EFFECT OF SOME NATURAL ADDITIVES 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 yeast, 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 1% yeast, 2% parsley and 1% yeast or 2% inulin and 1%parsley. The addition of yeast and inulin in laying hens' diets significantly increased the zinc content in the yolk eggs. The use of yeast and parsley in laying hens' diets 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: *Hens, heat stress, Antioxidants, polyphenols*

INTRODUCTION

The poultry industry is growing across the world to fulfill the increasing demands of poultry meat and eggs. Poultry meat contains a low amount of saturated fatty acids and is rich in protein, vitamins and minerals (Marangoni et al., 2015). 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 and Cahaner, 2002). For optimal performances of adult laying hens is between 19 and 22°C, if the temperature is outside this range, measures must be taken to condition the temperature in the household (Lin et al., 2006). High temperatures can lead to heat stress that causes decreased performance, altered blood chemistry, and increased mortality (Khan et al., 2011). High temperatures can lead to heat stress that causes decreased performance, altered blood chemistry, and increased mortality (Khan et al., 2011; Kilic and Simsek, 2013). Enhanced respiration rate during heat stress reduces blood CO^2 concentration. Respiratory alkalosis is a condition of the body when blood pH increases beyond the normal range which is often resulted from enhanced respiratory rates and change in bicarbonate to CO2 ratio (Franco-Jimenez et al., 2007). Antioxidants protect cells from the effects of lipid peroxidation. Lipid peroxidation is an indicator of cellular injury due to the generation of free radicals (Wu et al., 2016). Recently, dietary supplementation of

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probiotics, prebiotics and synbiotics has also been implemented in poultry to counteract the negative effects of heat stress (Lara and Rostagno, 2013) Inulin is currently the most widely used and studied prebiotic (Iraporda et al., 2019). As a feed additive in animal production, inulin had beneficial effects on improving growth performance and regulating the intestinal microflora of laying hens (Shang et al., 2010).

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. 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), inulin, 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.

MATERIAL AND METHOD

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 4 groups, control group (C), experimental group 1,(Y) experimental group 2 (YP),experimental group 3 (YI).

The recipes of the experimental groups differ from that of the control group as follows, group 1 (Y) 1% yeast, group 2 (YP) 1% yeast and 2% parsley, group 3 (YI) 1% yeast and 2% inulin. The hens were housed in an experimental hall with 30°C constant temperature, humidity 60% and 16 h light/ 8h 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 flflow 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. Brieflfly, 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). In order to evaluate the oxidation stability of the eggs, they were stored for 14, respectively 28 days at a temperature of 18°C and 50% relative humidity.

RESULTS AND DISCUSSIONS

Table 1 shows the antioxidant activity of the feeds used. Regarding the amounts of polyphenols, the highest amount expressed in mg/g GAE was recorded in the feed supplemented with yeast-parsley, and the lowest antioxidant activity was found in the feed supplemented with yeast.

Table 1 DPPH analysis of feed and feed additives

Group	mg/g GAE	mM echiv trolox
Control	1.601	8.024
Y	1.440	7.818
YP	1.733	8.974
YI	1.562	8.250

The laying percentage, egg weight and feed conversion rate were not influenced by the dietary treatments (Table 2). Other studies on broiler chickens show that the use of parsley in diet leads to an increase in feed consumption (Mohammed 2010). The diets were isoproteic and isocaloric for all groups (Table 3), the recipes of the experimental groups differ from that of the control group as follows, group 1 (Y) 1% yeast, group 2 (YP) 1% yeast and 2% parsley, group 3 (YI) 1% yeast and 2% inulin. It can also be observed that the experimental diets contain higher amounts of Xanthophyll compared to the diet of the control group. However, the highest amount of vitamin E in the diet is found in the Y group.

