Effect of phyto-additives on proximate composition and lipid profile of eggs stored for 6 weeks

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Received: June 21, 2024 DOI: 10.14295/bjs.v3i9.641

Accepted: July 22, 2024 URL: https://doi.org/10.14295/bjs.v3i9.641

Abstract

This study was conducted to examine the effect of supplementing layers' diet with three phyto-additives; Black seeds (BS), African Nutmeg (AN), and Negro pepper (NP) on the chemical composition of stored table eggs. *Isa Brown* pullets were used and allotted into 7 treatments of 4 replicates with 6 birds each to make a total of 24 birds per treatment in a completely randomized design experiment. The treatments were T1 - control (given antibiotics), T2 - 0.5% BS, NP T3 – 1.0% BS, T4 – 0.5% AN, T5 – 1.0% AN, T6 – 0.5% NP and T7 – 1.0%. Collection of data started eight weeks after the birds were placed on experimental diets; 3 eggs were selected from each replicate to make a total of 12 eggs per treatment. They were stored for 6 weeks before taking to the laboratory for chemical analysis. Eggs from birds given diets supplemented with 0.5% black seed had the highest dry matter (26.79%), crude protein (12.08%), crude fat (11.26%), ash (1.22%) and gross energy (1.47 Kcal/g^{-1}) while eggs of birds on Treatment 3 (0.5% African nutmeg) and Treatment 4 (0.5% Negro pepper) had the highest percentage carbohydrate (3.22 and 3.27%). Total cholesterol and low-density lipoprotein (LDL) were significantly ($p < 0.05$) reduced in eggs fed diet supplemented with 0.5% black seed and also gave the best high-density lipoprotein. It can be concluded from this study that supplementing layers diet with these phytoadditives enhanced the proximate composition and reduced the total cholesterol of stored eggs.

Keywords: layers, stored eggs, phyto-additives, proximate composition, lipid profile.

Efeito de fitoaditivos na composição centesimal e no perfil lipídico de ovos armazenados por 6 semanas

Resumo

Este estudo foi conduzido para examinar o efeito da suplementação da dieta das poedeiras com três fitoaditivos; Sementes pretas (BS), noz-moscada africana (AN) e pimenta negra (NP) na composição química de ovos de mesa armazenados. Foram utilizadas frangas Isa Brown distribuídas em 7 tratamentos de 4 repetições com 6 aves cada, perfazendo um total de 24 aves por tratamento em um experimento de delineamento inteiramente casualizado. Os tratamentos foram T1 - controle (administrado antibiótico), T2 - 0,5% BS, NP, T3 – 1,0% BS, T4 – 0,5% AN, T5 – 1,0% AN, T6 – 0,5% NP e T7 – 1,0%. A coleta de dados começou oito semanas após as aves terem sido colocadas nas dietas experimentais; 3 ovos foram selecionados de cada repetição para perfazer um total de 12 ovos por tratamento. Eles foram armazenados por 6 semanas antes de serem levados ao laboratório para análise química. Ovos de aves que receberam dietas suplementadas com 0,5% de semente preta apresentaram maior matéria seca (26,79%), proteína bruta (12,08%), gordura bruta (11,26%), cinzas (1,22%) e energia bruta (1,47 Kcal/g^{-1}).), enquanto os ovos das aves do Tratamento 3 (0,5% noz-moscada africana) e do Tratamento 4 (0,5% pimenta negra) apresentaram os maiores percentuais de carboidratos (3,22 e 3,27%). O colesterol total e a lipoproteína de baixa densidade (LDL) foram significativamente ($p < 0,05$) reduzidos em ovos

alimentados com dieta suplementada com 0,5% de semente preta e também proporcionaram a melhor lipoproteína de alta densidade. Pode-se concluir deste estudo que a suplementação da dieta das poedeiras com esses fitoaditivos melhorou a composição centesimal e reduziu o colesterol total dos ovos armazenados.

Palavras-chave: poedeiras, ovos armazenados, fitoaditivos, composição centesimal, perfil lipídico.

1. Introduction

Eggs are an important source of food due to their high biological value and nutritional benefits on human health (Selim; Hussein, 2020; Ojediran et al., 2022). Fatty acids and cholesterols in eggs are essential components from a health and consumption perspective for humans particularly in terms of polyunsaturated fatty acid (PUFA) and omega-3 fatty acids consumption (Attia et al., 2015; Batkowska et al., 2021). It is well known that hen's diet, particularly those containing fats/fatty acids, strongly influences egg composition (King et al., 2012; Kkang; Zivkovic, 2022).

