Annual Research & Review in Biology

Volume 37, Issue 9, Page 108-117, 2022; Article no.ARRB.93394
ISSN: 2347-565X, NLM ID: 101632869

Quality Evaluation of Eggs from ISA Brown as Influenced by Natural Antioxidants and Storage Time

A. O. Akinwumi [a*], O. A. Oshodi [b], R. A. Atandah [a], O. O. Okunlola [a], O. Adeosun [a], B. D. Odeleye [a], K. O. Abdulhameed [a] and B. S. Akinwumi [a]

[a] Department of Animal Nutrition and Biotechnology, Animal Products and Processing Technology Unit, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[b] Department of Animal Science, University of Queensland, Australia.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2022/v37i930555

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/93394

Original Research Article

Received: 20/07/2022
Accepted: 14/09/2022
Published: 15/09/2022

ABSTRACT

This study aimed to assess the effect of natural antioxidants on storage time, physical and chemical properties of egg. A total of 200 laying ISA brown birds were distributed into eight dietary groups, each dietary group with 25 birds (5 birds per replicate). They were fed with roselle, black pepper, green tea, combine (roselle + black pepper + green tea) at 0.5 g/kg and 1.0 g/kg in basal diet respectively and a control feed. At the end of eight (8) weeks of feeding trial, twelve eggs were collected from each dietary group (six eggs per dietary group were analyzed for the internal and external properties while the remaining six were stored). Data collected include; Egg shape index, egg weight, shell thickness, membrane weight, yolk index, haugh unit, meat and blood spot, yolk colour, lipid profile, lipid peroxidation and proximate analysis. Data generated were subjected to Analysis of variance using the General Linear Model for factorial within a completely randomized design. The natural antioxidants significantly (P<0.05) improves the proximate composition of the poultry eggs. Both Green tea and Black pepper have significant effect (P<0.05) on the yolk

**Corresponding author: E-mail: aoakinwumi@lautech.edu.ng;*

percentage. Black pepper increases (P<0.05) the Haugh unit while it shows to be lower (P>0.05) in the combination of the antioxidants. The inclusion levels of the natural antioxidants on the internal and external quality of egg reveals that there were no significant (P>0.05) differences in the two inclusion level of 0.5 g/kg and 1.0 g/kg of feed but they have numerically higher values in the external parameters and internal parameters. Eggs stored for 4 weeks had the lowest value (P>0.05) for the proximate and lipid profiles, though there are no significant (P>0.05) differences in their values. The fresh eggs show high moisture content (P<0.05) but the value for nitrogen free extract is low (P>0.05) in the fresh eggs. The natural antioxidants improved significantly (P<0.05) the proximate composition of the poultry eggs, with the green tea having the highest value (P<0.05) for Moisture contents and CP, black pepper the highest (P<0.05) for CP Based on the result obtained from this study, the natural antioxidants (black pepper, green tea and roselle) in layers' diet shows significant effects on the physical qualities of egg and the yolk color was also preferred with the inclusion of the natural antioxidants compared to the control. The chemical properties also deteriorate with the storage time. Natural antioxidants are hereby recommended for better and improved chemical qualities of eggs.

Keywords: Egg quality; physical properties; chemical properties; antioxidants.

1. INTRODUCTION

"Poultry is by far the largest group of livestock species [1] contributing about 30% of all animal protein consumed in the world" [2]. Poultry refers to all birds of economic value to man as source of meat, egg and fiber. This industry in Nigeria occupies a prominent position as a major source of animal protein supply to the citizens. The persistence short supply of protein for populace is the main problem which was compounded by the accelerated increase in human population especially in Nigeria and thus created pressure on every form of food supply [3]. According to Orji and Chukwuma [4] "the poultry eggs goes a long way in providing animal protein for the populace because it yields quickest returns and provides for egg in a very short time". "Eggs are major sources of animal protein in human diets. Eggs constituted an important part of human diets for centuries because of its high quality protein" [5].

