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UTILIZATION OF NATURAL ANTIOXIDANTS ON THE PERFORMANCE, SENSORY AND QUALITY OF ISA BROWN LAYER'S EGGS

Atandah R. A.[1], Olawuyi B. S.[2*], Akinwumi A. O.[1], Oyeyode A. T.[2], Adeoye O.[2] and Fasasi M.O.[3]

[1] Animal Products and Processing Unit, Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

[2] Department of Crop and Animal Science, Ajayi Crowther University, Oyo State, Nigeria.

[3] College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria

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***Corresponding author:** Olawuyi B. S.

Department of Crop and Animal Science, Ajayi Crowther University, Oyo State, Nigeria.

Abstract

Natural antioxidants as a potential for antibiotics alternative, have proven zero residue effect in poultry products. Three hundred (300) ISA Brown point of lay birds with 65% hen-day egg production (HDEP) were used for the experiment and randomly assigned to five (5) dietary treatments of three (3) replicates with twenty (20) birds each: T1= Negative Control (NC)- basal diet, T2= Positive Control containing basal diet + 0.1% oxytretracycline while T3-T5 contain basal diet supplemented with Oregano (ORG), Rosemary (RSM) and Nutmeg (NMG) respectively, each included at 5g/kg of basal diet. Data were collected on the production performance, sensory properties of egg and the egg qualities of Isa Brown Layer' Egg and subjected to One-way Analysis of Variance (ANOVA). The results revealed that significant variations exist in all production parameters, supplementation of NMG and ORG compared favourably ($p<0.05$) with NC in HDEP Percentage ($p=0.0003$), Total Number of Egg/week ($p=0.002$), Feed Conversion Ratio ($p=0.009$), egg diameter (43.80 and 43.89mm), yolk height (16.00 and 16.04mm), albumen height (7.60 and 7.32mm) and haugh unit (86.51 and 85.78). ORG had highest egg shape ($p>0.05$) and yolk index% ($p<0.05$) relative to both controls. Ease of peeling and shell colour were significantly influenced ($p=0.0001$) and overall acceptability was however similar ($p>0.05$) to controls. The shell, albumen and yolk colour were rated highest (slightly to moderately light) with RSM diet supplementation. It can be concluded that dietary supplementation of NMG and ORG improved production performance and egg qualities while RSM improved the sensory properties relative to both controls.

Keywords: Natural Antioxidants, Performance, Egg Quality, Sensory Properties, Isa Brown Layers.

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1.0 Introduction

The contribution of poultry to food security is significant providing protein and essential micronutrients for humans (Mottet and Tempo, 2017). Egg is a major source of animal protein in poultry chain and the production of quality eggs is dependent on the nutrition (Oluyemi and Robert 2000) and perhaps feed additives from natural sources. Organic feed additive is a promising alternative to synthetic antimicrobials which is unlikely to leave adverse residue in food industry and animal (Mandey and Sompie, 2021; Abd-El-Hack, *et al*., 2022). Utilization of natural feed additives as antioxidants in poultry has increased due to its wide acceptance and improvement of poultry production performance. These antioxidants are mostly used in food industries for the potential antioxidative and antimicrobial characteristics (Giannenas *et al.*, 2005). The bioactive substances in natural antioxidants necessitate its supplementation in the laying hens' diets as they improve egg quality and shelf-life of egg on storage (Zhang *et al.*, 2020; Vlaicu *et al.*, 2021).

Oregano, in previous study has been used as alternative for chemical antibiotics in poultry and livestock (Ri *et al*., 2017). Alagawany *et al*. (2018) highlighted that oregano extracts benefit the poultry when supplemented in diets improving feed intake and conversion, digestion, increase production performance, reduce disease incidence and economic loss. Rosemary is regarded as a strong antioxidant (Yildirim *et al*., 2018; Yang *et al*., 2021) because of its ability to improve technological aspects of food for consumers (Nieto *et al*., 2018), minimize oxidation of lipids and oxidative stress in animals, hence its application as feed additives. It also has a positive impact on egg production performance of poultry, egg development, egg quality properties (Gerzilov *et al.*, 2015). Nutmeg is also an aromatic natural extract that are added to poultry feed as antioxidants and possess the potential to improve production performance and quality meat and egg products of poultry (Reddy *et al.*, 2018).

