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EFFECT OF PARSLEY AND INULIN ON BIOPRODUCTIVE PARAMETERS AND ANTIOXIDANT NUTRIENTS OF EGGS PROVIDED BY LAYING HENS REARED UNDER HEAT STRESS CONDITIONS

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Abstract

The objective of this study was to examine the effects of dietary inclusion of parsley and inulin, as sources of natural antioxidants in poultry diets, on the enrichment of antioxidant nutrients in the egg yolk and on the susceptibility of the yolk to lipid peroxidation during storage. The experiment was conducted on 47-week-old TETRA SL LL laying hens, reared in high temperature (30°C). Experimental dietary treatments differed from control diet (C) by addition of 2% parsley or 2% inulin. The addition of parsley and inulin in laying hens' diets significantly decreased the iron content in the yolk eggs and increase the total polyphenol content, vitamin E, lutein and zeaxanthin and concentrations in the egg yolks. In regards to the oxidative stability parameters, a significant decrease in the concentrations of primary oxidation products formed in the egg yolk of experimental groups was seen, proving an efficient inhibition effect of the phytoadditives on peroxyl radical formation. A significant correlation was observed between oxidation products and total polyphenol content of the egg yolks, where lutein and zeaxanthin inhibit the formation oxidation products.

Key words*: antioxidants, heat stress, hens, polyphenols.*

INTRODUCTION

In recent years, heat stress has become a major environmental stressor that harms animals worldwide (Renaudeau et al., 2012). In the case of poultry, the thermal stress is much higher than in other animals, because modern genotypes of poultry have been suggested to produce more body heat due to their higher metabolic activity (Deeb & Cahaner, 2002).

The optimum environmental temperature for performance of adult laying hens is between 19 and 22°C, with temperatures above and below this range requiring thermoregulation (Lin et al., 2006). It is well known that exposure to temperatures above 30°C feed intake decrease (Xing et al., 2019). The decrease in feed consumption seems to be the origin of the most harmful effects caused by thermal stress in egg production. Consequently, this influences the endocrine system, resulting in acid-base imbalance and organ dysfunction, which leads to increased mortality, depressed food efficiency and reduced egg production and quality (Mashaly et al., 2004).

While poultry are exposed to high temperatures, they increase their breathing rate. Increased respiration rate reduces the partial pressure of carbon dioxide in the blood, changing the ratio of bicarbonate to carbon dioxide and eventually resulting in an increase in blood pH, a process known as respiratory alkalosis (Franco-Jimenez et al., 2007).

The increase in blood pH leads to a decrease in the bioavailability of calcium, by forming bonds with the proteins. By default, with a lower availability of calcium, the formation of the eggshell is affected (Etches et al., 2008). It has been observed that when poultry are subjected to high temperatures they have a lower density of red blood cells, this is due to increased water consumption and thus their dilution in the blood. According to some studies, the use of plants with antioxidant potential in laying hens' feed can increase the stability of eggs over the time (Untea et al., 2020).

This study was carried out of determine the effect of utilization of dry parsley (*Petroselinum crispum*) and yeast enriched with zinc in the diets on egg quality, feed consumption, feed conversion ratio, egg production, egg quality, hatchability blood parameters in the laying hens and egg stability.

MATERIALS AND METHODS

The feeding trial was conducted in an experimental hall at the Laboratory of Chemistry and Nutrition Physiology of the National Research Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to an experimental protocol. This protocol was approved by the Ethics Commission of the Institute.

A three-week experiment was conducted on 47-week-old TETRA SL laying hens, which were assigned to three dietary treatments with 20 birds each. They were sheltered in an environmentally verified space (temperature, humidity, ventilation, and light program).

At the age of 47 weeks, the hens were and assigned to 3 groups, control group (C), experimental group 1 (P), experimental group 2 (I). The hens were housed in an experimental hall with 30°C constant temperature, humidity 60% and 16 h light/8 h darkness.

Eggs were collected and weighed between 10:00 and 11:00 am each day. Egg production and egg mass, were all recorded individually on a daily basis and summarized over a 3-wk period.

After 3 weeks of the feeding trial, 6 hens form each group where randomly selected and blood samples were aseptically collected into 9-mL Vacutainer containing 14.3 U/mL of lithium heparin (Vacutest®, Arzergrande, Italy) for serum biochemical assessment on an automatic BS-130 Chemistry analyser (Bio-Medical Electronics Co., LTD, China).

Blood samples were prepared by centrifugation at 775 × g for 25 min at 4°C. The supernatant obtained was employed to analyse the following serum markers: glucose, cholesterol, triglyceride, total bilirubin, total protein, calcium, iron). The biochemical parameters were analysed using an automatic BS-130 Chemistry analyzer (Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Lutein and zeaxanthin were analyzed using a high performance liquid chromatograph (Perkin Elmer 200 series, Shelton, CT, USA) with a UV detector (445 nm). A stationary phase of 5 µm C18 reversed-phase column (250 × 4.60 mm i.d.) (Nucleodur, Macherey-Nagel, Germany) was used. Chromatographic analysis was carried out under isocratic conditions at a flow rate of 1.0 mL/min and a mobile phase of 13% water and 87% acetone was used.