"Antioxidants can be defined as any substance that, when present in low concentrations compared to that of an oxidisable substrate, delays or inhibits the oxidation of that substrate" [6]. "The important commercially available natural antioxidants are tocopherols (Vitamin E) ascorbic acid (Vitamin C) and Rosemary extracts" [7]. "The most abundant constituent of green tea extracts (GTE) is catechins which has antibacterial activities" [8], as well as anti-tumorigenic, anti-inflammatory, anti-proliferative, antiviral, anti-parasitic and anti-oxidative properties [9,10,11]. "Black pepper (*Piper nigrum*) is known as spices due to its pungent quality" [12]. "It is a flowering vine in the family *Piperaceae*, genus *Piper* and species *Piper nigrum*. Efficiency compounds of pepper consist: cupsaeesin, cupsisin and cupsantine that some of them allay rheumatic aches. Roselle (*Hibiscus sabdarifa*) is leave use to make a drink locally known as Zobo. The chemical constituents of the roselle include the flavonoids, gossypetine, and sabdaretine" [13]. In addition to their antioxidant capacity they have the possibility to be used as feed colorants [14].

However, this present study is to determine the effects of different natural antioxidants on performance and egg quality of ISA brown laying pullets.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out at the Poultry unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomosho, Oyo – State, Nigeria. Ogbomoso is situated in derived savannah zone of Nigeria and lies on longitude $4^0\ 15^1$ East of Greenwich meridian and $8^0\ 15^1$ North of equator. The altitude is between 300 and 610 mm above sea level, with the mean temperature and annual mean rainfall are 26.1^0 C and 1217 mm respectively [15].

2.2 Procurement of Test Ingredients

Dried roselle (*Hibiscus sabdariffa*), green tea (*Camellia sinensis*) and black pepper (*Piper nigrum* L.) were purchased from Oja jagun a local market in Ogbomoso, Oyo state. They were grounded into powdered form and stored for usage.

2.3 Experimental Animal and Management

Two hundred (200) laying birds were purchased from Amo farm Sieberer Hatchery Limited, Awe, Oyo state and used for the experiment. They were stabilized for two weeks before the commencement of the feeding trial. The birds were randomly allotted to eight (8) dietary treatments of 25 birds each in a Completely Randomized Design. On arrival, birds were fed diets and water mixed with vitamins and glucose to reduce transportation stress. Routine medication and vaccination programs were strictly followed as required for the birds.

They were fed with roselle, black pepper, green tea, combine (roselle + black pepper + green tea) at 0.5 g/kg and 1.0 g/kg in basal diet respectively and a control group fed the diet as indicated in Table 1. The feeding period lasted for eight weeks.

2.4 Experimental Layout

Table 1. Layout of the experiment

	Control	Black pepper		Green tea		Roselle		Combined
	T_1 (0 g/kg)	T_2 (0.5 g/kg)	T_3 (1.0 g/kg)	T_4 (0.5 g/kg)	T_5 (1.0 g/kg)	T_6 (0.5 g/kg)	T_7 (1.0 g/kg)	T_8 (1.0 g/kg)
Total of Birds	25	25	25	25	25	25	25	25

2.5 Data Collection

At the end of eight weeks of feeding trial, 30 eggs were collected from each dietary treatment with a total of 240 eggs in all. Six eggs per dietary group were analyzed for the egg quality as fresh while the remaining were stored and analyzed at 1,2,3 and 4 week for internal, external and chemical qualities. Data collected were as follows:

External quality parameter:

Egg shape index- The egg shape index is defined as the ratio of width to length of the egg [16].

$$\text{Egg Shape Index} = \frac{Egg\ Width}{Egg\ Lenght}$$

The egg width and the egg length are both measured using the vernier caliper.

Eggshell thickness- this can be measured by various methods, which may include destruction of the egg [17]. The thickness was measured with a micrometer screw gauge.

Egg weight- The eggs are placed on the sensitive scale to measure the weight of the eggs.

Internal quality parameter:

Yolk index- this is the ratio of yolk height to yolk length

$$\text{Yolk index} = \frac{yolk\ height}{yolk\ length}$$

Haugh unit- the use of haugh unit scores has been generally accepted as a measure of albumen quality in egg quality studies in recent years. The formula given by Haugh [18] for haugh unit is $HU=100Log\ (H + 7.57 – 1.7W^{0.37})$

Where HU is haugh unit, h is observed albumen height, W= weight of egg

Yolk color- this was evaluated visually by means of the usual La Roche Scale also known as DSM Yolk Color fan [19].

Yolk weight- the yolk was separated and weighed using a sensitive scale

Blood and Meat spot- the total number of stain in the inner part of the eggs were noted.

