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Effect of feeding artichoke (*Cynara scolymus* L.) on reproductive performance of laying hens

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Abstract
The study was conducted to evaluate the effect of a milled artichoke (*Cynara scolumus* L.) on some productive and reproductive performance of laying hens. In this experiment a total of 40 ISA Brown hens at the initial age of 38 weeks were randomly distributed into two groups for experimental period of 50 days. The only difference between both groups was that the herb was included in the diet of the experimental group. The artichoke level had no clear effect on productive performance and may not be enough to cause remarkable changes. Results showed that inclusion of 3 g/kg artichoke in diet increased significantly fertility and hatchability of eggs ($P< 0.05$) of experimental hens. The average ovaries weight and length were significantly higher in experimental group in comparison with control group ($P< 0.05$). Ovaries in experimental group established significant more small yellow follicles than that of the control group ($P< 0.05$). It can be concluded that 3 g/kg artichoke can be used in the diet of layer hens to get good reproductive performance.

Keywords: Artichoke, laying hens, performance, reproduction

Introduction
The physical characteristics of the egg play an important role in the process of embryo development and successful hatching. The most influential parameters of eggs are weight, shell thickness and porosity, shape index, content consistency [5]. Determining the quality of the egg, as a vital food product, has a great interest among researchers in recent years [12, 18, 9]. Probably for this reason, detailed descriptions characterizing reproductive organs and oocyte functioning in laying hens are rarely found in veterinary medical literature, especially after intake of natural substances with diet. Scientific studiy demonstrated that the organic minerals of natural sources can improve quality of eggs and chick yield [17]. Furthermore, the effect of minerals can be optimized due to synergy with other food additives, including phenolic compounds found in plant extracts, i.e. chelation between these compounds increases the absorption of minerals [20]. The main priorities of agriculture intensified in last years to found for alternative nutritional supplements, which aimed at ensuring optimal health and good quality of products. Therefore, the purpose of the present study was conducted to evaluate the effect of a milled artichoke (*Cynara scolumus* L.) on some productive and reproductive performance of laying hens.
Reproduction in hens deserves their attention, as it differs from other animal species.

2. Material and Methods
2.1 Birds and management
A total number of 40 ISA Brown hens 38 weeks of age were randomly divided in two groups in the Experimental poultry breeding center of Institute of animal science-Kostinbrod, Bulgaria. The body weight was similar between groups in the beginning (1790±37.30). The experiment period lasted 50 days (10 preparatory and 40 experimental periods). The poultry received 130 g/day compound feed for layers, which contained: metabolizable energy 2710.23 kcal/kg, crude protein – 164.43%, crude fat – 32.98%, crude fibers – 46.18%, lysine 7.9%, methionine 4.4%, calcium 37.32%, phosphorus 4.89%. The only difference between both groups was that the diet for the experimental group included 3g/kg of dried and milled artichoke (*Cynara scolumus* L.). The tested product, originated in Poland, is a dry mass of the above-ground part of the plant *Cynara scolumus* L., with total antioxidant activity 2928.7 mmol Trolox equivalent (TE)/ /100g by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assays [15]. The water was available all the time.

Live body weight was measured on electronic balance at the beginning and end of the experiment.

2.2 Egg production performance and hatching traits of eggs

Egg production was checked daily. Average egg weight was determed by weighing eggs laid by each group for the day.

Incubation traits of eggs were investigated at the end of experiment on 100 eggs collected from the whole period. The fertility was evaluated as the relative share of fertilized to eggs set in the incubators; the hatchability of eggs set (HS) – the relative share of hatched chickens from eggs set; the hatchability of fertile eggs (HF) – the relative share of hatched chickens from fertilized eggs, embryonic death rate - as relative proportions of dead embryos from fertilized eggs.

2.3 Gross parameters

At the end of the trial, birds of each group were humanely killed in accordance with Directive 2010/ 63/EC of the European Parliament. Ovaries and oviducts were grossly examined and following parameters were recorded: length, width (cm) and weight (g) of left ovary and length and weight of oviduct.

2.4 Histological studies

Samples were collected from ovary and different regions of oviduct and fixed in 10% neutral buffered formalin. Formalin-fixed tissues were routinely processed and embedded in paraffin blocks (IBIR-BAS). Five micrometers thick sections were cut by using rotary microtome (Leica 2125RT) and stained with Hematoxylin and Eosin for histomorphological observations. Sections were examined by light microscopy (Olympus BX51, Tokyo, Japan). Micrometry was done after calibration of ocular with stage micrometer scale. The following parameters were recorded in ovary: number of follicles, average diameter of follicles and thickness of follicular wall of antral follicles.

2.5 Statistical analysis

Analysis was carried out with SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). All the results were expressed as means and standard error. A difference of $P< 0.05$ was considered statistically significant.