Furthermore, the external (weight, shape index, shell thickness and color) and internal (yolk index, color, albumen, haugh unit) qualities are regarded as important for marketing and storage (Hrncar *et al.*, 2014). Hence the present investigation is aimed to assess the impact of selected natural antioxidants (Oregano, rosemary and nutmeg) on production performance, external and internal characteristics and sensory properties of Eggs from Isa Brown Layers.

2.0 Material and Methods

2.1 Site of the Experiment

The experiment was conducted at the Teaching and Research Farm Poultry Unit, Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria, situated in the derived Savannah zone and lies on Longitude 4^0 15^1 East of greenish meridian and Latitudes 8^0 81 North of the equator (Google Earth Map, 2024).

2.2 Experimental Animals and Management.

A total of Three Hundred (300) ISA Brown strain point of lay (POL) birds were bought from a reputable commercial farm and assigned to five (5) experimental diets of three (3) replicates each in a battery cage following a completely randomized design (CRD). Each replicate contained 20 POL birds and the Hen day egg production was allowed to attain 65% before experimental data were collected. Routine vaccination program was followed promptly with feed and water administered *ad-libitum* while the period of the experiment lasted for eight (8) weeks.

2.3 Source of test ingredients and dietary preparation.

The test ingredients (natural antioxidants) were purchased from a popular market in Ogbomoso metropolis. They were air-dried, grinded, supplemented to basal diet on batch – basis to avoid auto-oxidation and served as treatments diet on daily basis.

2.4 Experimental Diets/Treatments

Five (5) dietary treatments were used for the experiment that include T_1 = Negative Control (NC)- basal diet without any feed additive, T_2 = Positive Control containing 1g/kg diet of Oxytetracycline antibiotics), T_3 = Oregano, T_4 = Rosemary and T_5 = Nutmeg included at 0.5% rate per kg (equiv. 500mg/kg) of basal diet. All the experimental treatments were replicated thrice containing of 20 birds per replicate. And the basal diet was formulated according to NRC (1994) with 2,800 kcal ME/kg and 16.30% CP.

2.5 Experimental Design.

The experiment was arranged in a Completely Randomised Design (CRD) while the model of the experimental design is thus; $\mathbf{Y_{ij} = \mu + T_i + \varepsilon_{ij}}$. Y_{ij} = observation of the i^{th} and j^{th} effect, μ= common mean, T_i = fixed effect of i^{th} treatment, ε_{ij} = random experimental error.

2.6 Data Collection

Laying Performance **(**hen-day egg production, egg mass, egg weights), feed intake, feed efficiency were recorded daily throughout experiment, fifteen (15) egg samples from each treatment (5 from each replicate) were observed for external and internal egg qualities twice per week and sensory evaluation was also done accordingly.

i. **Hen-day egg production (%) =** $\frac{\text{Total no of eggs laid x 100}}{\text{Total no of live hens}}$

ii. **Egg mass (g) =** Hen-day egg production (%) x Average Egg Weight (g)

- **Average Egg weight =** Total Sum of Egg weight (g/day) / Number of egg laid.

iii. Feed intake

Feed intake (g/bird) = Feed served – Left over

iv. Feed Efficiency

- **Feed Conversion ratio**

Feed conversion Ratio = $\frac{\text{Total Feed Intake or Consumed (g)}}{\text{Total Egg Weight (g).}}$

- **Feed efficiency per dozen**

Feed efficiency per dozen was estimated by dividing total feed intake in kg by the number of egg laid for that day/week, then multiply by 12 factor.

Feed Efficiency per dozen = $\frac{\text{Total Feed Intake or Consumed (kg) x 12}}{\text{Total Number of Egg laid}}$

Egg Qualities

Egg external and internal characteristics were analyzed using randomly picked for egg diameter, length, shape index, shell weight and thickness, shell membrane weight, albumen and yolk weight, height, yolk diameter, color, indexes and Haugh units were estimated according to Yalçın *et al.* (2014)

- **Egg Shape Index (%) =** $\frac{\text{Egg width x 100}}{\text{Egg length}}$
- **Yolk index (%) =** $\frac{\text{Yolk height x 100}}{\text{Yolk length}}$

