Quality Assessment of Eggs from Laying Hens fed with a Plant Mixture Supplement

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Abstract

The aim of this study, carried out on 60 TETRA SL (32 weeks old) laying hens, was to determine the effect of dietary plant mixture (sea buckthorn meal (50%), walnut leaves (25%) and bilberry leaves (25%)) on egg quality. The birds were allocated in two experimental groups, 30 birds each. The control group (C) was fed a conventional diet, without the experimental additive. All diets had similar levels of protein (18.90% (C) and 18.55% (E)) and energy (3962 Kcal/Kg (C) and 4009 Kcal/Kg gross energy (E)). Diet of hens from experimental group was supplemented with 1.0% of plant mixture. In the experimental period, 18 eggs from each group were collected twice (week 2 and 4 of experiment). Eggs were analyzed for proximate composition contents. The experimental group additions of plant mixture increased the crude protein in egg yolk and decreased the crude fat. Our results show that addition of 1.0% plant mixture improved the quality of eggs and it can be recommended in feed for laying hens

Keywords: eggs, lipid degradation indices, plant mixture, proximate composition

1. Introduction

It was a common practice for decades to use of sub-therapeutic dose of antibiotics in animal feed to prevent animals diseases and to improve production performance in modern animal husbandry. In the meantime, concerns over the increasing emergence of antibiotic-resistant bacteria have prompted efforts to develop so-called alternatives to antibiotics [1]. Another concern was about development of antimicrobial resistance and about transference of antibiotic resistance genes from animal to human microbiota and for this reason European Union (EU) banned all antibiotic growth promoters in 2006 [2]. Unlike ruminants, poultry do not have a natural bacterial flora capable of degrading all nutrients. These animals are characterized by a resistor and a limited immunity against infection due to colonization by pathogenic microorganisms [3]. In this context, herbs, including essential oils and plant extracts are searched to be incorporated in poultry feed as growth promoters and proposed as antibiotic alternatives in farm animal nutrition [4].

In the last period, it was noticed an increasing interest of consumers regarding the quality of the products of animal origin, including eggs. Eggs constitute a valuable component of the human diet because its biological value in the protein content and other nutrients that are essential for vital functions like lipids, vitamins and minerals [5].

Sea buckthorn (*Hippophaė Rhamnoides L.*), an ancient crop with modern virtues has recently gained worldwide attention, mainly for its nutritional and medicinal value as the berries contain different kinds of nutrients and bioactive compounds including vitamins, fatty acids, free amino acids and elemental components [6]. The leaves and fruit residues of sea buckthorn are suitable for use in livestock and poultry fodders [7].

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Bilberry (*Vaccinium myrtillus L.*) is one of the richest natural sources of anthocyanins. These polyphenolic components give bilberry its blue/black color and high antioxidant content, and they are believed to be the key bioactives responsible for the many reported health benefits of bilberry and other berry fruits [8].

Walnut (*Juglans regia L.)* is a medicinal plant that has been widely used in traditional medicine for a wide array of ailments that include helminthiasis, diarrhea, sinusitis, stomachache, arthritis, asthma, eczema, scrofula, skin disorders, and various endocrine diseases such as diabetes mellitus, anorexia, thyroid dysfunctions, cancer and infectious diseases [9]. In the last decades studies have been completed on the content of phytoconstituents of the walnut.

The purpose of this study was to study the effect of a plant mixture, used in layer diets on nutritional quality of eggs.

2. Materials and methods

Experimental design

This study was carried out at the National Institute of Research-Development for Biology and Animal Nutrition - IBNA Balotesti, Romania. This study, carried out on 32 weeks old TETRA SL laying hens and the birds were allocated in two experimental groups, 30 birds each. The control group (C) was fed a conventional diet without the experimental additive. Diets of hens from experimental group were supplemented with 1.0% of plant mixture. In the laying period, 18 eggs from each group were collected twice (week 2 and 4 of experiment). The composition and chemical analyses of the experimental diets are presented in Table 1. For the shelf time determinations, egg samples (18 eggs/group) were collected randomly at the end of experiment and stored at 16^{o}C, for 28 days. Throughout the experimental period, the feed intake, egg production, egg weight and laying percentage, were recorded on a daily basis.