Vitamin E determination was performed according to the method described in EC Regulation no. 152/2009, using a high performance liquid chromatograph and a PDA-UV detector (HPLC Finningan Surveyor Plus, Thermo-Electron Corporation, Waltham, MA) at a wavelength of 292 nm. A HyperSil BDS C18 column, with silica gel, dimensions of 250 × 4.6 mm, and a particle size of 5 µm (Thermo Electron Corporation, Waltham, MA), was used. Chromatographic analysis was carried out under isocratic conditions at a flow rate of 1.5 mL/min and a mobile phase of 4% water, using 96% methanol.

The total phenol content of all extracts was measured spectrophotometrically according to the Folin–Ciocalteu method, as described by Conrad et al., 2001, with slight modifications. Briefly, the extract samples (0.5 mL of different dilutions) were mixed with 0.5 mL Folin-Ciocalteu reagent and 7 mL water, and then homogenized. The solution was kept at room temperature for 3 min before adding 2 mL of 20% sodium carbonate solution. After an hour in the dark, the absorbance was measured at 732 nm against a blank (solution with no extract added). The calibration curve of gallic acid was used to determine the total phenol content, and the results were reported as mg gallic acid equivalents per gram of dried sample (mg GAE/g).

RESULTS AND DISCUSSIONS

The usage of parsley and inulin diets had no effect ($p > 0.05$) on laying hens' performances compared to control group (Table 1).

Table 1. The effect of supplemental parsley and inulin on the productive parameters of the laying hens

Parameter/group	Control	Parsley	Inulin	SEM	p Value
Average daily feed intake (g)	81.05	78.93	81.97	6.3003	0.2797
Laying percentage (%)	85.71	84.21	89.42	10.8282	0.6147
Egg weight (g)	59.13	59.95	59.99	1.0974	0.014
Feed conversion rate (kg feed/kg egg)	1.69	1.77	1.64	0.2703	0.2891

The laying percentage, egg weight and feed conversion rate were not influenced by the dietary treatments. Other studies on broiler chickens show that the use of parsley in diet leads to an increase in feed consumption (Mohammed, 2010).

Table 2 shows the chemical composition of the feed diets used in this experiment. The additive inclusion rate was 2% for the parsley group (P) and 2% for the inulin group (I).

Table 2. Chemical analysis of feed

Parameter	Group	Control	Parsley	Inulin
DM		90.5	90.63	90.5
OM		75.97	75.86	75.81
CP		17.74	20.13	18.34
CF	%	4.23	3.83	3.99
Cel		3.75	3.72	4.51
NES		50.25	48.18	48.97
Ash		14.52	14.77	14.7
Xanthophyll	ppm	7.135	11.017	8.505
Vitamin E	ppm	67.941	65.382	95.317

DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, like most other free radicals (Kedare & Sing, 2011).

Antioxidants are the compounds, which combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process (Sindhi et al., 2013).

These antioxidants are also produced by biological system and occur naturally in many foods and the balance between oxidants and antioxidants decides the health (Kuźma et al., 2014).

Table 4 shows the physical parameters of hens eggs after 21 days from the administration of additives in their feed. Significant differences ($p<0.05$) were recorded for the weight of the yolk, where in group P it was lower than in group C and group I. Also, significant differences ($p<0.05$) were recorded for the weight of the albumen where the groups P and I had higher values compared to group C.

Table 3. DPPH analysis of feed and feed additives

Group	mg/g GAE	mM echiv trolox
Control	1.601	8.024
Feed (P)	1.947	8.457
Feed (I)	1.744	8.202
Parsley	7.71	13.05
Inulin	0.4	1.25

In the case of the amount of xanthophylls present in the yolk (Table 7), group P had a higher concentration but the difference is not statistically supported ($p>0.05$). An oxidized version of carotenoid called xantophyll constitutes a major part of carotenoids in nature. Xanthophylls are yellow pigments that are widely available in nature. Xanthophylls are well known for their benefits in human nutrition (Ribaya-Mercado et al., 2004). Also, in the case of vitamin E, a difference can be observed in the case of the experimental groups, being a larger amount in the group supplemented with inulin, but even here the difference is not statistically significant. The addition of parsley and inulin in laying hens' diets significantly decreased the iron content in the yolk eggs. Also, the addition of inulin in hens diets lead to decrease content of zinc in egg yolk.

Results presented in this study indicate that supplements added in diets of goups P and I were effective in increasing quality in 3 weeks of feeding by increasing polyphenols and antioxidant capacity in eggs. Moreover, it was clear that the supplements used showed high antioxidant properties by manipulating the poultry feed, which further promoted a significant increase in polyphenols and antioxidant compounds in the eggs of the laying hen in the experimental group compared to the eggs in the control group. Polyphenol concentration increased in both experimental eggs, in case of P group the increase was 27% and in group I was 9.5%.