2.6 Chemical Analysis

Proximate analysis

The proximate analysis of eggs was carried out according to the method of [20].

Lipid profile

Lipid profile of egg samples was determined as published by Richmond [21] to estimate the total cholesterol, low-density lipoprotein, high-density lipoprotein and triacylglyceride and fatty acid profile.

Lipid peroxidation

This is the oxidative degradation of lipids. Samples of yolk were analyzed for peroxide value [22].

Table 2. Effect of natural antioxidants on external, internal qualities and period of storage of layer's egg

Treatment	External qualities					Internal qualities							
	Egg Weight (g)	Egg Shape Index	Shell Weight (g)	Shell weight (%)	Shell Thickness (mm)	Albumen Height (mm)	Haugh Unit (%)	Yolk Color	Yolk Index	Yolk Weight (g)	Yolk %	Membrane Weight (g)	Membrane weight (%)
Natural antioxidants													
Control	49.92^{b}	0.83^{c}	4.24^{c}	8.60^{c}	0.52^{b}	7.83^{b}	95.99^{b}	3.23^{b}	0.66^{a}	13.09^{c}	26.55^{bc}	0.22^{ab}	0.45^{ab}
Green Tea	49.39^{b}	0.89^{a}	4.39^{b}	8.97^{b}	0.45^{c}	8.10^{ab}	96.01^{b}	3.93^{a}	0.56^{b}	13.40^{ab}	27.44^{a}	0.22^{ab}	0.44^{ab}
Black Pepper	50.23^{ab}	0.87^{b}	4.47^{b}	8.95^{b}	0.50^{b}	8.24^{a}	96.68^{ab}	3.42^{b}	0.58^{b}	13.66^{a}	27.42^{a}	0.21^{b}	0.42^{b}
Roselle	50.12^{ab}	0.89^{a}	4.25^{c}	8.56^{c}	0.45^{c}	8.08^{a}	96.09^{a}	3.17^{b}	0.64^{a}	13.39^{abc}	27.05^{ab}	0.18^{c}	0.35^{c}
Combine	51.04^{a}	0.87^{b}	4.70^{a}	9.23^{a}	0.55^{a}	6.88^{c}	89.15^{c}	3.24^{b}	0.50^{c}	13.27^{bc}	26.24^{c}	0.23	0.46^{a}
SEM	0.25	0.01	0.04	0.07	0.01	0.08	0.29	0.07	0.01	0.09	0.17	0.01	0.01
P.Value	*	*	*	*	*	*	*	*	*	*	*	*	*
Inclusion Level													
0.5	50.27	0.87	4.47	8.96	0.49	7.68	93.95	3.36	0.56	13.36	26.86	0.20	0.41
1.0	49.88	0.88	4.33	8.75	0.49	8.16	96.48	3.51	0.62	13.44	27.25	0.21	0.43
SEM	0.17	0.00	0.03	0.05	0.01	0.05	0.20	0.05	0.01	0.06	0.12	0.00	0.01
P. Value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Period of Storage (week)													
Fresh	53.36^{a}	0.78^{b}	4.74^{a}	8.96^{b}	0.40^{c}	11.67^{b}	95.74^{a}	2.19^{c}	0.38^{a}	13.30^{c}	25.08^{c}	0.14^{d}	0.26^{d}
1 Week	53.08^{ab}	0.78^{b}	4.44^{b}	9.80	0.37^{d}	5.25^{d}	89.01^{b}	1.77^{d}	0.16^{c}	14.22^{ba}	31.26^{a}	0.18^{c}	0.39^{c}
2 Week	52.46^{b}	0.78^{b}	4.13^{c}	9.11^{b}	0.38^{d}	8.75^{c}	73.05^{c}	5.42^{a}	0.24^{b}	13.92^{b}	30.63^{b}	0.21^{b}	0.47^{b}
3 week	42.55^{c}	1.27^{a}	4.18^{c}	8.00^{d}	0.40^{b}	13.66^{a}	69.20^{d}	5.38^{a}	0.17^{c}	12.48^{d}	23.89^{d}	0.25a	0.47^{b}
4 Week	40.53^{d}	0.78^{b}	4.50^{b}	8.47^{c}	0.66^{a}	1.09^{e}	52.92^{e}	2.52^{b}	0.07^{d}	13.12^{c}	24.70^{c}	0.27a	0.57^{a}
SEM	0.27	0.01	0.04	0.08	0.01	0.08	0.03	0.08	0.01	0.09	0.18	0.01	0.01
P.Value	*	*	*	*	*	*	*	*	*	*	*	*	*
Interactions													
Treatment* Inclusion	*	*	*	*	*	*	*	*	*	*	*	*	*
Treatment* Week	*	*	*	*	*	*	*	*	*	*	*	*	*
Inclusion level* Week	*	*	NS	NS	*	*	*	*	*	*	*	*	*
Treatment* inclusion*week	*	*	*	*	NS	*	*	*	*	*	*	*	*