Egg quality was determined by measurements on egg weight and on the weight of egg components: yolk, white, shell, using a Kern scale, 0.001 accuracy; egg freshness measured by the Haugh indicator and by the specific freshness levels (Egg Analyser TM, Bountiful, USA); eggshell thickness (Egg Shell Thickness Gauge, Bountiful, USA); eggshell breaking strength (Egg Force Reader, Bountiful, USA). The experimental results were processed statistically using StatView software for Windows (SAS, version 6.0).

Table 1. Composition and chemical analyses of the basal diets

Ingredients	Control group (%)	Experimental group* (%)
Corn	30	29
Wheat	31.46	31.46
Gluten	4	4
Soy Mill	21.2	21.2
Vegetable Oil	1.46	1.46
Lisyne	0.06	0.06
Methionine	0.13	0.13
Chalk	8.78	8.78
Phosphate	1.46	1.46
Salt	0.4	0.4
Choline	0.05	0.05
Plant mixture[1]	0	1
Premix[2]	1	1
Total	100	100

[1] Experimental group - Plant mixture (sea buckthorn meal (50%), walnut leaves (25%) and bilberry leaves (25%))

[2] Content per Kg diet: vitamin A: 13,500 IU; vitamin D3: 3,000 IU; vitamin E: 27 mg; vitamin K3: 2 mg; vitamin B1: 2 mg; vitamin B2: 4.8 mg; pantothenic acid: 14.85 mg; nicotinic acid: 27 mg; vitamin B6: 3 mg; vitamin B7: 0.04 mg; vitamin B9: 1 mg; vitamin B12: 0.018 mg; vitamin C: 25 mg; manganese: 71.9 mg; iron: 60 mg; copper: 6 mg; zinc: 60 mg; cobalt: 0.5 mg; iodine: 1.14 mg; selenium: 0.18 mg

Plant materials

The studied plants used in experiment were obtained from local producers (Stefmar, RamnicuValcea, Romania; E-Prod SRL, Teleorman, Romania), dried, grounded and packed. The choice of the plant mixture (sea buckthorn meal (50%), walnut leaves (25%) and bilberry leaves (25%)) was based on the properties known and their use in the traditional medicine: sea buckthorn (*Hippophaė rhamnoides L.*) meal, bilberry (*Vaccinium myrtillus L.*) leaves, walnut (*Juglans regia L.)* leaves.

Chemical analysis

Chemical analysis were used to determine the concentration of the main nutrients from feeds according to Regulation (EC) no. 152/2009: gravimetric method for dry matter determination; Kjeldahl method for crude protein determination; the crude fat was determined by extraction in organic solvents; the crude fibre was determined by successive hydrolysis in alkali and acid environment and gravimetric method for crude ash determination. The fat degradation indices (peroxide value, fat acidity and Kreiss reaction) were determined by the volumetric method, according to STAS 12266-84. The samples were analyzed for Ca concentrations applying flame atomic absorption spectrometry (FAAS) after the microwave digestion. The phosphorus content was determined by UV-Vis spectrophotometry according to Regulation (CE) nr. 152/2009.

3. Results and discussion

Laying hens performance values showed no significant differences between groups. There was no significant effect (P>0.05) of supplemental mixture on productive parameters: average daily feed intake (g/layer/day), feed conversion ratio (g feed/g egg), laying percentage, egg production. The results are presented in Table 2.

Table 2. Main effects and standard errors of egg production parameters, as affected by dietary treatment of hens

	C	M	SEM	P value
Average daily feed intake (g/hen)	108.29	107.97	0.582	0.7865
Feed conversion ratio (g feed/g egg)	1.83	1.86	0.014	0.2824
Egg weight (g)	60.43	60.81	0.115	0.0970
Laying percentage	98.22	97.72	0.391	0.5339

In table 3 it can be seen that introduction of plant mixture in laying hens diets (1% in the experimental group) impacted zootechnical parameters.

