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Lipid Profile, Microbial Load and Specific Gravity of Stored Eggs Obtained from Hens fed Three Commercial Layers Feed

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Abstract: This research examined the lipid profile, microbial load and specific gravity of stored eggs obtained from hen that were fed different commercial layers feeds. A total of 108 ISA brown laying hens were used for the study. The hens were assigned to three treatments (FT1 – FT3) which consisted of four replicates each in a completely randomized design. These replicates had nine hens each. Each commercial layer's feed was fed to the hens in each treatment for 12 weeks. Twenty-one (21) eggs of similar weight were collected from each replicate within 72 hours at the end of the experiment. Three (3) of the freshly laid eggs from each replicate were analyzed on the day of collection while the remaining were stored for 42 days and analyzed weekly to study the cholesterol, triglyceride, outer and inner microbial load and the specific gravity. The cholesterol level and specific gravity decreased significantly ($P > 0.05$) in all the treatments as the storage period increased while the outer microbial load decreased ($P > 0.05$) up to day 28 and progressively increased thereafter. The inner microbial load gradually accumulated from day 14 in all the treatments and significantly increased ($P < 0.05$) as the duration of the of storage increased. It was also observed that the quality of the eggs obtained from FT1 and FT2 deteriorated after day 35 such that they could not be analyzed on day 42 while those from FT3 were still intact and were analyzed on day 42. It was concluded that all the three feeds were useful as layers feed since their consumption by the hens resulted in a good level of egg cholesterol up to day 28 of storage and uniform triglyceride level across the treatment groups. These may subsequently support high-density lipoprotein, monounsaturated and polyunsaturated fatty acids on consumption and give useful information on nutrient/food intake.
Keywords: Commercial feeds; Cholesterol; Microbial load; Hens, Specific gravity, Triglyceride.

INTRODUCTION

Commercial feeds play crucial role in poultry production in Nigeria because the poultry industry uses up to 90 % of the total quantity of commercial feeds produced. It helps to bridge the gap between the high demands (70 – 80 %) for feed in the industry. Any deviation in the nutrient content and quality of the feeds from the recommended level of nutrient will, therefore, affect the performance of the birds and the quality of the eggs produced [1]. Poultry feed is normally formulated from a combination of ingredients such as grains, cereal by-products, vitamins and mineral supplements, amino acids and feed additives. However, [2] noted that commercial poultry feeds were deficient in protein, calcium and phosphorus below the recommended level and even far less than the declared content indicated on the bag labels when broiler finisher, pullet grower and layers mash were subjected to proximate analysis.

Egg quality is usually influenced by the composition of the feed and it is important in determining the income of any poultry venture [3]. The quality of the egg affects its acceptability by the buyer [4]. Thus, [5] recommended that eggs meant for the retail market should be labeled as "keep refrigerated', transported in refrigerated vehicles and stored under refrigerated conditions or at room temperature that is about 7.2 ^{0}C (45 ^{0}F). This recommendation cannot be met by most Nigerians even in this 20th century due to the irregular supply of power in most parts of the country. As a result, eggs are mostly stored at room temperature. Deterioration of the internal quality of egg during storage period depends on the shell and the internal content [6] and on the environmental factors, for example, temperature [7]. Since most poultry farmers and egg consumers in Nigeria store eggs at ambient temperature both in urban and rural areas, several studies had been conducted to determine the effect of such storage on external and internal qualities

of the eggs. There is, however, lack of information on the effect of storage on the lipid composition (cholesterol and triglyceride), microbial load and specific gravity of eggs when stored at ambient temperature. This study therefore focused on the effect of storage on lipid profile, microbial load and specific gravity of the eggs when different commercial feeds were fed to the laying hens.

MATERIALS AND METHODS

The research was conducted at the University of Port Harcourt Teaching and Demonstration Farm, Choba, Port Harcourt, Rivers State.

The design of the study was the completely randomized design (CRD). One hundred and eight (108) ISA brown laying hens which were 34 weeks old were used for the 12 weeks' study. The birds were collected from the same farm and were randomly assigned to three treatments which were marked as FT1, FT2, and FT3 with four replicates each. Nine hens were randomly assigned to each of the replicate. Three types of commercial layers feed commonly sold in the area were bought from a sales outlet (within the week when they were supplied) and served in each of the treatment. The content of the commercial layers' feeds as shown in the labels attached to bags of feed are shown on Table 1. Water was provided *ad libitum* while all routine activities were observed.

On termination of the study, 21 eggs with similar weight were randomly collected within 72 hours from each replicate (84 eggs per treatment). Three (3) eggs from each replicate were analyzed on the day that they were collected while the remaining were analyzed weekly for the lipid profile, microbial load and specific gravity on day 7, 14, 21, 28, 35 and 42. The eggs from each replicate were weighed, hard boiled and allowed to cool. Thereafter they were carefully cracked and the albumen and yolk separated. The yolks were weighed and 1g extracted. Samples were homogenized as well as filtered and the procedure of analyzing cholesterol using the Randox test kit was followed, adding $CaCl_2$ to the filtrate to aid the extraction of aqueous phase of non-lipid materials. After the extraction, Randox reagent was added to the extracted samples. The extracted lipids were incubated in a laboratory oven at 37 ^{0}C for 5min and the absorbance of sample done using spectrophotometer. From the result obtained, the total cholesterol (HDL and LDL) and total triglycerides was calculated using spectrophotometer.

