EFFECTS OF DIETARY SUPPLEMENTATION OF NETTLE FLOUR (*URTICA DIOICA* L) ON THE PERFORMANCE OF LAYING HENS AND QUALITY OF EGG

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RESEARCH ARTICLE

Abstract

The purpose of this study was to investigate the effect of the use of nettle (Urtica dioica) flour in the feed of laying hens on production performance and the quality of consumption eggs. A total of 60 laying hens from the hybrid (Tetra SL) at the age of 42 weeks were distributed in a completely randomized design consisting of two experimental groups, respectively two dietary treatments: the control group (C) fed with a standard diet and the experimental group (E) - fed with a diet in which nettle flour (Urtica dioica) was introduced in a proportion of 2% (% of the weight of the feed. The hens were housed in separate pens at a density of 6 birds/m^2. Experimental period lasted 4 weeks. The obtained results demonstrated that the introduction of nettle flour in a proportion of 2% (% of the feed weight) in the feed of laying hens does not affect egg production (laying intensity and egg weight), consumption and the degree of utilization of the feed nor the quality of the obtained eggs, except for the color of the yolk which improved significantly. Thus, the use of nettle flour in the feed of laying hens would improve the quality of the eggs and could satisfy the consumer preferences for the more intense color of the yolk.

Keywords: nettle flour, laying hens, performance, egg quality .
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INTRODUCTION

Recently, there has been an increase in the demands of consumers regarding the quality of agri-food products of animal origin, which must not only be a source of nutrients for human food, but must also provide the consumer with an important intake of bioactive compounds with a positive effect on human health . In this context, nutritionists have paid special attention to medicinal plants, which used in animal feed can lead to a significant improvement in the nutritional and blood quality of animal production. Medicinal plants are accepted in animal feed as possible substitutes for feed antibiotics, being accepted by consumers because they are considered natural sources of bioactive compounds with the effect of promoting animal production as well as antioxidant effects (Mousavi et al., 2017; Kheiri and et al., 2018; Moula et al., 2019). One of these medicinal plants is nettle (*Urtica dioica*) (Sharma et al., 2018), which has been less studied in terms of its use in bird feed and its effect on the production and quality of eggs for consumption (Moula et al., 2019).

Nettle (*Urtica dioica* L., family *Urticaceae*) is a wild herbaceous perennial plant, frequently found in the spontaneous flora of our country, being characterized by a high content of nutrients (vitamin C, carotenoids, minerals), as well as tannins, formic acid, salicylic acid, thymol and carvacrol (Gülçin et al., 2004). Nettles have antioxidant, antimicrobial, antifungal and antiviral properties (Upton, 2013). Nettle is a plant rich in leaves and produces a fluid of formic acid and histamine, which in humans causes blisters upon entering the skin (Adhikari et al., 2016). Rutto et al., (2013) and Said et al., (2015) concluded that nettle leaves are rich in proteins, fats, carbohydrates, vitamins, minerals and trace elements (mainly Fe). The protein content (30% of DM) and amino acid profile is clearly superior to other leafy plants (Rutto et al., 2013). In addition, in organic egg production it is necessary for the hens to have access to and consume green fodder such as alfalfa plants, clovers or nettles (Zheng et al., 2019). The use of nettle flour in the feed of broilers improved growth performance and hematocrit value (Hashemi et al., 2018). Research by Avci et al., (2006) demonstrated that mice fed a diet supplemented with nettle meal showed

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significant reductions in triglycerides and total cholesterol content in blood plasma. Also Grigorova et al. (2022) demonstrated that supplementing the diet of laying hens with nettle (0.3-0.5% of diet) led to a significant improvement in yolk pigmentation, a decrease in cholesterol concentration in the yolk, as well as blood sugar and total serum cholesterol content. The purpose of this study was to investigate the effect of the use of nettle (*Urtica dioica*) flour in the feed of laying hens on production performance and the quality of consumption eggs.