Eggs with high unsaturated fatty acid content are preferred by customers due to low cholesterol (hypocholesterolemic). However, eggs with greater polyunsaturated fatty acid are susceptible to peroxidation and thus enriching such eggs with antibiotics could decrease lipid peroxidation and improve the quality of the eggs (Laudadio, 2015). Egg lipids are confined to the yolk and account for about 30% of the fresh weight of the yolk and 60% of the yolk dry matter (Leskanish; Nobel, 1997; Ariza et al., 2021; Kralik et al., 2021). The fatty acid composition of eggs in laying hens can be influenced predictably by the fatty acid composition of the diet (Beynen, 2005).

Phyto-additives are natural medicinal products derived from herbs and spices used in animal nutrition to enhance performance (Windisch et al., 2008; Vlaicu et al., 2021) and improve the quality of their products. Because they are considered natural products, consumers may willingly accept them to be included in poultry feeds. Comprehensive investigations on phytogenic plants have revealed their growth-promoting, antimicrobial, antioxidant, and anti-inflammatory potentials (Windisch et al., 2008; Gheisar; Kim, 2017).

Jing et al. (2024), characterized phytogenic feed additives as having natural properties with multiple functions, reduced or no side effects, non-resistance, and leaving no residues in livestock products. These may have enhanced their wide acceptability as a new alternative to age-long synthetic antibiotics and growth promoters which poses economic burden and health risks to poultry (Ojediran et al., 2017). Black seeds, African nutmeg, and Negro pepper could be utilized in livestock diets and serve as phytoadditives to enhance the antioxidant capacity of animal products (Idowu et al., 2024).

Therefore, this study aims to examine the effect of supplementing brown egg-type commercial layers' diet with black seeds, African Nutmeg, and Negro pepper on proximate composition and lipid profile of stored table eggs.

2. Materials and Methods

2.1 Description of the experimental site

The experiment was carried out at the Poultry unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. Ogbomoso is located in the derived savanna zone that lies on longitude $4^{0}10$ East of Greenwich meridian and latitude $8^{0}10$ North of the Equator. The latitude ranges from 300m and 600m above sea level while the mean temperature and annual rainfall are 27 ºC and 1247 mm (Google Earth Map, 2022).

2.2 Preparation of test ingredients

The black seed, African nutmeg, and Negro pepper were purchased from reputable and reliable markets within the Ogbomoso metropolis. The shells of African nutmeg were removed, and the seeds, Black seed, and Negro Pepper were ground with Eurolex Mixer/Grinder model MG1153 (a domestic blender) into the powdery form of 0.05mm and stored in air-tight containers.

2.3 Experimental diets

Seven experimental diets were formulated for the study such that Diet 1 (T1) serves as the control that neither contained black seed, African nutmeg, and negro pepper. T2 - 0.5% BS, T3 – 1.0% BS, T4 – 0.5% AN, T5 –

1.0% AN, T6 – 0.5% NP and T7 – 1.0% NP. The gross composition of the experimental diets is presented in (Table 1).

Table 1. Gross composition of the experimental diets (kg^{-1}).

Ingredients	T1	T2	T3	T4	T5	T6	T7
Maize	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Soya bean meal	19.80	19.80	19.80	19.80	19.80	19.80	19.80
Wheat offal	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Fish meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Palm kernel cake	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
VMP*	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100
Black seed	-	+	++	-	-	-	-
African nutmeg	-	-	-	+	++	-	-
Negro pepper	-	-	-	-	-	+	++
Calculated Analysis							
Crude protein (%)	17.16	17.27	17.37	16.49	16.56	16.51	16.59
ME ($Kcal/kg^{-1}$)	2598.50	2622.31	2646.12	2615.24	2631.98	2615.59	2632.68
Crude fibre(%)	3.72	3.75	3.78	3.73	3.74	3.75	3.78
Ether extract (%)	3.42	3.61	3.80	3.45	3.48	3.48	3.54
Calcium (%)	4.05	4.05	4.05	4.05	4.05	4.05	4.05
Phosphorus (%)	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Lysine (%)	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Methionine (%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40

Note: *VMP contain vitamin and mineral, 2.5 kg of premix contains Vitamin A (14,000,000 I.U), Vitamin D3 (3,500,00 I.U), Vitamin E (20,000 I.U), Vitamin K (2,400 mg), Vitamin B1 (1,800 mg), Vitamin B2 (5,000 mg), Vitamin B6 (1,800 mg), Vitamin B12 (12 mg), Niacin (18,400 mg), Panth Acid (6,000 mg), Folic Acid (700 mg), Biotin (50 mg), Choline (240,000 mg), Manganese (96,000 mg), Zinc (60,000 mg), Iron (40,000 mg), Copper (8,000 mg), Iodine (1,400 mg), Selenium (240 mg), Cobalt (250 mg) and Antioxidant (125 mg). *ME – Metabolizable energy, + is 0.5 inclusion level, ++ is 1.0% inclusion level. Source: Authors, 2024.

