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Original scientific paper

AN INVESTIGATION ON POSSIBILITY OF *CYNARA SCOLYMUS* USE AS DIET INGREDIENT OF ISA BROWN LAYERS

Svetlana Grigorova[1], Desislava Abadjieva[2], Mariya Todorova[1]

[1]*Agricultural Academy, Institute of Animal Science, 2232 Kostinbrod, Bulgaria*
[2]*Bulgarian Academy of Sciences,*
Institute of Biology and Immunology of Reproduction,
"Acad. Kiril Bratanov", 1113 Sofia, Bulgaria
svetlanagr_61@abv.bg

A b s t r a c t: Current study aimed to assess the effect of adding dried and milled artichoke (*Cynara scolymus* L.) to the diet of laying hens on morphological and incubation traits of their eggs, as well as on the cholesterol content in the blood serum and egg yolk. A trial was conducted with 40 layers from ISA Brown breed at the age of 38 weeks, randomly allocated into two groups (control and experimental, 20 hens each). The compound feed was formulated to contain 17.64% crude protein, 4.08% crude fat, 5.08% crude fiber and metabolisable energy 2560 kcal/kg. The poultry from experimental group received as daily dose 0.22% dried and milled artichoke with the diet for a period of 32 days. It was found significantly lower cholesterol content ($P<0.05$) in the yolk of the hens from the experimental group in comparison to the control group. The hens from experimental group had higher egg fertility (96%) and egg hatchability (79%) compared to the control group (92% and 70%, respectively).

Key words: *Cynara scolymus*; laying hens; diet ingredient; morphological traits; incubation traits

ИСТРАЖУВАЊЕ НА МОЖНОСТА ЗА УПОТРЕБА НА *CYNARA SCOLYMUS* КАКО ДОДАТОК ВО ИСХРАНАТА НА НЕСИЛКИ ОД РАСАТА ISA BROWN

А п с т р а к т: Оваа студија имаше за цел да го процени ефектот од додавање сушена и мелена артичока (*Cynara scolymus* L.) во исхраната на кокошки несилки врз морфолошките и инкубационите особини на нивните јајца, како и врз содржината на холестерол во крвниот серум и жолтокот од јајцето. Беше спроведено испитување со 40 кокошки несилки од расата ISA Brown на возраст од 38 недели, по случаен избор распределени во две групи (контролна и експериментална, по 20 кокошки во секоја). Комбинираната храна беше формулирана така да содржи 17,64% сурови протеини, 4,08% сурови масти, 5,08% сурови влакна и метаболичка енергија од 2560 kcal/kg. Живината од експерименталната група во период од 32 дена како дневна доза во исхраната примала 0,22% сушена и мелена артичока. Утврдена е значително помала содржина на холестерол ($P<0,05$) во жолтокот на кокошките од експерименталната група во споредба со контролната група. Кокошките од експерименталната група имале поголема плодност на јајцата (96%) и лупливост (79%) во споредба со контролната група (92% и 70%, соодветно).

Клучни зборови: *Cynara scolymus*; кокошки несилки; диететски состојки; морфолошки особини; инкубациски карактеристики

INTRODUCTION

In order to avoid the possible risk of developing resistant pathogens, as well as to meet the public pressure of antibiotic free animal products, antibiotic use in the poultry compound feed was totally banned in European Union (Puvača et al., 2013). Various alternatives of phyto additives have been investigated in order to maximize the growth performance of poultry diets without antibiotics (Puvača et al., 2018). Interest in plant extracts and derived phytochemicals (botanicals) as alternative of nutritive antibiotics in poultry feedstuffs has increased during last decades (Wallace et al., 2010). Herbs are a rich source of biochemicals, which are known to exert various positive biological effects (Varzaro et al., 2015). Artichoke (*Cynara Scolymus* L., Asteraceae) is widely used in culinary and pharmaceutical industries. This plant contains a number of biologically active substances such as: polyphenols (cinarin, apigenin, luteolin, coffee acid, tannins); fructans (inulin, oligofructose); MUFA and PUFA (Ceccarelli et al., 2010) and is also of interest to the feed industry (Grigorova et al., 2017). Over the past decades, a number of studies have been conducted to prove the influence of artichoke addition on growth, egg production and reproduction in poultry (Abadjieva et al., 2020). Bonomi (2001) reported that replacement of alfalfa meal with 4% meal from dehydrated artichoke leaves in the laying hens diet improved their egg production and egg yolk colour. Radvan et al. (2004) found that artichoke leaves meal can be used at 8% of laying hens diet to get good economic efficiency, productive and reproductive performance as well as low cholesterol eggs.

The present work aimed to evaluate the effect of *Cynara scolymus* supplementation to the laying hens' diet on morphological and incubation traits of their eggs, as well as on the cholesterol content in the blood serum and the egg yolk.