Table 3. Proximate composition and mineral concentrations of plant mixture and combined feeds

Sample	Dry matter	Crude protein	Crude fat	Crude fibre	Crude ash	Ca	P
	%	%	%	%	%	%	%
Plant mixture[1]	90.68	12.20	6.70	22.80	4.97	0.39	0.30
NC M	90.29	18.90	2.83	4.06	13.40	3.11	0.72
NC E	90.10	18.55	2.89	3.74	12.69	3.10	0.68

[1]Plant mixture - (25% Walnut leaves, 25% Bilberry leaves, 50% Sea buckthorn meal)

Supplementing with plant mixture to the basal diet increased egg weight and decreased the laying percentage and average daily feed intake but only numerically. Shaker et al. [10], showed that replacement of 5% of the feed wheat with sea buckthorn fruit residues in laying hens diets, significantly affected the total number of laid eggs as well as the egg yolk colour. Biswas, A. et al. [11] observed that sea buckthorn berry flour represents a rich source of carotenoids for the hens feed and by adding (2.5% and 4%) in hens diet leads to an increase in production of eggs.

The proximate composition and mineral concentrations of dietary supplement and combined feeds used in experiment are presented in Table 3.

The results obtained for plant mixture chemical profile are in accordance with the data published in scientific literature, showed that sea buckthorn fruit residues has crude protein–18.3%, crude fat

–12.7%, Ca–0.31%, P–0.15% [12]; walnut leaves has crude protein 16.62%, crude fat 5.63 %,crude fibre 14.92%, ca 187 mg/kg [13]. The proximate composition of combined feeds respects the physiological requirements of animals.

In table 4 are presented data regarding lipid degradation of feeds during 28 days, meaning peroxide value (ml thiosulphate 0.01N/g fat), acidity fat (mg KOH/g fat) and KREISS reaction.

Table 4. Lipid degradation indices

		CF (C)	CF (E)
Peroxide value (ml thiosulphate 0.01N/g fat)	0 days	0.48	0.44
	14 days	0.59	0.58
	28 days	0.85	0.85
Acidity fat (mg KOH/g fat)	0 days	13.75	13.43
	14 days	16.52	16.19
	28 days	18.93	18.70
The Kreiss reaction	0 days	negative	negative
	14 days	negative	negative
	28 days	negative	negative

The values of the lipid degradation indices for the control compound feed and for the experimental compound feeds (Table 4) are below the admitted level that shows that the experimental compound feeds can be used in laying hens diet.

In tables 5 and 6 are presented data regarding physical parameters of eggs collected after 2 respective 4 experimental weeks.

Table 5. Physical parameters of the eggs (average values)

Specification		C	E	Sem	p-value
Egg weight, g	2 weeks	59.76	59.56	0.322	0.7623
	4 weeks	60.68^{b}	63.21^{a}	0.524	0.0135
Egg white weight, g	2 weeks	36.48	36.50	0.375	0.7336
	4 weeks	37.24^{b}	38.74^{a}	0.438	0.0337
Egg yolk weight, g	2 weeks	15.44	15.35	0.220	0.8319
	4 weeks	15.24	15.93	0.247	0.1640
Eggshell weight, g	2 weeks	7.84	7.71	0.081	0.4261
	4 weeks	8.20^{b}	8.54^{a}	0.726	<0.0001
Eggshell thickness, mm	2 weeks	0.37	0.36	0.013	0.0127
	4 weeks	0.38	0.37	0.002	0.1371
Eggshell breaking strength, kgF	2 weeks	3.97	3.87	0.118	0.6669
	4 weeks	3.93^{b}	4.57^{a}	0.094	0.0002
Egg white pH	2 weeks	8.51	8.49	0.061	0.8727
	4 weeks	8.19	8.20	0.003	0.2400
Egg yolk pH	2 weeks	6.17^{b}	5.93^{a}	0.046	0.0100
	4 weeks	6.18	6.18	0.003	0.1970
Egg yolk Haugh units	2 weeks	68.86	71.90	1.564	0.3384
	4 weeks	74.96	77.58	1.637	0.4314

The measurements of the quality parameters (Table 5) showed that the egg, egg white and eggshell weights, significantly increased at the end of experiment for E group compared to C. The eggshell thickness didn't differ between groups, while eggshell breaking strength was significantly ($P \leq 0.05$) higher in group C compared to the group E, at the end of the experiment. In terms of egg yolk Haugh units there have been a slightly increased values, but the differences are only numerically. Chand, N., et al. [14] found out that eggshell thickness was not significantly ($p>0.05$) affected by sea buckthorn seed supplementation. The results showed that egg production and egg yolk weight was significantly ($p<0.05$) higher at the end of the study in the experimental group (supplemented with sea buckthorn seed powder 3 g/kg of feed) compared to the control.