2.4 Experimental animals and management

Ninety-six (168) *ISA Brown* strains of layers at eighteen (18) weeks old were used. The birds were weighed before the commencement of the experiment and distributed into four treatment groups of four (4) replicates having six (6) birds to make a total of twenty-four (24) birds per treatment. The birds were stocked at the rate of 3 birds per cell (measuring 42 cm x 36 cm x 38 cm). Feed and water were provided *ad libitum* throughout the experiment and medications were administered solely to the control group when necessary.

2.5 Data collection

On the 8th week of the experiment, three eggs per replicate were selected to make a total of 12 egg samples per

treatment. The eggs were weighed using a sensitive scale and stored for 6 weeks (42 days). Eggs were stored at room temperature where the relative humidity and temperature were monitored.

2.5.1 Percentage of egg weight loss

The egg samples were weighed every week to determine the percentage of egg weight loss using the following equation:

$$\text{Percentage egg weight loss} = \frac{(\text{Initial egg weight} - \text{weight of stored egg})}{\text{Initial egg weight}} * 100$$

2.5.2 Chemical composition of eggs

After 6weeks of storage, the egg samples were analyzed for proximate and lipid composition according to the official methods of analysis described by the Association of Official Analytical Chemists (AOAC, 2012).

2.6 Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS version 25, and significant means were separated using *Duncan's* multiple range test of the same software. The significance difference was determined at $p < 0.05$.

3. Results and Discussion

The proximate composition of black seed (*Nigella sativa*), African nutmeg (*Monodora myristica*), and Negro pepper (*Xylopia aethiopica*) presented in (Table 2), shows that Black seed and Negro pepper had higher crude protein (21.03 vs 17.43%), crude fibre (6.90 vs 5.88%), ether extract (37.91 vs 11.67%) and ash (7.42 vs 7.24%) than African nutmeg (13.54, 2.20, 6.77 and 4.59%) respectively.

Table 2. Proximate composition of Black seeds, African nutmeg, and Negro pepper.

Parameters	Black seeds	African nutmeg	Negro pepper
Dry matter (%)	95.37	91.88	93.70
Crude protein (%)	21.03	13.54	17.43
Crude fibre (%)	6.90	2.20	5.88
Ether extract (%)	37.91	6.77	11.67
Ash (%)	7.42	4.59	7.24
NFE (%)	25.70	64.69	51.49
ME (Kcal/kg^{-1})	2890.05	3145.05	3500.00

Note: Where: NFE- Nitrogen Free Extract, ME – Metabolizable Energy. Source: Idowu et al. (2024).

The relative humidity and temperature of the environment where the eggs were stored from weeks 1 to 6 are shown in (Table 3). The average relative humidity for the storage period is 71.99% while the average temperature is 27.54 ºC.

Table 3. The environmental condition of stored eggs.

Weeks of storage	Relative humidity (%)	Temperature (ºC)
1	74.00 ± 0.89	28.10 ± 0.13
2	72.01 ± 1.12	27.56 ± 0.11
3	70.72 ± 0.15	28.11 ± 0.56
4	72.57 ± 0.93	26.05 ± 0.01
5	72.01 ± 1.14	28.42 ± 0.53
6	70.62 ± 1.35	27.01 ± 0.32
Mean	71.99 ± 1.01	27.54 ± 0.41

Source: Authors, 2024.

The percentage of egg weight loss at 6 weeks of storage is shown in (Table 4). At the end of the storage period, the minimal percentage of egg weight loss (5.60 and 5.69%) was observed in the eggs of hens fed a diet supplemented with black seed while the highest percentage of egg weight loss (12.08%) was observed in the eggs of hen on the control diet.

Table 4. Percentage of egg weight loss at 6 weeks of storage.

