicates reduction of lipid peroxidation in eggs. The antioxidant capacity of nettle (*Urtica dioica*) is achieved mainly by in vitro approaches (Gulcin et al., 2004).

Grigorova et al. (2020) confirms a significant reduction of the lipid peroxidation in egg yolk by feeding laying hens with rosehip rich with lycopene. In the current experiment, there is no significant influence on content of malondialdehyde (MDA), which is the main indicator of lipid peroxidation because of the lack of lycopene in nettle (Table 5). The literature data from investigations about the influence of the nettle powder or extract used in the diet on lipid peroxidation in the egg yolk are very scarce. Nettle was usually used as supplement for poultry meat colour (yellow colour of meat) and

Table 5. Effect of dietary nettle powder on yolk lipids, yolk cholesterol, and lipid oxidation (X ± SE)

Indices \ Groups	Control	Dietary nettle powder supplementation (%)	
		0.3	0.5
Total lipids g / 100 g yolk	36.00 ± 0.59	35.36 ± 0.46	35.34 ± 0.40
Total cholesterol, mg / 100 g yolk	1536.78 ± 39.90	1382.10 ± 31.12 A**	1501.73 ± 44.72
Malondialdehyde (MDA), μg g^{-1}			
At the end of the experiment	0.31	0.38	0.31
Storage 30 days in fridge	1.14	0.91	0.85

Significance by: ** – $p \leqslant 0.01$
A – control group / experimental group 1

improvement of the colour of the yolk with nature colourants. The investigation established with broilers by Keshavarz et al. (2014) revealed that thiobarbituric acid reactive substances (TBARS), as an indicator for meat lipid oxidation after storage, were not influenced by adding nettle powder and nettle extract in broiler diets. Loetscher et al. (2013b) have reported that nettle supplementation in a diet did not improve oxidative stability (TBARS), but strongly intensified skin yellowness compared with the control treatment. In our experiment, the nettle supplementation did not significantly influence reduction of the lipid peroxidation in egg yolk, but there was a slight reduction of the lipid oxidation value compared with the control group.

Serum biochemical parameters

The influence of nettle powder supplementation on some serum biochemical parameters of laying hens is shown in Table 6. The addition of 0.3% dried nettle to the diet significantly decreased serum concentrations of total cholesterol ($p < 0.001$) and triglycerides ($p < 0.01$). The supplementation of nettle powder at 0.5% in the diet decreased ($p < 0.001$) total cholesterol, without affecting triglycerides. The level of the glucose was reduced significantly in the serum of laying hens fed with 0.3% supplemented diet ($p < 0.01$) and 0.5% ($p < 0.05$)

Mansoub (2011) have reported that the serum total cholesterol, triglycerides and LDL concentration were significantly reduced in the group supplemented with 2% nettle powder compared with the control group ($p < 0.05$), but the concentration of serum HDL and glucose were not significantly reduced in the groups compared with the control group. Moula et al. (2019) who studied the nettle effect in quails have reported reduced quail serum total cholesterol and serum triglyceride levels at the level of 6%.

The results of a significant reduction of the level of cholesterol in serum lead to the cholesterol reduction in egg yolk, which is reported in this study.

Conlussions

Our results demonstrated a positive effect of 0.3% and 0.5% nettle addition to the layers diet on yolk colour pigmentation. A significantly lower egg yolk cholesterol content was found in hens from experimental group 1 ($p < 0.05$). Nettle supplementation also reduced significantly blood serum glucose ($p < 0.01$ and $p < 0.05$ in experimental groups 1 and 2, respectively) as well as the total serum cholesterol content ($p < 0.001$). However, laying hens' performance did not alter by dietary nettle supplementation. The results also suggest that nettle could be added to the diets of laying hens to improve egg quality and the serum biochemical profile. Nettle proved unsuitable as a dietary antioxidant in the applied form and concentration, yet the colouring effect on the egg yolk could be interesting for egg production in certain cultures.

Table 6. Effect of dietary nettle powder on some biochemi cal parameters in blood serum of layers (X ± SE)

Indices \ Groups	Control	Dietary nettle powder supplementation (%)	
		0.3	0.5
Glucose, mmol/L	9.22 ± 0.44	7.07 ± 0.64 A**	7.66 ± 0.58 B*
Triglycerides, mmol/L	11.95 ± 1.54	5.59 ± 1.11A**	8.46 ± 1.11
Total cholesterol, mmol/L	2.75 ± 0.17	1.86 ± 0.13 A ***	1.87 ± 0.10 B ***

Significance: * – $p \leqslant 0.05$; ** – $p \leqslant 0.01$; *** – $p \leqslant 0 = 001$
A – control group / experimental group 1
B – control group / experimental group 2

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