3. Results and discussion

The analysis of results showed that the body weight of hens did not differ significantly (P>0.05) between both groups. At the end of the experiment, the body weight was 1936±28.34 and 1927±33.91, respectively for control and experimental hens. The livability of experimental birds was not affected by the dietary treatment throughout the experimental period. Similarly, dietary supplementation with either 50 mg/kg of green tea leaves [1] and 50 or 100 mg/kg of essential oils [7] had no effect on body weight of the birds. Radwan *et al*. [16] reported that oregano and rosemary at an inclusion level of 5 g/kg diet and thyme and turmeric in general had no effect on body weight gain in laying hens.

The dietary effects of artichoke on the egg production performance of hens are shown in Table 1. No significant differences were observed in the hen-day egg production and egg weight between the both groups.

The hatching characteristics of hens were affected by dietary treatment (Table 1). Specifically, the supplemented diet significantly increased fertility and Hatchability compared with the control diet ($P< 0.01$). The embryonic death rate was not influenced in this study.

Table 1: Egg production performance and hatching traits of hens

Parameters	control group (n = 20)	experimental group (n=20)	P value
Egg production (%), hen-day 71.5±0.14 73.3±0.94			0.111
Average egg weight (g)	64.10±0.81	62.39±0.77	0.134
Fertility (%)	94.70±0.45	96.40±0.71 *	0.045
Hatchability /fertile eggs/ %	85.61±0.48	95.33±0.75*	0.0001
Hatchability /set eggs/ % 79.10 ±0.74 91.05±0.82*			0.0001
Embryonic mortality (%)	0.20±0.06	0.11±0.05	0.251

* - statistically significant differences ($P< 0.05$)

The artichoke levels may not be sufficient to cause remarkable effects on egg production. Similar result was reported by Bozkurt *et al*. [3] who indicated that a diet supplemented with essential oil mixture had no beneficial effects on egg production and egg weight in laying hens. However, contrasting results have been reported by Ma *et al*. [10] who found that a diet supplemented with herbal medicine (Ligustrum lucidum and Schisandra chinensis) significantly improved egg production and FCR in laying hens. There are only few *in vivo* studies that focus on the use of herbs in laying hen diets. Conclusions of studies with different birds tend to vary. Cetingul *et al.* [4] reported that the inclusion level of 20 g/kg oregano increased fertility in laying quail, but above that level of the supplement (50 g/kg) fertility was reduced and the hatchability was not affected. In another study, different levels of black cumin and parsley were added to the diets of laying quail and did not observe any beneficial effect on hatchability percentage, however, high level of each or combination of these two plants decreased the hatchability [21]. The reason for these contradictory results is unclear, it may be related to variety of content of herbal product, stability of substances or the period of experiments.

Gross observation revealed that left ovary was elongated in the form of bunch of grapes with mature follicles in all groups. Large follicles extend from the surface of the ovary to short handles while the small follicles stand like a protrusion from the surface (Fig. 1).

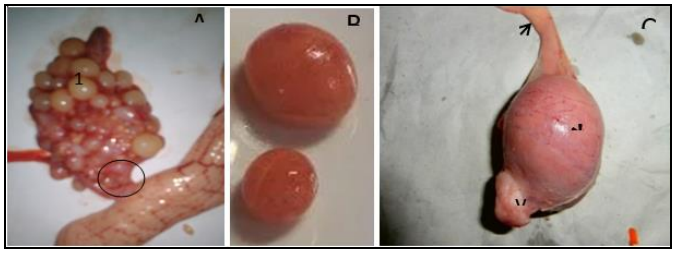

Fig 1: Photograph showing: A. Reproductive hen tract, with many follicles (1); the nerve and blood vessels surrounded by mesothelium are marked, which enter the *hilus ovarii* (in circle); B. Large antral follicles; C. The oviduct (↑) growing in the uterus, also referred to as a shell-forming gland (*) ending with the vagina (v).

In experimental group mean weight and length of the ovary were significantly higher ($P< 0.05$) than that of the control group. Table 2 shows the length of different parts of oviduct (infundibulum, magnum, isthmus, shell gland and vagina) of hens. Both the groups did not show definite pattern of changes in length.