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- **Haugh unit**- According Haugh (1937) formula; **HU = 100Log (H+7.57-1.7W$^{0.37}$).**

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

- **Yolk color**- using usual La Roche Scale also known as DSM Yolk Color fan (Bovskova *et al.,* 2014).
- **Yolk Percentage – Yolk weight (g)/Egg weight (g) x100**
- **Albumen Percentage – Albumen weight (g)/Egg weight (g) x100**

v. Sensory Analysis

Twenty (20) eggs sample were randomly selected from each treatment weekly for organoleptic properties and served to 20 semi-trained sensory panelists to assess the shell colour/mottleness, ease of peeling, albumen color, yolk color, smell, taste, texture, residue after chewing and overall acceptability using a 9 point hedonic scale for scoring.

vi. Data Analysis

All data collected from the study were subjected to One Way Analysis of variance (ANOVA) using SAS (2003). Means were separated by Fisher's Least Significant Difference (LSD) test option of the same statistical software for significant differences. The probability of 5% was considered significant (P<0.05)

3.0 Result and Discussion

3.1 Production Performance:

Table 1 showed the effect of natural antioxidants on the production performance of Isa Brown layers. Significant variation exists in all the production parameter except mortality rate. Supplementation of nutmeg and oregano improved (p<0.05) the Average Egg Weight and Average Egg Number (per/bird/week) relative to the Negative Control (NC). Highest significant (p<0.05) hen-day egg production (HDEP) was noted for group fed nutmeg (74.43%) although similar to NC, the potential of nutmeg is evident in improving production performance and diminish pathogenic bacteria in egg products with its bioactive compounds (Myristicin, safrole, eugenol, sabinene etc) present (Dhama *et al*., 2015; Reddy *et al.,* 2018). Otherwise to the study, Daramola *et al.* (2018) observed no variation in HDEP% and Feed intake (FI), however variation (p<0.05) observed for feed efficiency in the researcher's study concurred with this study when natural additives were compared. The supplementation of natural antioxidant had effect (p<0.05) on the feed intake and this is contrary to Radwan *et al.* (2008) and Cho *et al.* (2014) studies on natural antioxidants and phytoadditives on poultry. Rosemary supplemented in Isa Brown layer's diet does not result to significant improvement in HDEP% as the NC contradicting the study of Kedir *et al.* (2023) who observed significant improvement in egg production% as the rosemary meal levels increase relative to control. Oregano supplementation to layers' diet resulted into significant improvement in the Average Daily egg weight (59.49g) and Egg Mass (332.36g/bird/week), however in the study of Abdel-Wareth *et al.* (2013), the egg weight was not statistically influenced when oregano and thyme were supplemented a different level. The PC (antibiotic) group had the least egg number and weight, meaning that the supplemented antioxidants can potentially improve production performance. This is evident in Alagawany and El-Hack (2015) study who reported that addition of rosemary powder improved both parameters. Better Feed Conversion Ratios (FCR) and feed efficiency (per kg of Feed per dozen of Egg) were observed in the layers groups fed nutmeg (2.55 and 1.79kg/dozen) and Oregano (2.52 and 1.80kg/dozen) comparably with the NC (2.41 and 1.68kg/dozen) with no statistical difference amongst them. The improvement in the FCR by natural antioxidants supplementation agreed with Abdel-Wareth *et al.* (2013).

3.2 Egg Qualities:

The results in Table 2 revealed that Egg weight, albumen weight, yolk height and yolk index were significantly (p<0.05) affected by the supplementation of natural antioxidants while other parameters were not affected (p>0.05). Albumen weight affected by the natural antioxidants and the values of albumen and yolk weight, albumen height and haugh unit for rosemary in our study were all in support of Kedir *et al.* (2023) who compared varied levels of rosemary leaf meal (RLM) in relation to group fed no RLM (control). Albumen Weight was found highest (p<0.05) in laying hens fed dietary supplementation of nutmeg (35.42g) and rosemary (34.71g) compared with least (p<0.05) in Positive Control (31.75g). Daramola *et al.* (2018) observed no significant (p>0.05) influence of herbal supplementation on the haugh unit, yolk index and albumen height, same observation was also noted in our current study. Haugh unit is measure of egg quality dependent on the albumen height and egg weight and USDA (2000) classified haugh unit value into AA (100-72), A (71-60), B (59-30) and C (below 30). Although haugh unit was not significantly (p>0.05) affected, it was improved by nutmeg and oregano supplementation, indicating addition of natural antioxidants to laying hens diet improve egg quality. Furthermore, Akinwumi *et al.* (2022) reported considerable improvement of egg weight and shape index of natural antioxidants supplemented group and this conformed to the present investigation. Nutmeg supplementation mostly improved the egg, length (54.51mm) and yolk color (4.63), proportion of yolk (27.81%) and albumen (67.05%) while oregano improved (p>0.05) the egg shape (81.88%) and yolk (42.76) index. According to Cufadar *et al.* (2018), rosemary as feed additives improves eggshell quality, rosemary addition in this study also enhanced the shell thickness (0.78mm) relative than the two controls. Kedir *et al.* (2023) also noted the improvement of shell thickness with rosemary leaf meal supplementation.