***NOTE**: SEM: Group standard error of mean; ^{a,b,c,d}: Means with different superscripts on the column are significantly different (P<0.05).*

Table 3. Effect of natural antioxidants on chemical properties of egg

Treatment	Proximate					Lipid Profile				Lipid Peroxidation
	M%	C.P%	E.E%	T.A%	NFE%	CHO (mg/dL)	TAG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	MDA (U/L)
Natural antioxidants										
Control	67.87^{b}	11.29^{b}	17.09^{a}	1.19^{c}	3.90^{a}	6.07^{a}	633.13^{a}	30.80^{b}	524.18^{a}	22.39^{d}
Green tea	68.61^{a}	11.59^{c}	15.77^{a}	1.24^{b}	2.79^{b}	647.26^{a}	490.07^{b}	25.07^{d}	450.34^{b}	31.79^{a}
Black pepper	68.14^{b}	13.31^{a}	14.41^{d}	1.29^{a}	2.09^{c}	521.33^{b}	521.48^{b}	28.67^{c}	388.36^{c}	25.21^{c}
Roselle	67.73^{b}	12.48^{b}	15.17^{b}	1.33^{a}	4.05^{a}	638.81^{a}	441.76^{c}	31.10^{a}	519.36^{a}	21.01^{d}
Combine	66.97^{c}	12.68^{b}	14.73^{c}	1.30^{a}	1.96^{c}	4.71^{b}	489.21^{b}	19.33^{e}	354.43^{c}	27.31^{b}
SEM	0.08	0.08	0.08	0.01	0.08	15.22	3.10	0.09	14.69	0.48
P.Value	*	*	*	*	*	*	*	*	*	*
Inclusion level										
0.5	67.72	12.56	15.14	1.27	3.41	6.23	5.23	27.97	4.89	24.66^{b}
1.0	68.24	12.48	15.49	1.28	2.53	5.51	4.82	26.98	4.27	26.87^{a}
SEM	0.06	0.06	0.06	0.01	0.06	11.09	2.24	0.06	10.7	0.36
P. Value	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Storage Period										
0 week (Fresh)	68.63^{a}	12.60^{a}	15.17^{b}	1.27	2.34^{b}	5.39^{c}	4.47^{b}	24.68^{c}	4.25^{b}	26.99^{b}
2 week	67.32^{C}	12.62^{a}	15.58^{b}	1.28	3.19^{a}	6.41^{a}	6.01^{a}	27.05^{b}	4.94^{a}	31.72^{a}
4 week	67.98^{b}	11.12^{b}	15.19^{a}	1.28	3.37^{a}	5.80^{b}	4.63^{b}	30.70^{a}	4.57^{b}	18.59^{c}
SEM	0.07	0.07	0.07	0.01	0.07	12.88	2.7	0.08	12.43	0.44^{a}
P. VALUE	*	*	*	NS	*	*	*	*	*	*
Interactions										
Treatment* Inclusion	*	*	*	*	*	*	*	*	*	*
Treatment* Week	*	*	*	*	*	*	*	*	*	*
Inclusion level* Week	*	*	NS	NS	*	*	*	*	*	*
Treatment* inclusion*week	*	*	*	*	NS	*	*	*	*	*

***NOTE**: SEM: Group standard error of mean; ^{a,b,c,d}: Means with different superscripts on the same column are significantly different (P<0.05). M= Moisture content, C.P= Crude protein, E.E- ether extract, T.A= Total Ash, N.F.E= Nitrogen Free Extract, CHO= Cholesterol, TAG= Triacylglycerides, HDL= High density lipoprotein, LDL= Low Density lipoprotein, MDA= Malondialdehyde*