MATERIAL AND METHOD

This study was approved by the ethics committee of the Faculty of Environmental Protection of the University of Oradea (3 of 06/01/2021). A total of 60 laying hens from the hybrid (Tetra SL) at the age of 42 weeks were distributed in a completely randomized design consisting of two experimental groups, respectively two dietary treatments: the control group (**C**) fed with a standard diet and experimental group (**E**) - fed with a diet in which nettle flour (*Urtica dioica*) was introduced in a proportion of 2% (% of the weight of the feed. The birds were randomly assigned to the two dietary treatments (30 hens/group). The hens were housed in separate pens at a density of 6 birds/m^2, provided with nests for laying eggs (8 nests/pen). During the experimental period, the specific technological conditions for raising laying hens were ensured (temperature, humidity, ventilation, light schedule, etc.) The experimental period lasted 4 weeks, from the age of the chickens 42 weeks to 46 weeks of age. The nettle plants (*Urtica dioica*) were harvested from the spontaneous flora, in the month of June - 2022, before flowering, they were naturally dried in a thin layer in the shade after which they were chopped and then ground with a mill with chop by passing through the mill several times to obtain a mass made of the finest particles. During the experimental period (28 days), feed consumption, egg production (number of eggs) and egg weight were recorded daily, separately by group (treatments), by weighing them individually.

Nettle flour samples for laboratory analyzes were taken in compliance with the SR EN ISO 6498:2012 standard. Thus, three laboratory samples were constituted, which were squared in jars with rubbed stoppers until the chemical analysis was carried out. The crude chemical composition of nettle flour was performed in accordance with the AOAC International (2005) technique, determining: dry matter (DM) (Method 934.01); crude ash by calcination (Met. 942.05); crude protein by the Kjeldahl method (Met. 954.01); crude fat (ether extract - EE) by the Soxhlet method (Met. 920.39); crude cellulose by acid hydrolysis followed by basic hydrolysis of the sample (Met. 978.10) and organic matter by difference (OM = 100 − crude ash). In the last experimental week, in 3 consecutive days, 20 eggs/treatment were collected and analyzed for: weight of morphological components (10 eggs / treatment), yolk color (5 eggs / treatment) and chemical composition (5 eggs / treatment). The analyzed eggs were collected in the morning, taking into account the eggs without shape anomalies and with a weight close to the average weight of the eggs of the batch from which they come. To determine the morphological components of the eggs, the weight of the yolk and the mineral shell was determined by weighing, and the weight of the white was determined by the difference from the whole egg. The three morphological components of the whole egg were analyzed and statistically interpreted as absolute values (g) or as weight in the structure of the whole egg: % white, % yolk, % shell. The assessment of the quality of fresh eggs based on the relevant physical indices (white index, yolk index and Haugh unit) was carried out on a number of 5 eggs/treatment/day, respectively 15 eggs/treatment, using the following calculation relationships (Moula et al., 2019):

$$\text{Albumen index} = \text{Albumen height}/(\text{Albumen length} + \text{Albumen width}) \times 100. \quad (1)$$

$$\text{Yolk index} = \text{Yolk height}/\text{Yolk diameter} \times 100. \quad (2)$$

$$\text{Haugh unit} = \log(\text{Albumen height} + 7.57 - 1.7 \times \text{Egg weight}^{0.37}) \times 100. \quad (3)$$

The height and diameter of the yolk and white were measured with a digital caliper (Stainless, precision 0.01 mm). The color intensity of the yolk was determined using the Roché Scale (on a scale of 1 – 15 points). The raw chemical composition of the eggs was determined after separating the yolk and white

from the shell and homogenizing them immediately after harvesting the eggs, which were then stored at -80°C until the chemical analyses. From the egg samples (white + yolk), the content in protein, fat and crude ash was determined, according to the procedures of AOAC International (2005), similar to those described in the case of nettle flour analysis. The Tukey test was used to compare the differences between the mean values of applied treatments. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSIONS

Nettle flour (*Urtica dioica*) is characterized by a relatively high protein content, which is on average 25.64% (% of DM) with variation limits between 21.7% and 29.6% respectively (table 1). The fat content is relatively low (1.84 - 3.09%) but it has a high crude cellulose content (10.3 - 14.7%), which could limit the use of nettle flour in large quantities in bird feed.

Egg-laying intensity (%), average egg weight (g/egg) and average daily production of egg mass (g egg mass/day/hen) were not significantly influenced by the introduction of nettle flour in the feed of laying hens, although it can be finds a slight increase in these indicators of productive performance, compared to the control group (table 3). Thus, in the four experimental weeks, the average laying intensity was 90.73-91.91%, with an average egg weight of 61.96-61.66 g/egg and respectively a daily production of egg mass of 56.21-56.67 g/day/hen (table 3). During the experimental period, the percentage of eggs with defects (eggs with cracked shells, eggs that are too small or too large, deformed eggs, eggs with incompletely formed shells) was small (0.43 - 0.51%) and was not influenced by the experimental factor, namely the introduction nettle flour in food (data not shown).