3.3 Egg Sensory Properties

The results (Table 3) showed that there were significant differences (p<0.05) in the shell colour and ease of peeling egg. Although positive control (antibiotics) facilitated the ease of peeling to be very easy (8.00), meanwhile nutmeg supplemented group (6.30) that had the least rating (slight easy) was not reported to be difficult in peeling. The laying hens egg fed dietary supplementation of rosemary was rated higher (p>0.05) in yolk (7.30) and albumen (7.40) in colour and followed oregano (7.50) in shell colour/ mottled (6.30) than other additives, this informs the clarity of egg content and shell of egg supplemented with rosemary. It may also imply that rosemary contained less or no pigment compounds that may be responsible for the hue (dark) coloration. Rosemary also enhanced the taste (7.80) and smell/odor (7.30) perceptibility of the egg which was desirable mostly and it also compared favourably (p>0.05) with both control in terms of the overall acceptability. Rosemary is used as flavoring agent in food industry (Hussain *et al*., 2010) and this might be good reason for the improved values observed for taste and smell/odor of rosemary supplemented in this study. The general improvement in the sensory qualities of rosemary supplemented diets supported Nieto *et al*. (2018) that rosemary have technological benefits to consumers when used as food preservative.

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Table 1: **Production Performance of Isa Brown Layers as Affected by Natural Antioxidants.**

Treatments	Total Avrg Egg Weight (g/week/bird)	Avrg Egg Number (per bird/week)	Hen Day Prod (%)	Avrg Egg Weight (g)	Egg Mass (g/week/bird)	Avrg Feed Intake (g/bird/day)	FCR	Feed Efficien /kg/dozen	Mortality
NC	284.41^{ab}	4.91^{a}	74.12^{a}	57.89^{b}	330.09^{a}	103.52^{ab}	2.41^{c}	1.68^{b}	0.00
PC (Antibiotic)	215.33^{c}	3.79^{c}	57.91^{c}	56.73^{b}	253.21^{d}	96.86^{b}	3.06^{a}	2.08^{a}	1.00
Oregano	288.48^{a}	4.87^{a}	72.63^{ab}	59.49^{a}	332.36^{a}	106.62^{ab}	2.52^{bc}	1.80^{ab}	0.00
Rosemary	252.92^{b}	4.36^{bc}	63.11^{bc}	58.06^{ab}	317.41^{b}	102.35^{ab}	2.88^{ab}	2.01^{a}	2.00
Nutmeg	288.46^{a}	4.96^{a}	74.43^{a}	58.22^{ab}	309.56^{c}	110.05^{a}	2.55^{bc}	1.79^{ab}	0.00
P-Value	0.0002	0.002	0.0003	<0.0001	<0.0001	0.006	0.009	0.04	0.21
R-Square	0.38	0.33	0.37	0.51	0.45	0.29	0.28	0.22	0.15

^{abcd} Means along the same column having different superscripts are significantly different at 5% probability (P<0.05) using Fisher's LSD.

NC- Negative Control, PC – Positive Control, P-value –Probability value, FCR - Feed Conversion Ratio.

Table 2: Egg Quality Indices of Fresh Isa Brown Layers Egg as Affected by Natural Antioxidants.