Table 6. Egg freshness (average values)

Egg freshness		C (%)	E (%)
2 weeks	AA	33.33	38.89
	A	55.56	55.56
	B	11.11	5.56
4 weeks	AA	72.22	83.33
	A	16.67	16.67
	B	11.11	0.00

The data regarding the egg freshness, showed increased percentage of AA category for E group in both collecting time and at the end of experiment, no B freshness was registered for E group.

In the Figures 1-4 are presented the values of proximate composition of egg yolk and egg white (dry matter, crude protein, crude fat, crude ash) and Figure 5 are presented the values of the crude ash and Ca content in eggshell.

It can be seen from Figure 1 that significant differences were noticed in the eggs collected at 4 weeks in the experimental group from the control group. The dry matter increased in egg yolk while decreased in egg white.

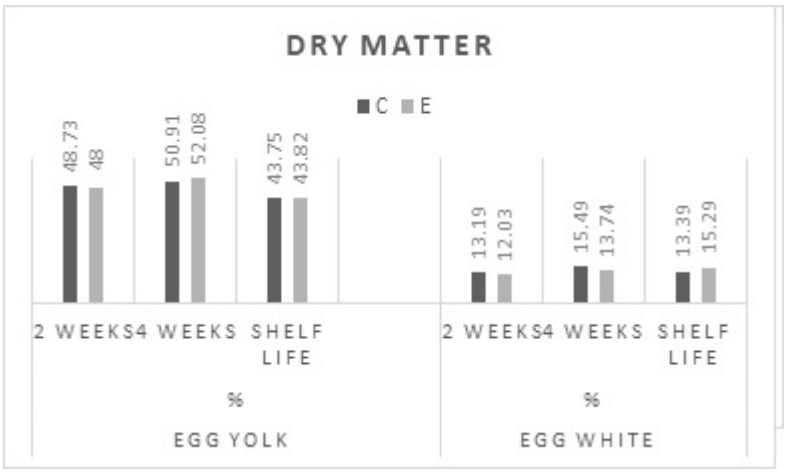

Figure 1. The dry matter content in egg yolk and egg white

In the Figure 2 are presented the values of the crude protein content in egg yolk and egg white. The crude protein has not recorded significant changes in egg yolk. In egg white the crude protein concentrations recorded a significant decrease after 4 experimental weeks.

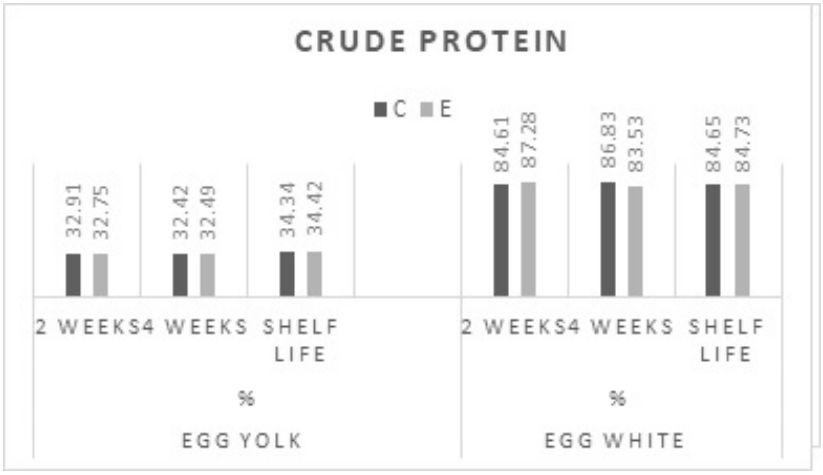

Figure 2. The protein content in egg yolk and egg white

In the Figure 3 are presented the values of the crude fat content in egg yolk and egg white. The crude fat has recorded a slight increase in egg yolk in the experimental group from control group and a slight decrease in egg white, but not significant differences were calculated.