Table 2 The effect of supplemental on the productive parameters of the laying hens

Parameter/group	Control	Y	YP	YI	SEM	p Value
Average daily feed intake (g)	81.05	81	81.97	83.21	7.82	0.2797
Laying percentage (%)	85.71	84.52	86.34	82.66	10.57	0.6147
Egg weight (g)	59.13	60.02	59.91	59.41	1.01	0.014
Feed conversion rate (kg feed/kg egg)	1.69	1.65	1.67	1.95	0.3	0.2891

Table 3 Chemical analysis of feed

Parameter	Group	Control	Y	YP	YI
DM	%	90.5	90.51	90.45	90.61
OM		75.97	75.93	76.12	75.56
CP		17.74	19.24	19.20	20.00
CF		4.23	3.21	4.15	3.85
Cel		3.75	3.82	4.32	3.96
NES		50.25	49.66	48.45	47.74
Ash		14.52	14.58	14.34	15.05
Xanthophyll	ppm	7.135	9.182	8.398	9.718
Vitamin E	ppm	67.941	87.433	48.536	52.33

DM-Dry matter, OM- Organic matter, CP-Crude protein, CF- Crude fat, Cel- Celulose, NES- non-nitrogenous extractive substances.

In the case of the amount of xanthophylls present in the yolk (Table 4), groups Y and YP had a significant ($p<0.0001$) higher concentration than groups C and YI. An oxidized version of carotenoid called xantophyll constitues 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 significant difference ($p<0.05$) can be observed between the experimental groups, where in the case of supplementation with yeast and parsley (YP) the values were the highest. The addition of parsley (YP) and inulin (YI) to the diet of laying hens significantly ($p<0.05$) decreased the iron content of the egg yolk. Also, the addition of inulin and yeast to the hens' diets leads to a significant increase ($p<0.05$) in the zinc content of the egg yolk. 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 YP and YI 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 significant increased in experimental eggs, in case of YP group the increase was 45%, in group Y was 21% and in group YI was 1%

Table 5 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 eggshell, where in group Y it was lower than in group YI. Also significant differences ($p <0.05$) were recorded for the weight of the egg white pH where in the groups YP and YI had higher values compared to group C. Regarding to HU unit, significantly lower values ($p<0.05$) were recorded for the YP and YI groups compared to the C group.

Table 4. Polyphenols in eggyolk after 21 days of treatment

Parameter	Control	Y	YP	YI	SEM	P value
mg/g GAE	0.715	0.8	0.84	0.76	0.1339	0.6664
mM echiv trolox	2.5cd	2.5c,d	2.97a,b	2.96a,b	0.4924	0.0022
Xanthophylls (ppm)	4.243b,c	5.13a,c,d	6.176a,b,d	4.3,b,c	0.999	<0.0001
Vitamin E (ppm)	80.212c	74.578c	100.878a,b,d	77.8193c	12.67	<0.0001
Iron in yolk (ppm)	154.888 c,d	154.369c,d	145.885a,b	142.657a,b	6.46	<0.0001
Zinc in yolk (ppm)	75.02b,c,d	78.803a,d	78.730a,d	79.971a,b,c	1.97	<0.0001

*where a, b, c show significant (P≤0.05) differences from C, Y, YP and YI

Table 5. Physical and chemical (at the end of experiment) parameters of the egg after 21days on heat stress

	Control	Y	YP	YI	SEM	p Value
Egg weight (g)	60.6	60.91	98.83	60.55	2.8084	0.7557
Egg white (g)	37.4	38.1	37.03	37.51	2.3254	0.7703
Egg yolk (g)	15.55	15.35	15.29	15.25	1.0949	0.827
Eggshell (g)	7.65	7.47d	7.5	7.79b	0.580447	0.1876
Egg white pH	9cd	9.16	9.29a	9.26a	0.2623	0.0074
Eggyolk pH	6.58	6.58	6.5	6.54	0.131	0.4482
HU unit	97.52c,d	97.96	93.04a	92.97a	6.7534	0.0415

*where a, b, c show significant (P≤0.05) differences from C, Y, YP and YI

Table 6 shows the physical parameters of hens eggs after 14 days from the administration of additives in their feed. Significant differences ($p < 0.05$) were recorded for HU unint, the YP group having the highest value compared to all other groups. Regarding yolk weight, significant differences ($p < 0.05$) were recorded in the case of the control group compared to YI group.

In table 7 are presented physical parameters of the egg after 28 days of storage.

In this case, no significant differences were recorded between batches of the physical parameters followed.