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM	P-value
Initial weight (g)	52.97^{b}	60.33^{a}	56.95^{ab}	55.00^{ab}	60.09^{a}	57.68^{ab}	59.61^{a}	1.41	0.04
Stored egg weight (g)	46.51^{c}	56.95^{a}	53.71^{b}	51.76^{b}	56.42^{a}	54.34^{b}	56.21^{a}	0.90	0.04
Egg weight loss (g)	6.46^{a}	3.38^{c}	3.24^{c}	3.25^{c}	3.67^{b}	3.34^{c}	3.40^{c}	0.15	0.01
% Egg weight loss	12.01^{a}	5.60^{c}	5.69^{c}	5.91^{b}	6.11^{b}	5.79^{bc}	5.79^{bc}	1.02	0.03

Source: Authors, 2024.

The proximate composition of 6 weeks of stored eggs laid by hens fed diets supplemented with black seed, African nutmeg, and Negro pepper is shown in (Table 5). Eggs from birds given diets supplemented with 0.5% black seed had the highest dry matter (26.79%), crude protein (12.08%), crude fat (11.26%), ash (1.22%), and gross energy (1.47Kcal/g^{-1}) while eggs of birds on Treatment 4 (0.5% African nutmeg) had the highest percentage carbohydrate (3.22%). However, the dietary treatments did not have a significant ($p > 0.05$) effect on Nitrogen-free extract.

Table 5. Proximate composition of 6 weeks stored eggs laid by hens fed diet supplemented with three Phytoadditives.

Parameter	T1	T2	T3	T4	T5	T6	T7	SEM	P-value
Dry matter (%)	25.03^{c}	26.79^{a}	26.43^{ab}	25.74^{abc}	25.74^{abc}	25.37^{bc}	25.22^{c}	0.17	0.02
Crude protein (%)	10.78^{c}	12.08^{a}	12.00^{ab}	11.13^{bc}	11.37^{abc}	10.84^{c}	10.84^{c}	0.33	0.01
Crude fat (%)	10.28^{c}	11.26^{a}	11.00^{ab}	10.46^{bc}	10.65^{abc}	10.26^{c}	10.26^{c}	0.09	0.05
Ash (%)	1.23^{a}	1.22^{a}	1.24^{a}	0.91^{b}	1.00^{ab}	1.21^{a}	1.21^{a}	0.14	0.02
GE (kcal/g)	1.44^{b}	1.47^{a}	1.48^{a}	1.46^{a}	1.46^{a}	1.47^{a}	1.47^{a}	0.05	0.01
Carbohydrate (%)	2.23^{c}	$2{\cdot}99^{ab}$	2.18^{c}	3.22^{a}	2.84^{ab}	2.92^{ab}	2.92^{ab}	0.27	0.03
NFE	77.71	75.44	75.76	77.51	76.98	77.90	77.69	1.02	0.09

Note: ^{abc} Means within each row with different subscripts are significantly different ($p < 0.05$) SEM: Standard Error of Mean, GE: Gross Energy, NFE: Nitrogen Free Extract. Source: Authors, 2024.

The lipid profile of the 6 weeks stored eggs laid by hens fed diets supplemented with black seed, African nutmeg, and Negro pepper is shown in Table 6, Total cholesterol and low-density lipoprotein (LDL) were significantly ($p < 0.05$) reduced in eggs fed diet supplemented with black seed and also gave the best high-density lipoprotein. However, triglyceride was not significantly affected ($p > 0.05$) by the dietary treatments.

The proximate composition reveals the nutritive value of feedstuffs. The crude protein contents of black seed, African nutmeg and Negro pepper ranging between 17.43 – 21.03% were consistent with percentage reported by other authors (Dike, 2010; Bouba et al., 2012; Osabor et al., 2015; Borquaye et al., 2017 and Kanu; Onuegbu, 2020) but higher than the value (9.22%) reported by Oso & Oladiji (2019). The crude fibre and the lipid contents were relatively higher compared to those reported for other phytoadditives such as ginger and garlic (Okolo et al., 2012) but were within the range reported by Mamun & Absar, (2018). Differences in the proximate compositions may be due to differences in soil characteristics, growth conditions, climatic conditions at the locations where the ingredients were cultivated, and differences in analytical procedures.

The hens on T2 (0.5% black seed) produced eggs with the lowest percentage of weight loss at the end of 6 weeks of storage. As the duration of storage increased from 1 to 6 weeks, the weight of stored eggs decreased, and the percentage of egg weight loss increased.