2.7 Statistical Analysis

The data collected was analyzed using a completely randomized design [23]. Significant differences among treatments were separated by Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effects of natural antioxidants and storage time on physical properties of eggs

The effects and interactions of treatment of different natural antioxidants on both the internal and external egg quality were shown in Table 2. Result showed that green tea (*Camellia sinesis*) improves (P<0.05) the yolk color of the egg than the other. Both Ggreen tea and black pepper have significant effect (P<0.05) on the yolk percentage. Black pepper increases (P<0.05) the Haugh unit while it shows to be lowered (P>0.05) in the combine group. The inclusion levels of the natural antioxidants on the internal and external quality of egg revealed that there were no significant (P>0.05) difference in the two inclusion level of 0.5 g/kg and 1.0 g/kg of feed but have numerically higher values in the external and internal parameters. Compared to 0.5 g/kg inclusion level, 1.0 g/kg Inclusion level has high effect (P<0.05) on egg shape index, haugh unit, yolk index, yolk color, membrane weight, membrane % and blood spot. There were significant difference (P<0.05) in all the treatment stored at different weeks. Egg weight reduced (P<0.05) as the storage week increased while the reversed (P<0.05) was noted for membrane weight. Yolk colour was improved (P<0.05) at week 2 and 3 but dropped (P<0.05) again at week 4 of storage.

3.1.2 Effects of natural antioxidants and storage time on chemical properties of ggg

Table 3 shows the effect of natural antioxidants on chemical properties of egg. The natural antioxidants significantly (P<0.05) improves the proximate composition of the poultry eggs. Green tea shows minimal (P>0.05) influence on crude protein, though the combination of the natural antioxidants have a significant effect (P<0.05) on the ether extract. The different inclusion levels of the antioxidants have no effect (P>0.05) for both proximate and lipid profile except in the lipid peroxidation where 1.0 g gave higher values (26.87) than 0.5g (24.66). Eggs stored for 4 weeks had the lowest value (P>0.05) for the proximate and lipid profiles, though there are no significant (P>0.05) differences in their values. The fresh eggs show high moisture content (P<0.05) but the value for nitrogen free extract is low (P>0.05) in the fresh eggs. The natural antioxidants significantly (P<0.05) improves the proximate composition of the poultry eggs, with the green tea at 1.0 g having the highest value (P<0.05) for Moisture contents, black pepper 0.5 g having the highest (P<0.05) for CP and combine 1.0 g having the highest value (P<0.05) for E.E. The lipid profile was also improved significantly (P<0.05) by the inclusion levels of the natural antioxidants. The storage periods had a significant (P<0.05) effect on the Moisture content and CP value of the eggs. The Lipid profile was not significantly (P>0.05) affected either. The chemical analysis result shows that the storage periods of the 1.0 g inclusion, eggs does not have significant (P>0.05) effect on the proximate composition of the poultry egg.

3.2 Discussion

Results of this study showed that all the egg quality parameters, such as egg weight, egg shape index, yolk color, were significantly (P<0.05) affected by the inclusion of the natural antioxidants (roselle, green tea, black pepper) in the layer's diet. Present investigation showed that the egg weight was affected by dietary inclusion of green tea and black pepper (0.5 g/kg of diet) during the whole experimental compared to other treatment group. This is in contrary to the research carried out by Melo et al. [24], who reported "no significant differences in egg weight between control and black pepper supplemented groups. Conversely, inclusion of black pepper in laying birds diet in the current study increased egg weights beyond the control".

Haugh unit is a measure of the egg quality inside the shell. Haugh unit value obtained from the relationship between height of albumen and egg weight (albumen quality). Inclusion of roselle in laying birds diet had the highest Haugh unit percentage compared with control and other treatment groups. However, Saki et al. [25] observed "no significant effect on Haugh unit from roselle supplementation". Hilmi et al. [26] reported that "supplementation of piperine in quail diets at a level of 30 mg/kg body weight increased Haugh unit". In contrary with the

current findings, Lokaewmanee et al. [27] found that “Haugh unit was not influenced by dietary supplementation of 0.5% black pepper in laying birds diets”. According to Mohammed Al-harti [28] “green tea had a beneficial effect on haugh unit value of stored eggs and yolk cholesterol of fresh eggs when compared to control which is in accordance with the result of this study”. “The use of phytogenic additives with antioxidant properties, such as black pepper and roselle, may improve albumen quality”, as previously reported by Bozkurt et al. [29]. “In addition, the bio-active ingredients of herbal plants were shown to protect magnum and uterus, and encourage the albumen secretion in laying birds” [30].