Table 1.

Chemical composition of nettle powder (% of DM)

Items	Mean	Min.	Max.
Dry matter (DM)	90.82	90.45	91.83
Organic matter (OM)	85.92	81.65	94.17
Crude protein (CP)	25.64	21.72	29.61
Crude fat (EE)	2.75	1.84	3.09
Crude fibre (CF)	12.01	10.33	14.76
Bross energy (kcal/kg)	3440	3267	3679
Crude ash	14.08	5.83	18.35

Table 2.

Ingredient and nutrient compositions of the experimental diets

	Treatments			Treatments	
	Control (C)	Experienced (E)		Control (C)	Experienced (E)
Ingredients (%)			**Calculated analyses (%)[2]**		
Corn (8%)	54.44	55.24	ME (kcal/kg)	2761	2773
Nettle powder (23.2%)	***0.00***	***2.00***	Crude protein (%)	17.20	17.26
Sunflower oil	2.60	2.60	Lysine (%)	0.84	0.84
Sunflower meal (35%)	15.00	12.20	Methionine + cystine	0.71	0.72
Soybean meal (46%)	16.50	16.50	Threonine (%)	0.61	0.61
Limestone	8.70	8.70	Tryptophan (%)	0.20	0.20
Dicalcium phosphate	1.75	1.75	Linoleic acid (C18:2 n-6)	2.58	2.56
Salt	0.30	0.30	Calcium (%)	3.70	3.70
Premix vitamin-mineral[1]	0.50	0.50	Available phosphorus (%)	0.38	0.38
Lysine HCl (78.8%)	0.11	0.11	Sodium (%)	0.21	0.21
DL-methionine (99.9%)	0.10	0.10			

[1]Provides 1 kg of feed: Mn - 80 mg; Zn - 50 mg; Fe - 60 mg; Cu - 7 mg; Co - 0,4 mg; I - 2,0 mg; Se - 0,10 mg; choline hydrochloride - 230 mg; vitamin A - 11000 IU; vitamin - D_3 - 2500 IU; vitamin E - 50 mg; vitamin K_3 - 3.3 mg; vitamin B_1 - 3 mg; vitamin B_2 - 5 mg; niacin - 25 mg; calcium pantothenate - 11 mg; vitamin B_6 - 6 mg; vitamin B_{12} - 0.08 mg; folic acid - 1.7 mg.
[2]Source: Nutrient Optimiser, 2022; Nutrition Value.org, 2022. ME: Metabolizable energy

The average daily feed consumption, compared to the entire experimental period, did not differ significantly between the two groups of chickens, although there was a tendency to increase consumption in the case of chickens fed with the diet supplemented with nettle flour (113 g/day vs. 110 g/day). The feed conversion ratio was 121-123 g

feed/egg, respectively 1.96-1.99 g feed/g egg mass (table 4). The introduction of nettle flour in the feed of laying hens did not affect the morphological structure of the eggs nor their chemical composition (table 5).

Table 3.

Effect of nettle powder (Urtica dioica) on egg production (mean ± SE)

Week	egg production (%)		egg weights (g)		amount of eggs (g/day)	
	C	**E**	**C**	**E**	**C**	**E**
1	93.27[b] ± 1.59	96.34[a] ± 1.85	60.85 ± 0.42	60.41 ± 0.57	56.75 ± 0.31	58.20 ± 0.43
2	88.46[b] ± 2.54	94.45[a] ± 2.68	61.85 ± 0.45	61.60 ± 0.64	54.71 ± 0.40	58.18 ± 0.51
3	91.90[a] ± 2.72	88.69[b] ± 2.47	62.41 ± 0.50	61.80 ± 0.60	57.35 ± 0.32	54.81 ± 0.40
4	89.32 ± 2.31	88.15 ± 2.76	62.75 ± 0.47	62.85 ± 0.71	56.04 ± 0.45	55.40 ± 0.59
Mean	90.73 ± 2.62	91.91 ± 2.97	61.96 ± 0.53	61.66 ± 0.67	56.21 ± 0.40	56.67 ± 052
p-value	0.22	0.18	0.12	0.16	0.21	0.20

[a, b]Within a row, mean values with different letters were significantly different (P < 0.05).

Table 4.