Treatments	External Qualities					Internal Qualities										
	Egg Weight (g)	Egg Diamet (mm)	Egg Lengt. (mm)	Shell thickness (mm)	Egg Shape Index %	Yolk height (mm)	Yolk diameter (mm)	Yolk weight (g)	Yolk Color	Yolk Index %	Yolk %	Albumen Height (mm)	Albumen Weight (g)	Albumen %	Haugh Unit	Shell Memb Wght (g)	Shell Membr %
NC	56.99^{ab}	43.00	54.41	0.69	79.10	15.68^{ab}	38.20	14.60	4.17	41.16^{ab}	26.00	7.08	32.68^{abc}	58.06	83.76	0.63	1.11
PC (Antibiotic)	55.08^{b}	42.74	53.71	0.77	79.72	15.84^{a}	38.76	14.06	4.20	41.13^{ab}	25.97	6.84	31.75^{c}	58.59	82.54	0.68	1.25
Oregano	58.21^{a}	43.89	53.59	0.69	81.89	16.04^{a}	38.00	14.12	4.36	42.76^{a}	25.36	7.32	32.62^{abc}	58.53	85.78	0.66	1.18
Rosemary	57.26^{ab}	43.30	54.14	0.78	79.95	14.88^{b}	39.68	14.24	4.16	37.57^{b}	25.47	6.84	34.71^{ab}	61.60	83.18	0.62	1.10
Nutmeg	57.96^{a}	43.80	54.68	0.78	80.17	16.00^{a}	39.96	14.61	4.63	40.17^{ab}	24.89	7.60	35.42^{a}	60.17	86.51	0.64	1.09
P-Value	0.01	0.07	0.12	0.54	0.15	0.03	0.35	0.29	0.77	0.03	0.45	0.93	0.002	0.30	0.94	0.83	0.67
R-Square	0.35	0.05	0.14	0.11	0.20	0.16	0.16	0.13	0.08	0.13	0.08	0.05	0.36	0.17	0.05	0.07	0.09

^{abc} Means along the same column having different superscripts are significantly different at 5% probability (P<0.05) using Fisher's LSD.

NC- Negative Control, PC – Positive Control, P-value –Probability value.

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Table 3: **Sensory Properties of Isa Brown Layers' Egg as Affected by Natural Antioxidants.**

Treatments	Shell Color/ Mottledness	Ease of Peeling	Albumen Color	Yolk Color	Smell	Taste	Texture	Residue after chewing	Overall Acceptability
NC	2.90[e]	7.10[b]	6.50	5.40[b]	7.10	7.60	7.80	5.90	8.30
PC (Antibiotic)	4.00[d]	8.00[a]	6.50	6.20[ab]	6.80	7.40	7.50	6.70	8.20
Oregano	7.50[a]	7.10[b]	6.60	6.10[ab]	6.60	6.90	6.80	6.30	7.60
Rosemary	6.30[b]	7.10[b]	7.40	7.30[a]	7.30	7.80	7.60	6.90	8.20
Nutmeg	4.9[c]	6.0[c]	6.20	6.50[ab]	6.70	6.80	6.70	7.10	7.70
P-Value	<0.0001	<0.0001	0.43	0.08	0.97	0.67	0.60	0.97	0.23
R-Square	0.86	0.66	0.09	0.16	0.02	0.06	0.07	0.02	0.12

[abcdef] Means along the same column having different superscripts are significantly different at 5% probability (P<0.05) using Fisher's LSD.

NC- Negative Control, PC – Positive Control, P-value –Probability value.

4.0 Conclusion and Recommendation

The dietary supplementation of natural antioxidants impacted the performance, quality and sensory properties of Isa brown egg positively. Inclusion of Nutmeg and Oregano compared favourably with basal diets without additive (Control) in Hen Day Egg Production Percentage, Number of Eggs and Feed Conversion Ratio. Furthermore, Nutmeg and Oregano improved egg diameter, yolk height, albumen height and haugh unit, Oregano had highest egg shape and yolk index while Rosemary improved the shell thickness relative than both controls. Lastly, the smell and taste were more desirable and shell mottledness, albumen and yolk colour were more enhanced with rosemary supplementation in layer diet. Hence, incorporation of these natural antioxidants in layer's diet is hereby advocated for sustainable and increase production of egg protein from poultry.

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6.0 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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8.0 Conflicts of Interest

The authors declare no conflicts of interest.

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