“The egg yolk colour score was significantly increased by the addition of green tea in laying hen diets compared with the other treatment groups. The egg yolk colour was stated to be influenced by the consumption of zeaxanthin, lutein, alpha-carotene, beta-carotene, and carotenoids” [31]. Santos-Bocanegra et al. [32] and Lokaewmanee et al. [27] reported that “capsanthin improved egg yolk color and was responsible for the deep red color of the egg yolk. The yolk colour score (yellowness of egg yolk) was increased in the layers fed at 2.0 % green tea diet compared with that of the control diet”. Later, Uuganbayar et al. [33] reported that “the eggshell thickness and shape index were significantly reduced in layers fed at 1.0 % or 2.0 % Japanese green tea diets compared to that of the control”.

The percentage of composition for moisture, crude protein, ether extract and ash were affected by the antioxidants as there were significant difference between the antioxidants and the control. This corresponds with Tounkara et al [34] which also stated that there were significant differences between Roselle seeds with respect to total ash. The result shows that the inclusion of green tea at 1.0 % had the highest value for moisture content, showing a significant ($P<0.05$) effect on the moisture content of the egg. This result is close to agreement with a previous result from a research by Kojima and Yoshida [35] and Wei et al. [36] who suggested that green tea treatment could affect the egg quality and egg production performance [37], but the results were inconsistent and even very different from each other. The moisture content of the eggs without antioxidants (control) increase as the storage period increases, however the moisture contents of the stored egg containing antioxidants did not increase as the storage period increases, but remain almost stable with slight reduction as the storage period increases. The total ash was higher in the natural antioxidants compared to the control thou there were no significant differences in the inclusion level and the storage time. There was significant difference between the storage periods on nitrogen free extract (NFE).

Lipid oxidation is one of the main reasons for foods deteriorating and causes a significant reduction in their nutritional value and taste [38]. Triacylglyceride are the main constituents of body fats which have to be kept in low levels; addition of antioxidants increased the triacylglyceride levels in black pepper while there was a decrease in the levels of triglycerides in the combination of the antioxidants (black pepper, green tea and Roselle). The result of this study were in accordance to Melo et al. (24) who also observed that Triacyglycerides level increased significantly ($P< 0.05$) with increase inclusion level of black pepper in the diet. Chen et al. [39] result also shows that triacylglycerides decreases in green tea. The levels of high density lipoprotein (HDL), the good type of cholesterol in roselle compared to the combination of Roselle, green tea and black pepper while the levels of low density lipoprotein (LDL) were significantly increased in green tea. Though green tea significantly increased high-density lipoprotein and no significant difference was observed in the low-density lipoprotein (LDL) according to [39]. Malondialdehyde is an indicator of oxidative stress. Its level was significantly increased in green tea and low in Roselle. In the study of [39] MDA in stored eggs numerically decreased, but not significantly, by dietary antioxidant supplementation, this is because storage period was relatively short. The combined inclusion at 1.0 had the lowest value for Cholesterol with significant difference ($P>0.05$). Green tea supplementation of layer diets reduced the cholesterol content of the egg yolk [40,41,42]. Biswass and Wakita [40] also observed that “feeding green tea extract or powder improved FCR and decreased feed intake, low density lipoprotein (LDL) and blood cholesterol and reduced triacylglycerides and yolk cholesterol and increased HDL to cholesterol or LDL ratio”. “The beneficial effects of Green tea could be attributed to its contents of flavonoids, phenolic extracts, catechins and beta-carotene” [28].

4. CONCLUSION

Conclusively, no particular trend was observed with the influence of the various natural antioxidants on both external and internal characteristics of eggs from ISA brown. However, the yolk color was preferred with the inclusion of the natural antioxidant compared to the control.