Effect of nettle powder (*Urtica dioica*) on feed intake and feed conversion ratio

Week	Feed intake (g/day/hen)		Feed conversion ratio			
			g feed/egg		g feed/g egg	
	C	**E**	**C**	**E**	**C**	**E**
1	101 ± 5	104 ± 4	108 ± 3	108 ± 6	1.78	1.79
2	116 ± 7	117 ± 8	131 ± 9	124 ± 5	2.12	2.01
3	118 ± 7	122 ± 8	128 ± 6	137 ± 11	2.06	2.23
4	106 ± 6	110 ± 7	118 ± 8	125 ± 9	1.89	1.98
Mean	110 ± 7	113 ± 5	121 ± 8	123 ± 11	1.96	1.99
p-value	0.20	0.31	0.09	0.15	0.24	0.27

Table 5.

Effects of dietary nettle power supplementation on the egg quality and chemical composition of eggs from laying hens

Items		Treatments	
		C	E
Egg quality parameters			
- Albumen,	g	40.05	39.75
	%	64.64	64.46
- Yolk,	g	15.41	15.38
	%	24.88	24.95
- Shell,	g	6.32	6.23
	%	10.20	10.10
Albumen index[1], %		6.23	6.47
Yolk index[2], %		40.46	40.15
Haugh unit[3]		72.11	72.25
Yolk color[4]		5.27	11.73
Chemical composition			
Crude protein (CP, %)		11.62	12.40
Fat crude (EE, %)		8.23	7.69
Ash (%)		0.74	0.79

[1]Albumen index = Albumen height/[Albumen length + Albumen width] x 100;
[2]Yolk index = Yolk height/Yolk diameter x 100;
[3]Haugh unit = log (Albumen height + 7.57 - 1.7 x Egg weight$^{0.37}$) x 100;
[4]points on the La Roche scale.

Also, albumen index, york index and haugh unit, which characterize the quality of consumption eggs, did not register significant differences between the two groups of hens. Yolk color was significantly improved by supplementing the diet of laying hens with nettle meal; the color intensity of the yolk being 2.2 times higher than in the control group (fig. 1).

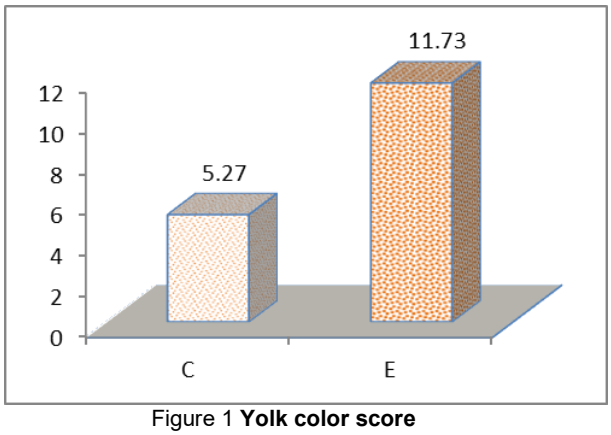

Figure 1 **Yolk color score**

The obtained results demonstrated that the introduction of nettle flour (Urtica dioica) in a proportion of 2% (% of the feed weight) in

the feed of laying hens does not affect egg production (laying intensity and egg weight), consumption and degree of utilization of the feed (fig. 2) and neither the quality of the obtained eggs, with the exception of the color of the yolk, which improved significantly.

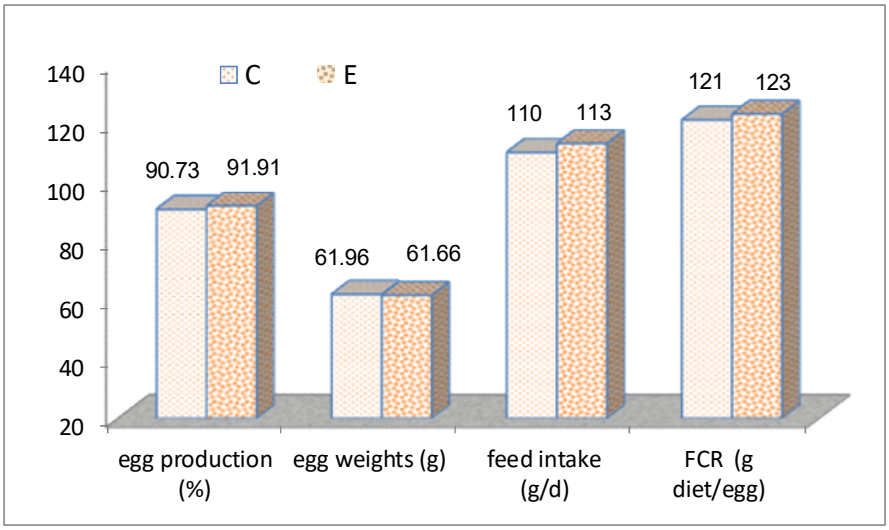

Figure 2 **Effect of nettle powder on egg production, consumption and utilization of the feed**

Nettle plants have a relatively high content in nutrients (Marchetti et al., 2018) but also in different biocompounds capable of reducing serum cholesterol in animals (Avci et al., 2006).