Table 4. Physical and chemical (at the end of experiment) parameters of the egg after 21days on heat stress

	Control	Parsley	Inulin	SEM	p Value
Egg weight (g)	60.60	60.14	61.02	2.4168	0.5568
Egg white (g)	37.40	38.25	38.14	2.3313	0.4988
Egg yolk (g)	15.55^{b}	14.56^{a}	15.30	1.2245	0.0375
Eggshell (g)	7.65	7.33	7.58	0.5511	0.1823
Egg white pH	9.00^{bc}	9.25^{a}	9.27^{a}	0.2710	0.0180
Eggyolk pH	6.58	6.59	6.58	0.1415	0.9679
HU unit	97.52^{c}	97.63^{c}	93.38^{ab}	5.5348	0.0286

*where $^{a, b, c}$ show significant (P≤0.05) differences from C, P and I

Table 5. Physical parameters of the egg after 14 days of storage

Parameter	Control	Parsley	Inulin	SEM	p Value
Egg mass (g)	60.10	62.14	59.62	3.4273	0.4735
Egg white (g)	34.82	38.45	35.76	2.8247	0.0848
Egg yolk (g)	17.56	16.31	16.35	1.2295	0.1433
Eggshell (g)	7.72	7.38	7.51	0.6323	0.6837
Egg white pH	8.36	8.55	8.55	0.2959	0.4822
Eggyolk pH	6.00	5.90	5.96	0.3045	0.8729
HU unit	84.57	81.12	84.01	5.5149	0.2890

Table 6. Physical parameters of the egg after 28 days of storage

Parameter	Control	Parsley	Inulin	SEM	p Value
Egg weight (g)	59.17	56.09	59.22	3.0364	0.1202
Egg white (g)	36.17	33.86	35.26	2.7382	0.3600
Egg yolk (g)	15.45	15.32	16.69	0.9479	0.0118
Eggshell (g)	7.55	6.91	7.27	0.6418	0.2329
Egg white pH	9.69	9.63	9.68	0.1457	0.7534
Eggyolk pH	6.68	6.73	6.62	0.1627	0.5312
HU unit	78.00	77.25	79.56	5.9843	0.1107

Table 7. Polyphenols in egg yolk after 21 days of treatment

Parameter	Control	Parsley	Inulin	SEM	P value
mg/g GAE	0.715	0.908	0.783	0.1339	0.0532
mM echiv trolox	2.500^{bc}	3.296^{a}	3.386^{a}	0.4924	0.0141
Xanthophylls (ppm)	4.2430	6.9900	4.2333	1.5646	0.1160
Vitamin E (ppm)	80.212	90.347^{c}	110.9677	19.2069	0.1255
Iron in yolk (ppm)	154.88^{c}	153.10^{c}	146.01^{ab}	5.4645	0.0041
Zinc in yolk (ppm)	75.02	75.36^{c}	74.55^{b}	0.6371	0.0818

$^{a, b, c}$ show significant (P≤0.05) differences from C, P and I

Table 8. Plasma blood parameter after 21 days of heat stress

Parameter/Group	Control	Parsley	Inulin	SEM	P value
Hematocrit	25.6	26.83	27.67	2.0775	0.2729
Leukocyte (WBC) K/µL	18.92	14.77	16.7	4.01	0.2432
Heterophiles, %	47.4	45	44.33	5.9068	0.7003
Lymphocytes, %	47	51.83	52.5	5.1228	0.1641
Monocytes, %	5.5	3	2.17	2.8718	0.1975
Eosinophils, %	1.5	1.4	1.25	0.6504	0.8811
Heterophiles K/µL	8.97	6.69	7.43	2.2562	0.2525
Lymphocytes K/µL	8.86^{b}	7.6	8.7	1.9168	0.5058
Monocytes K/µL	1.05	0.45	0.4	0.6013	0.217
Eosinophils K/µL	0.28	0.2	0.19	0.0943	0.4069
Uric acid (mg/dL)	4.9	4.93	4.02	1.4354	0.4825
Urea (mg/dL)	1.67^{b}	1.5	1.67	0.6077	0.8476
Urea nitrogen (mg/dL)	0.79^{bc}	0.66^{ac}	0.89^{ab}	0.2405	0.2543

[a, b, c] show significant (P≤0.05) differences from C, P and I.

The use of dietary parsley and inulin on the hematological parameters (Table 8) of laying hens showed no significant effect (p>0.05) across groups.

CONCLUSIONS

Antioxidants from plants can also be transferred to chicken eggs.
The use of parsley and inulin in the diet of laying hens increases the amount of zinc present in the egg yolk.
The use of natural additives in the feed of laying hens does not negatively affect the egg laying percentage and feed consumption.

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