Green tea improves the yolk color better than others. Black pepper greatly improves the CP, E.E and the lipid profile of the eggs. The inclusion levels have no influence on the proximate and the lipid profile. The chemical properties deteriorate with the storage time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. Statistical database. Food and Agriculture Organization of the United Nations. 2000; Rome, Italy.
2. Perin A. Pedersen G. Problems related to poultry production at village level. In: possibilities for the smallholder poultry projects in Eastern and Southern Africa, (edited by Pietta, P. G. 2000. Flavonoids as antioxidants. Journal of National Products. 2000; 63 (7):1035 – 1042.
3. FAO: Statistical Database - available at : http://faostat.fao.org. 2011; Rome, Italy 34.
4. Oji UO, Chukwuma AA. Technical Efficiency of Small- Scale Poultry Egg Production in Nigeria: Empirical Study of Poultry Farmers in Imo State, Nigeria. Medwell Research Journal of Poultry Science. 2007; 1(3-4): 16-21.
5. Forson A, Ayivor JE. Banini GK, Nuviadenu C, Deborah SK,. Evaluation of some elemental variation in raw egg yolk and egg white of domestic chicken guinea fowl and duck eggs, Annals of Biological Research. 2011; 2(6): 676-860.
6. Murthy K. Evaluation of antioxidant activity of pomegranate (Punicagranatum) and grapes (Vitis *vinifera*), thesis, Rajeev Gandhi University of Healh Science; 2001.
7. Trombino S, Seini S, Di Nicuolo F, Celleno L. Ando S. Picci N. Calviello G. Palozza P. . Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with alpha-tocopherol, beta carotene, and ascorbic acid. Journal of Agricultural Food Chemistry. 2004; 52(8): 2411-2420.
8. Cao LN, Lin ZB. Regulatory effect of Ganoderma lucidum polysaccharides on cytotoxic T-lymphocytes induced by dendritic cells in vitro. Acta Pharmacologica Sinica. 2003;34:312326.
9. Crespy V, Williamson G. “A Review of the Health Effects of Green Tea Catechins in Vivo Animal Models,” The Journal of Nutrition. 2004;134(12):3431S-3440S.
10. Fujiki H Suganuma M. Okabe S. Sueoka E. Suga K. Imai K. Nakachi K. A new concept oftumor promotion by tumor necrosis factor-alpha, and cancer preventive agents (-)epigallocatechin gallate and green tea--a review. Cancer Detect Prev. 2000; 24(1) :91-99.
11. Jang IS, Ko YH, Kang SY, Moon YS, Sohn SH. Effect of dietary supplementation of ground grape seed on growth performance and antioxidant status in the intestine and liver in broiler chickens. Korean J. Poultry Science. 2007; 34 (1):1-8.
12. Hassan MSH Abo TAM Wakwak M. Yousef BA. Productive, physiological and immunological effects of using some natural feed additives in Japanese quail diets. Egyptian Poultry Science Journal. 2007; 27(11): 557-588.
13. Pieta PG. Flavonoids as antioxidants. Journal of National Products. 2000;67(7):1035-1042.
14. Böttcher S, Steinhauser U, Drusch S. Off-flavour masking of secondary lipid oxidation products by pea dextrin. Food Chemistry. 2015;169:492–498.
15. BATC. British American Tobacco Company Meterological site. Ogbomoso. Nigeria; 2012
16. Duman M. Sekeroglu A. Yildirim A. Eleroglu H. Camci, O. Relation between egg shape index and egg quality characteristics. European Poultry Science. 2016;80:1-9.
17. Roberts JR, Factors Affecting Egg Internal Quality and Egg Shell Quality in Laying Hens. The Journal of Poultry Science. 2004;41(3):161-177.
18. Haugh RR. The Haugh unit for measuring egg quality. U. S. Egg Poultry Mag. 1937;43:552-555.
19. Bovskova H. Mikova, K. Panovska Z. Evaluation of Egg Yolk Colour. Czech Journal of Food Science. 2014; 32(3):213-217.