MATERIALS AND METHODS

Experimental birds and housing

The experiment was conducted at the Poultry Experimental Center of the Institute of Animal Science – Kostinbrod, Bulgaria, with a total of 40 layers (38 weeks old) and 6 cocks from ISA Brown breed, randomly allocated in control and experimental groups (20 hens and 3 cocks each).

Experimental diets and tested product

The used in the trial compound feed for laying hens had following composition: maize, wheat, sunflower meal, soybean meal, sunflower oil, limestone, monocalcium phosphate, salt, mineral premix, and vitamin premix. The poultry from experimental group received as daily dose 0.22% dried and milled artichoke with the diet for a period of 32 days. The compound feed was formulated to contain 17.64% crude protein, 4.08% crude fat, 5.08% crude fiber and metabolisable energy 2560 kcal/kg.

The tested in this study product (Origin-Poland) is a dry mass of the above-ground part of the plant artichoke. The following analysis of this additive was made: crude protein, crude fats, crude fibers (by Weende analysis); the content of both Ca and P (AOAC, 2007); total flavonoids' content (Kivrak et al., 2009); total polyphenols' content by the Folin-Ciocalteu method (Blainski et al., 2013).

Analysis of eggs

Thirty eggs from each group, laid within two consecutive days were taken at the beginning and at the end of the trial for evaluation of individual morphological traits as followed: the weight of the egg, yolk, albumen and egg-shell weight (measured with balance within 0.001g); shape index (measured by index meter); yolk index (determined by the formula: YI (%) = (h/d)·100); albumen index (determined by the formula: I_{al} (%) = (($\underline{h}$/[D+d])/2)·100). Haugh units (measured with index meter), egg-shell thickness (mm) without the shell membrane (mesured at three locations by a micrometer Ames 25EE with precision of 0.0001 mm). Visually were measured the egg yolk color (according to the Roche Color Fan) and albumen color as well as the presence of any blood stains and other not typical inclusion in them. At the end of the treatment ten eggs per group were collected for determination of total lipid content of egg yolk (Bligh and Dyer, 1959) and total yolk cholesterol (Sperry and Webb, 1950), also the total phenolic content (n = 6 eggs per group) was established using the Folin Ciocalteu reagent.

Blood analysis

At the end of the trial blood samples were taken from the *Vena cutanea ulnaris*. Serum was separated by centrifugation at 3000 rpm for 10

minutes. Serum total cholesterol content was determined by commercial kit (Biosystems S.A., Costa Brava, Spain) using biochemical analyzer Biosystems BTS-350.

Eggs incubation

At the end of the trial 100 eggs per group were collected for control of egg incubation traits – fertility, embryonic death and hatchability.

Statistical evaluation

The results obtained in the present research were statistically processed by subjected to one-way analysis of variance (ANOVA) followed by *t*-test to determine the level of significance among mean values. The results are presented as mean ± SEM. The significant differences among mean values were determined by Duncan's multiple range test at significance level $P < 0.05$.

RESULTS AND DISCUSSION

When analyzing the tested in this work dried and milled artichoke, the following results were obtained (in 100 g product): 90 g of dry matter; 10.49 g of crude protein; 2.07 g of crude fats; 15.12 g of crude fibers; 0.83 g of Ca; 0.30 g of P; 371 mg QE (quercetin equivalents)/100 g of total flavonoids; 1347.3 mg GAE (equivalents of gallic acid) /100 g of total polyphenols. Results from the investigation of egg parts' weights are given in Table 1.

Table 1

Egg parts' traits of hens from control and experimental groups (X ± SE)

Group / Traits, g	At the beginning		At the end	
	Control *n* = 30	Experimental *n* = 30	Control *n* = 30	Experimental *n* = 30
Egg weight	64.97±0.95	63.60±1.16	65.80±0.81	66.45±1.08
Yolk weight	15.50±0.24	15.14±0.24	15.47±0.26	15.62±0.22
Albumen weight	41.88±0.90	41.32±0.98	42.53±0.60	43.44±1.00
Egg-shell weight	7.72±0.11	7.48±0.14	7.80±0.11	7.49±0.11

There were no statistically significant differences ($P<0.05$) about egg weight as well as the weights of albumen, yolk and egg-shell. At the end of the experiment, the egg weight increased slightly in both groups (mainly due to the weight of the egg white), by 2.85 g in the experimental group and by 0.83 g in the control group. The addition of 0.22% dried artichoke to the diet of laying hens did not chance significantly the form index, yolk index, Haugh units and eggshell thickness (Table 2). Radvan et al. (2004) established similar results in Mandarah hens. They studied the effect of artichoke extract (in daily doses of 50,75 and 100g/ton diet).