However, there is only limited research on the effects of feeding with *Urtica dioica* in laying hens. Therefore, the aim of the current research was to investigate whether production performance (laying rate, egg weight, feed consumption and feed efficiency) and egg quality (physical quality indices, chemical composition and yolk color intensity) obtained by in laying hens can be affected by supplementing the diet with nettle meal. The results obtained indicate that the addition of this non-conventional feed resource in the feed for laying hens can contribute to the production of eggs with a higher yolk color intensity, which is highly appreciated by consumers, being associated with the free-range system of raising hens and with a "healthier egg" (Mierlita, 2020). In addition, nettle meal did not affect egg production, egg chemical composition, or feed efficiency in the hens in the present study.

The studies previously carried out by Moula et al. (2019), demonstrated that supplementing the diet of laying quails with 3% nettle flour did not significantly change the production performance of the birds (egg-laying intensity, egg weight, consumption and degree of feed utilization); these results being confirmed by the study carried out by us in laying hens. However, the introduction of nettle flour in the feed of laying quails in a proportion of 6% in the feed structure led to a significant decrease in egg production (laying intensity), without affecting the average weight of the eggs, the consumption and the degree of utilization of the feed (Moula et al., 2019).

Recently Zhang et al. (2020) came to the conclusion that the inclusion of Urtica cannabina (belongs to the botanical family *Urticacea* - nettles) in the feed of laying hens leads to an increase in the thickness of the egg shell, the concentration of omega-3 fatty acids in the yolk PUFA and the color intensity of the yolk and to the decrease in the concentration of cholesterol in yolk and blood serum without any negative impact on egg health or performance. The color of the yolk is a very important indicator of egg quality and appreciated by consumers. In our study, yolk color was significantly improved by supplementing the diet of laying hens with nettle meal, being in agreement with the results reported by other authors who supplemented the diet of birds with nettle meal (Loetscher et al., 2013; Moula et al., 2019; Zhang et al., 2020).

Nettle caused an increase in the color intensity of yolk greater than alfalfa (Zhang et al., 2020) and similar to that determined by synthetic pigments (Carophyll®Yellow and Carophyll®Red) used in chicken feed (Loetscher et al., 2013). However, the use of synthetic pigments is not allowed in organic farming (Codex Alimentarius, 2013), and is discouraged in intensive poultry rearing

systems. In addition, consumer preferences tend towards natural additives, in favor of synthetic ones (Loetscher et al., 2013).

Nettle is rich in yellow xanthophylls (lutein, β-carotene and zeaxanthin) (Kregiel et al., 2018). Furthermore, studies by Wen et al., (2019) demonstrated that polar xanthophylls such as lutein and zeaxanthin are absorbed in the digestive tract and deposited in subcutaneous fat and yolk. Thus can be explained the more intense color of the yolk in the case of hens additionally fed with nettle flour in our study. In addition, β-carotene is almost completely transformed into vitamin A in birds (Hencken et al., 1992), which implies an improvement in egg quality by increasing the concentration of retinol and improving antioxidant activity. It was found that only polar xanthophylls, i.e. carotenoids that include at least one oxygen-containing group, are effective in the pigmentation of yolk and skin in broilers, while non-polar carotenes do not contribute to coloration (Hencken, 1992), so that β- carotene has no influence on yolk pigmentation.

CONCLUSIONS

The obtained results demonstrated that the introduction of nettle flour (*Urtica dioica*) in a proportion of 2% (% of the feed weight) in the feed of laying hens does not affect egg production (laying intensity and egg weight), consumption and the degree of utilization of the feed and nor the quality of the eggs obtained, with the exception of the color of the yolk, which improved significantly. Thus, the use of nettle flour in the feed of laying hens would improve the quality of eggs and could satisfy consumers' preferences for a more intense color of the yolk.

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