Table 2: Range and mean of different parameters of ovary and oviduct in ISA Brown hens

Parameters	control group (n = 20)	experimental group (n=20)
Ovary		
Weight (g)	40.45±1.61	50.89±3.65 *
Length (cm)	3.92±0.14	5.05±0.08 *
Width (cm)	3.83±0.19	3.65±0.24
Ovarian weight to body weight (%)	2.15±0.40	2.73±0.51
Oviduct		
Weight (g)	50.12±5.29	51.09±2.65
Length (cm)	54.80±7.29	53.60±10.4
Oviduct weight to body weight (%)	2.67±0.28	2.74±0.78
Infundibulum length (cm)	8.15±0.52	8.28±0.15
Magnum length (cm)	23.05±1.18	22.87±1.97
Isthmus length (cm)	9.23±0.49	9.23±0.72
Shell gland length (cm)	5.38±0.16	5.50±0.09
Vagina length (cm)	4.50±0.19	4.52±0.21

* - statistically significant differences ($P< 0.05$)

It was observed that in experimental ovarian sections, the cortical zone of the ovarian parenchyma had a large number of growing follicles. The follicles were categorized into three groups as small yellow follicles, preovulatory follicles and regressing post-ovulatory follicle. The follicles less than 100 µm diameter were lined by single layer of flattened cells without yolk in the follicle.

Small yellow follicles were scattered in the cortex, whereas primary follicles - in the deeper part of the ovary (Fig.2, A). The preovulatory follicles were covered with large layer of granulosa cell. Inside was found a growing yolk-loaded oocyte (Fig. 2, D). The average thickness of follicular epithelium with 200 µm diameter was almost equal (25.36 µm in control and 24.38 µm in experimental group).

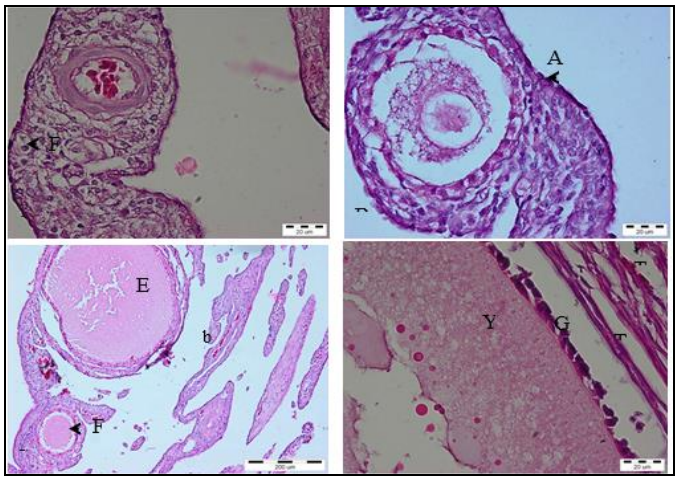

Fig 2: Photomicrographs (H&E) of ovary showing: A. Developing follicles (F) (x100); B. atretic follicle in experimental group (x100); C. Follicle (F) and ovoplasm of the oocyte (egg-E), blood vessel (bv), (x20); D. granulosa layer (Gr), theca interna (Tin), theca externa (Te) and yolk (Y) in a large follicle (x100);

The cortical zone of the experimental ovary had a large number of growing and mature follicles, it could be assumed that this was a direct effect of artichoke. The artichoke is known for its chemical composition, which includes phenolic acids as a cynarin, flavonoids, glucosides, phytosterols [6, 7]. One of the assumptions is the proliferation of the germinal epithelium. It was establihed that the cells migrating from germinal epithelium into the cortical area [19].

The number of small yellow follicles were significant higher in experimental group ($P<0.05$) than that of the control group (Table 3). The present research demonstrated that artichoke addition has a positive effect on folliculogenesis in hens.

Table 3: Percentage occurrence of follicles as per size of hens' ovary

Parameters (%)	Control group (n = 20)	Experimental group (n=20)
Small yellow follicles	7.8±2.28	10.0±2.12*
Preovulatory follicles	86.2±9.84	84.9±8.56
Regressing post-ovulatory follicle	6.0±2.07	5.1±1.41

* - statistically significant differences ($P< 0.05$)

Qualitative characteristics of eggs are affected by several factors, including age, hen genotype and feeding [2]. For example, Penkov and Nikolova [14] found that consumption of the bioadditive *Tr. terrestris* in a dose of 10 mg/kg body weight of guinea fowl for 12-weeks decreased significant the feed/egg conversion ratio.

The same authors found significant increase of ovary weight in quails treated with same additive for period of 10 weeks. The ovary core part contained a much lower number of mature follicles in comparison to control group [13]. It can't be hypothesized how the artichoke (C. scolymus) extract had an impact on folliculogenesis, because there is no available literature. But one of the recent studies over the additive artichoke gives evidence of a turbulent change in lipid metabolism, resulting in changes in pregnancy for both the mother and the fetus [8].

This puts a new point of view that requires subsequent investigations of folliculogenesis.

4. Conclusion

In conclusion, the morphometrical and histological observations from this study provides a baseline data for the female reproductive tract of laying hens. It is found that the supplementation pushing folliculogenesis. The artichoke meal (*Cynara scolumus* L.) can be used at 3 g/kg diet of layer hens to get good reproductive performance as fertility and hatchability of eggs.

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