20. AOAC. Official Methods of Analysis (18th edition) Association of Official Analytical, Chemists International, Maryland, USA; 2005.
21. Richmond W. Preparation and Properties of a Cholesterol Oxidase from Nocardia sp. and Its Application to the Enzymatic Assay of Total Cholesterol in Serum. Clinical Chemistry. 1973;19:1350-1356
22. Association of Official Analytical Chemists (AOAC) Official Methods of Analysis. 17th Edition, Association of Official Analytical Chemists, Arlington; 2002.
23. SAS. Statistical Analysis System User's Guide. Version 8, SAS Institute, Cary; 2000.
24. Melo RD, Cruz FG, Feijo JD, Rufino JR, Melo LD, Damasceno JL. Black Pepper in diets for laying hens on performance, egg quality and blood biochemical parameters. Acta Scientiarum, Animal Science. 2016; 38(4):405-410.
25. Saki AA, Aliarabi H, Hosseini Siyar SA, Salari J and Hashemi M). Effect of a phytogenic feed additive on performance, ovarian morphology, serum lipid parameters and egg sensory quality in laying hen. Veterinary Research Forum. 2014; 5(4):287-293.
26. Jancso G, Kiraly E, Jansco-Gabor A. Pharmacologically induced selective degeneration of chemosensitive primary sensory neurons. Nature. 1997;270:741–743.
27. Lokaewmanee K., Yamauchi K. Okuda N. Effects of dietary red pepper on egg yolk colour and histological intestinal morphology in laying hens. Journal of Animal Physiology and Animal Nutrition. 2013; 97:986-995.
28. Mohammed A. Al-Harthi The Effect of Natural and Synthetic Antioxidants on Performance, Egg Quality and Blood Constituents of Laying Hens Grown Under High Ambient Temperature, Italian Journal of Animal Science. 2014; 13:2:3239.
29. Bozkurt M. Kucukyilmaz, K. Catli A. U. Cinar M. Bintas E. Coven, F. . Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poultry Science. 2012; 91(6):1379-1386.
30. Nadia IR, Hassan RA, Qota EM, Fayek HM. Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. Int.J. Poult. Sci. 2008; 7:134-150.
31. Hammershoj M, Kidmose U, Steenfeldt S. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (Daucus carota) varieties as forage material for egg-laying hens. Journal of the Science of Food and Agriculture. 2010; 90: 1163–1171.
32. Santos-Bocanegra E. Ospina-Osorio X. Oviedo-Rondon E.O. Evaluation of xanthophylls extracted from Tagetes erectus (Marigold Flower) and Capsicum Sp. (Red Pepper Paprika) as a pigment for egg-yolks compare with synthetic pigments. International Journal of Poultry Science. 2004; 3:685-689.
33. Uuganbayar D. Shin I.S. Yang C.J. Comparative performance of hens fed diets containing Korean, Japanese and Chinese green tea', Asian-Australasian Journal of Animal Sciences. 2006;19(8): 1190–1196.
34. Tounkara F, Amadou I, Le G Shi Y. Effect of boiling on the physicochemical properties of Roselle seeds (*Hibiscus sabdariffa* L.) cultivated in Mali. African Journal of Biotechnology. 2011; 10(79): 18160-18166.
35. Kojima S Yoshida Y. Effects of green tea powder feed supplement on performance of Hens in the late stage of laying. International Journal of Poultry Science. 2008;7:491-496.
36. Wei Y Qu X. Cai C. "Effects of green tea powder on performance, egg quality and yolk cholesterol of green shell laying hens. China Feed. 2012;22:22-24.
37. Uuganbayar D. Bae IH. Choi KS. Shin IS. Firman JD. Yang CJ. Effects of green tea powder on laying performance and egg quality in laying hens. Asian Australia Journal Animal. 2005; 18:1769-1774.
38. Chen IJ, Liu CY, Chiu JP, Hsu CH. Therapeutic effect of high-dose green tea extract on weight reduction: A randomized, double-blind, placebo-controlled clinical trial. Clin Nutr. 2016;35(3):592–599.
39. Renfan Xu, Ke Yang, Sui Li, Meiyan Dai, and Guangzhi Chen. Effect of green tea consumption on blood lipids: A systematic review and meta-analysis of randomized controlled trials. Nutrition Journal. 2020;19: 48.
40. Biswass MA, Wakita M. Comparison of two dietary factors, green tea powder feeding

and feed restriction, influencing laying performance and egg quality in hens. Bulletin of the aculty of Bioresources, Mie University. 2001b; 25/26:55-61.

41. Biswas AH. Wakita M. Effect of dietary Japanese green tea powder supplementation on feed utilization and carcass profiles in broilers. Journal of Poultry Science. 2001;38:50-57.

42. Biswas MAH, Miyazaki Y, Nomura K, Wakita M. Influences of long-term feeding of Japanese green tea powder on laying performance and egg quality in hens. Asian-Australasian Journal of Animal Sciences. 2000;13:980–985.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/93394