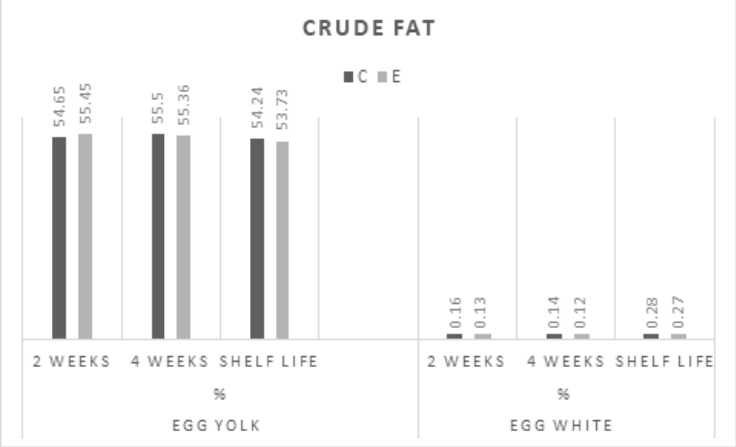

Figure 3. The crude fat content in egg yolk and egg white

The crude fat has recorded a slight increase in egg yolk in the experimental group from control group and a slight decrease in egg white, but not significant differences were calculated.

In the figure 4 are presented the values of the crude ash content in egg yolk and egg white. The crude ash has recorded significant decrease at 2 weeks harvest in egg yolk, in the experimental group from control group.

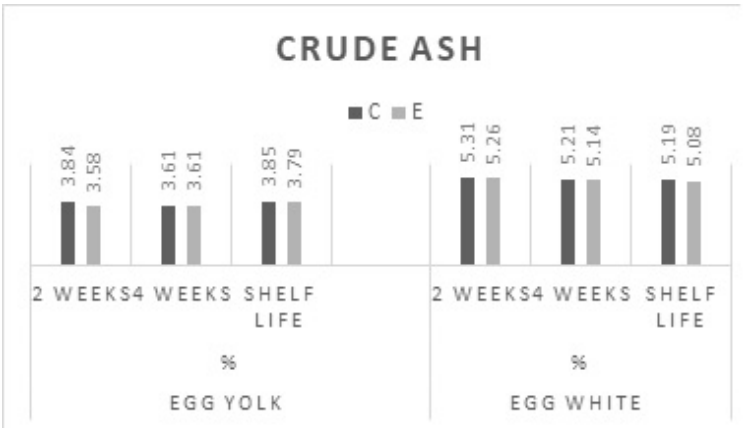

Figure 4. The crude ash content in egg yolk and egg white

As shown in figure 5 the values of the crude ash and Ca content in eggshell there have been no significant changes in the experimental group compared control group.

Rawdan, N., et. al. [15] found out that different herbs can improve productive performance, improve egg production, egg mass and feed conversion on laying hens and had a positive effect in oxidative stability of shell eggs during storage. Krejcarová, J., et. al. [16] observed that using sea buckthorn in animal nutrition as an additive to feed mixtures has a positive effect on the quality of farm animal products and on animal health. Biswas, A. [17] showed that the weight and egg rate of poultry are increased after feeding poultry with the leaves, seeds, and fruit residues of sea buckthorn and concluded that the leaves and fruit residues of sea buckthorn are suitable for use in livestock and poultry fodders. The content of active substances in these products can vary greatly depending on what part of the plant is used (grains, leaves, roots, bark, flowers, or buds), the harvest season and geographical origin [18].

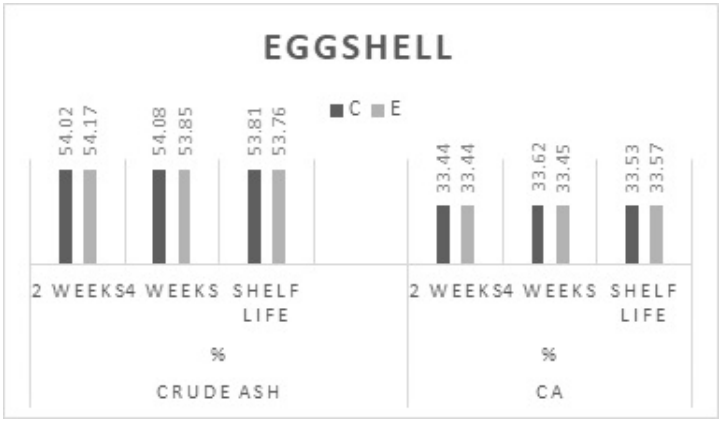

Figure 5. The crude ash and Ca content in eggshell

4. Conclusions

The experimental results showed that addition of 1.0% plant mixture improved the quality of eggs in terms of eggs weight and eggshell breaking strength and it can be recommended in feed for laying hens.

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