The crude protein (10.78 – 12.08%) of 6 weeks of stored eggs from laying hens fed experimental diets supplemented with black seed, African nutmeg, and Negro pepper was similar to 11.29 – 12.68% CP reported by Akinwumi et al. (2022) who evaluated the quality of eggs from ISA brown as influenced by natural antioxidants and storage time. However, the 10.78 – 12.08% CP obtained in this study is higher than the 6.64 – 8.48% CP observed by Ogunwole (2018) who investigated the effect of supplementing laying hen diets with different proprietary vitamin-mineral premixes on eggs stored for 28 days. The percentage of crude fat (10.26 – 11.26%) obtained in this study is lower than (14.73 – 17.09%) reported by Akinwumi et al. (2022). Meanwhile, the percentage of ash compares well with the result (Ogunwole, 2018; Akinwumi et al., 2022). Proximate analysis of eggs reveals their nutritional content, variation in the proximate composition of 6 weeks of stored eggs in this study could be due to storage methods, length of storage, diets fed, and the strain of birds used.

Total cholesterol is made up of high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). The values of total cholesterol obtained range between (394.00 - 415.10 mg/100 g^{-1}) which is higher than 183-386 mg/100 g^{-1} reported by Leke et al., (2022) from birds fed a diet with papaya skin flour, the highest total cholesterol level (415.10) was recorded in eggs from birds fed control (T1) and the lowest value was obtained in eggs from birds fed 0.5% black seed (T2). Also, in contrary to 198-212 mg/100 g^{-1} reported by Omri et al. (2019) from birds fed standard diets. The high-density lipoprotein (HDL) is a good type of cholesterol which has been established to protect against heart disease by mopping up circulating cholesterol units in the system (Narahari, 2003).

The high-density lipoprotein (HDL) and triglycerides were not affected but the low-density lipoprotein and very low-density lipoprotein were observed to reduce in the dietary treatments compared to the control which might be due to the oxidative properties of the Phytoadditives. The observed trend is by the report of Akinwumi et al. (2022) who reported a decrease in LDL in the eggs of layers-fed diets supplemented with natural antioxidants and Renfan et al. (2020) who also reported that green tea decreases low-density lipoprotein (LDL). Orsoni et al. (2016) and Kontus, (2015) reported that favorable lipoproteins profile including high-density, low-density, and total cholesterol can reduce the risk of atherogenic dyslipidemia. Triglycerides are the most common types of fat that exist in food and the body (Ologhobo et al., 2008). The triglycerides values obtained in this study were similar across all treatments which ranged between 56.55 - 62.35 mg/100 g^{-1}. The result is contrary to Melo et al. (2016) who reported that triglyceride levels increased significantly ($p < 0.05$) with the increased inclusion of black pepper in the diet.

Table 6. Lipid profile of 6 weeks stored eggs laid by hens fed diet supplemented with three phyto-additives.

Parameter	T1	T2	T3	T4	T5	T6	T7	SEM	P-value
Total cholesterol	415.10^{a}	394.10^{b}	390.80^{b}	401.87^{ab}	401.87^{ab}	399.77^{c}	393.40^{c}	1.91	0.04
HDL (mg/100 g^{-1})	110.58	112.43	110.58	113.23	113.90	111.88	111.00	1.82	1.01
LDL (mg/100 g^{-1})	222.73^{a}	209.23^{b}	209.28^{b}	215.40^{ab}	215.90^{ab}	210.90^{b}	210.03^{b}	3.33	0.01
VLDL (mg/100 g^{-1})	13.63^{a}	11.83^{b}	11.63^{b}	13.40^{a}	13.60^{a}	12.68^{ab}	12.58^{ab}	0.59	0.01
Trig. (mg/100 g^{-1})	62.35	61.33	54.33	59.10	56.55	64.33	59.93	3.62	1.02

Note: ^{abc} Means within each row with different subscripts are significantly different ($p < 0.05$), SEM: Standard Error of Mean, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, Trig. -Triglyceride. Source: Authors, 2024.

4. Conclusions

From the results of this study, it can be concluded that the inclusion of the three phytoadditives (black seed, African nutmeg, and Negro pepper) enhanced the proximate composition, increased the good cholesterol (HDL) and reduced the bad cholesterol that is, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDP) of the egg. It is hereby recommended that supplementing the diets of brown egg-type chickens with (black seed, African nutmeg, and Negro pepper) at 0.5% could be used to improve the chemical composition of stored eggs.

5. Acknowledgments

Special thanks to all the Lecturers in the department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

6. Authors' Contributions

Idowu Adijat Oyeyemi: conceptualized the study and wrote the article. *Ojebiyi Olusegun Ojeniyi*: designed the study and proofread the writing. *Shittu Muritala Daniel*: Proofread the writing. *Ojediran Taiwo Kayode*: Proofread the writing. *Abolaji Oluwaseun Ezekiel*: experimented. *Salahudeen Yusirat Oladunni*: experimented.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable

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Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.