Table 8 shows the blood plasma parameters after 21 days of administration experimental diets. In the case of serum magnesium, significant differences ($p < 0.05$) were recorded between the YI, C and YP groups. Regarding GPT, significant differences ($p < 0.05$) were registered between groups C, Y and groups YP and YI. In the case of Triglycerides, the YI group had the highest recorded value, being significant differences ($p < 0.05$) compared to the Y group. In the case of phosphorus present in the blood plasma, the Y, YP, YI groups had significant differences ($p < 0.05$) higher compared to group C.

Table 6 Physical parameters of the egg after 14 days of storage

	Control	Y	YP	YI	SEM	p Value
Egg weight (g)	60.098	57.893	58.933	58.263	3.1488	0.6649
Egg white (g)	34.818	34.000	35.240	35.050	2.2768	0.8149
Egg yolk (g)	17.558 d	16.295	16.280	15.483 a	1.3157	0.0406
Eggshell (g)	7.722	7.598	7.413	7.730	0.6049	0.806
Egg white pH	8.358	8.492	8.577	8.572	0.2782	0.5181
Eggyolk pH	6.000	6.140	6.120	6.238	0.3492	0.7295
HU unit	77.997c	79.503c	87.100 a,b,d	78.829c	6.0152	0.0224

*where a, b, c show significant (P≤0.05) differences from C, Y, YP and YI

Table 7 Physical parameters of the egg after 28 days of storage

	Control	Y	YP	YI	SEM	p Value
Egg weight (g)	59.17	58.75	57.36	56.25	3.3455	0.4362
Egg white (g)	36.17	35.46	33.95	33.64	2.8819	0.3874
Egg yolk (g)	15.45	16.03	15.99	15.52	0.9415	0.6207
Eggshell (g)	7.55	7.26	7.41	7.10	0.6622	0.6969
Egg white pH	9.69	9.62	9.73	9.71	0.1156	0.3761
Eggyolk pH	6.68	6.72	6.66	6.66	0.1707	0.9308
HU unit	84.57	79.16	84.68	83.70	5.3758	0.2522

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

Parameter	Unit	C	Y	YP	YI	SEM	P value
Calcium	mg/dL	15.2280	15.3980	15.2450	16.7800	2.2536	0.6185
Magnesium	mg/dL	1.62d	1.6820	1.583d	1.903a,c	2.2536	0.0832
Iron	mg/dL	171.0680	196.5100	179.9400	156.1983	2.2536	0.3813
Total protein	mg/dL	3.354 c	2.8380	2.6116 a	2.9150	2.2536	0.195
Bilirubin	mg/dL	0.1280	0.1540	0.1283	0.1450	2.2536	0.2459
GPT	U/L	9.97c,d	9.278c,d	5.8583a,b	6.4966a,b	2.2536	0.0002
GOT	U/L	103.7880	117.8560	113.2700	101.8750	2.2536	0.4243
Alkaline phospathase	U/LL	660.814b	1319.918a,c,d	709.729b	872.33b	2.2536	0.0145
GGT	U/L	97.316c	86.6900	81.9766,a	93.9400	2.2536	0.1209
Glucose	mg/dL	206.5460	214.804c	186.9816b	173.5350	2.2536	0.0934
Cholesterol	mg/dL	94.4220	97.0000	89.0200	121.3567	2.2536	0.3786
Triglyceride	mg/dL	955.3920	804.564d	909.8850	1285.405b	2.2536	0.1906
Albumin	mg/dL	1.2000	1.4000	1.5000	1.5000	2.2536	0.7673
Creatinin	mg/dL	0.116d	0.126d	0.115 d	0.1433a,b,c	2.2536	0.0032
Urea	mg/dL	4.7060	4.6700	4.5767	4.4333	2.2536	0.9333
Phosphorus	mg/dL	2.788d	3.02c	2.95166b	3.63166a	2.2536	0.0535

*where a, b, c show significant (P≤0.05) differences from C, Y, YP and YI

CONCLUSIONS

The use of yeast, parsley and inulin in hens feed improves the quality of eggs by decreasing the amount of iron in the yolk and increasing the amount of zinc in eggs.

Polyphenols present in the feed of laying hens can be transferred to the egg.

The use of these additives in feeding laying hens does not affect production parameters

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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