Table 2

Eggs' morphological traits of the hens from control and experimental group (X ± SE)

Group / Traits	At the beginning		At the end	
	Control *n* =30	Experimental *n* = 30	Control *n* = 30	Experimental *n* = 30
Shape index, %	79.95±0.40	80.17±0.39	80.62±0.44	80.45±0.44
Yolk index, %	43.84±0.75	44.50±0.81	45.81±0.91	45.83±0.70
Albumen index, %	9.12±0.40	9.15±0.41	9.96±0.42	9.27±0.44
Haugh unit, %	80.28±1.78	79.04±1.49	82.97±1.36	80.73±1.43
Egg-shell thickness, mm	0.39±0.005	0.38±0.007	0.39±0.005	0.38±0.006

The visual evaluation of yolk and albumen color did not establish any deviations from the normal color of the hens' eggs in both groups. The eggs from control and experimental groups did not have any blood stains and other not typical inclusions. The yolk color intensity into the groups varied in close range during the experiment – from 5.68 to 5.95 points on the Roche Color Fan (Table 3). In contrast to our study, Bonomi (2001) observed an increase of egg yolk color by the addition of 4% dried artichoke leaves to the layers' diet.

The dates about yolk total lipids, cholesterol content in the blood serum and yolk as well as total polyphenols in yolk are pointed in Table 4.

Table 3

Yolk colour intensity – Roche (X±SE)

Group / Periods	Control	Experimental
At the beginning of the trial *n* = 30/group	5.68±0.22	5.79±0.24
At the end of the trial *n* = 30/group	5.82±0.37	5.95±0.36

Table 4

Egg yolk total lipids, total cholesterol content in blood serum, and yolk and total polyphenols in yolk of control and experimental groups (X±SE)

Parameters / Group	Control	*n*	Experimental	*n*
Egg yolk total lipids, g/100 g yolk	35.26±0.38	10	35.08±0.65	10
Total cholesterol in blood serum, mmol/l	3.55±0.23	10	3.12±0.21	10
Total cholesterol in egg yolk, g/100 g yolk	1482.74±39.53	10	1372.36±18.45*	10
Total polyphenols' content in egg yolk, mg/GAE/100 g yolk	9.70±1.03	6	10.01±0.87	6

*Significant P<0.05

Concerning the content of the yolk total lipids the difference between the groups was insignificant (P>0.05). This result obtained was in agreement with some treatments (with artichoke leaves meal) published by Radvan et al. (2004). There is a tendency to reduce the total cholesterol in the blood of the hens from the experimental group compared to the control (P>0.05). The content of total cholesterol in the egg yolk of the experimental birds was significantly lower (P<0.05) than that of those in the control. Artichoke polyphenol compounds especially flavonoid cynaryn (one of the main active substances of this plant) affect cholesterolemia in two different biochemical mechanisms: on the one hand, they modulate cholesterol absorption and, on the other hand, delay the synthesis of endogenous cholesterol by inhibiting HMG-CoA reductase (Nazni et al., 2006). In addition, these substances may also increase the production of bile acids and exhibit antioxidant activity in the liver and blood serum. The addition of 0.22% artichoke did not change significantly the value of the total polyphenols in egg yolk.

The egg fertility and hatchability were significant higher in the experimental group (96% and 82%, respectively) compared to the control group (92% and 71%, respectively) (Table 5).

Table 5

Egg incubations traits of the hens from control and experimental groups

Parameters / Group	Control	Experimental
Total number of eggs set	100	100
% fertile eggs	92	96
Embryonic death up to the 7th day	10	7
Embryonic death up to the 18th day	11	10
Hatchability of total eggs set, %	71	79
Hatchability of fertile eggs, %	71.17	82.29*

*Significant P<0.05

Improvement of fertility (90%) and hatchability are also observed by adding 8% artichoke leaf meal to the hen's diet (Radvan et al., 2004). Enhancement the percentage of fertility, hatchability and semen quality in artichoke treatments may be due to contents of cynarin and others flavonoids, which have been classified as antioxidants (Wang et al., 2003). The importance of antioxidants in this regard was explained by Kelso et al. (1996) who reported that poultry spermatozoa are characterized by the presence of high PUFA concentration within the phospholipids. The presence of such PUFA requires an efficient antioxidant system to protect sperm membrane against peroxidative damage.

CONCLUSIONS

In the conditions of our study, artichoke addition in the daily dose of 0.22% to the laying hens' diet had no negatively effect on egg morphological traits. It was found significantly lower cholesterol content ($P<0.05$) in the yolk of the hens receiving the tested product in comparison to the control group. The treated hens had higher egg fertility (96%) and egg hatchability (79%) compared to the control group (92% and 70%, respectively). In conclusion, artichoke may be an alternative to nutriational antibiotics and hormones in compound feed of poultry. It is harmless to humans, farm animals and poultry and meet modern requirements for quality and safe food for human.

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