The eggshell surface was swabbed to determine the total microbial load. The swabs were placed in test tubes containing 10ml of lactose peptone water dilution, shacked up and 1ml inoculated into each petri-dish, then incubated at 37 ^{0}C for 48 hours and the result were taken thereafter. For analysis of the internal content of the egg, the eggs were washed with water and detergent, drained to remove excess water, dip in 70 % alcohol for 10min and then flamed (sterilized). After this, the eggs were cracked and the content poured into a sterile vial and homogenized for 1-2minutes. The sample was withdrawn (10ml) and discarded into a flask containing 90 mL of peptone water dilution to 10^{-1} from which 1 mL was inoculated into each petri-dish and incubated at 37 ^{0}C for 48 hours reading was be taken thereafter.

The specific gravity of the egg was determined at room temperature using Archimedes' method. The eggs were weighed in air on a mettle weighing balance. The weight of the water displaced by the eggs was determined by submerging the eggs in a beaker at room temperature. Specific gravity was then calculated as:

$$SG = \frac{\text{Egg weight in air}}{\text{Displaced water x temperature correction}}$$

All the data collected were subjected to statistical analysis of variance using the SAS software [8] while significant differences between the means were determined accordingly.

RESULTS

The influence of storage on the weekly lipid profile, microbial load and specific gravity of the stored eggs is presented in Table 2. The storage period significantly affected the cholesterol concentration, microbial load and specific gravity ($P < 0.05$) of the eggs while the triglyceride was not affected. The concentration of the lipid, outer microbial load and specific gravity were higher within the first 14 days of age of the eggs and declined as the eggs' age advanced. There was no microbial accumulation on the inner part of the eggs during the first 7 days across the treatment groups. There was, however, gradual buildup of microbes in the inner parts of the eggs after the 7th day which increased significantly ($P < 0.05$) as the age of the eggs increased. The specific gravity of the eggs was significantly higher ($P < 0.05$) during the first 7 days of storage and decreased as the duration of storage increased. The three commercial layers' feed however did not have any effect on the parameter studied as shown in Table 3. There were significant differences ($P < 0.05$) due to the storage period in the lipid, microbial load and specific gravity which followed similar trend as in the detail in Table 2.

Table-1: Declared nutrient values of three commercial layers feed on dry matter basis

Nutrients, %	FT1	FT2	FT3
Moisture	NA	NA	NA
Ash	NA	NA	NA
Crude protein	16.5	16.5	15.0
Fat/Oil	5.00	5.00	5.00
Crude fibre	6.00	7.00	6.50
ME	2500	2500	2400
Calcium	3.60	3.50	1.00
Phosphorus	0.45	0.45	0.40

ME - Metabolizable energy declared in Kcal/kg

Table-2: Effect of storage on lipid profile, microbial load and specific gravity of stored eggs

Treatments/Duration (Days)	Lipid Profile, mg/dL		Microbial Load		Specific gravity
	Cholesterol	Triglyceride	Outer	inner	
FT1					
Day 0	60.18^{a}	118.94	1242.50^{a}	0.00	0.19^{a}
7	51.33^{b}	123.52	1086.00^{a}	0.00	0.24^{a}
14	50.91^{b}	122.60	315.00^{b}	5.00^{c}	0.018^{b}
21	50.86^{b}	137.66	510.50	1.50^{c}	0.030^{b}
28	50.82^{b}	127.75	81.50^{c}	42.50^{b}	0.020^{b}
35	48.03^{c}	125.44	385.00^{b}	106.00^{a}	0.015^{b}
SEM	1.68	19.89	197.50	25.83	0.12
FT2					
Day 0	60.92^{a}	113.86	1264.81^{a}	0.00	0.18^{a}
7	50.87^{b}	118.62	1179.80^{a}	0.01	0.22^{a}
14	51.01^{b}	120.50	651.70^{b}	2.35^{c}	0.02^{b}
21	50.67^{b}	124.01	628.11^{b}	6.56^{c}	0.02^{b}
28	50.72^{b}	123.64	102.92^{c}	60.72^{b}	0.03^{b}
35	48.22^{c}	121.68	565.77^{b}	98.71^{a}	0.02^{b}
SEM	2.05	10.96	92.05	5.21	0.08
FT3					
Day 0	62.45^{a}	109.60	1257.12^{a}	0.00	0.20^{a}
7	54.18^{b}	114.27	1124.02^{a}	0.00	0.19^{a}
14	52.79^{b}	117.20	582.11^{b}	2.17^{c}	0.017^{b}
21	52.81^{b}	121.22	570.02^{b}	5.51^{c}	0.019^{b}
28	51.82^{b}	123.55	118.75^{c}	47.11^{b}	0.02^{b}
35	46.11^{c}	120.42	492.82^{b}	102.05^{a}	0.02^{b}
42	44.18^{c}	120.40	580.34^{b}	132.34^{a}	0.02^{b}
SEM	4.70	14.40	155.61	5.71	0.06

^{a,b,c} - Means within the same column with different superscripts differ significantly ($p <$ 0.05)

Table-3: Effect of commercial layers feeds and storage on lipid profile, microbial load and specific gravity of stored eggs

	Factors	Lipid Profile (mg/dL)		Microbial Load		Specific Gravity
		Cho.	Trigly.	Outer	Inner	
Commercial Feeds	T1	56.32	119.51	828.51	6.21	0.04
	T2	54.82	120.06	811.34	5.98	0.03
	T3	55.31	109.93	821.12	5.83	0.03
	SEM	1.25	11.21	18.19	0.57	0.04
Days	0	61.18^{a}	114.13	1254.81^{a}	0.00	0.19^{a}
	7	52.13^{b}	118.80	1129.94^{a}	0.00	0.22^{a}
	14	51.57^{b}	120.10	516.27^{b}	3.17^{c}	0.025^{b}
	21	51.43^{b}	127.63	569.54^{b}	4.53^{c}	0.023^{b}
	28	51.12^{b}	124.98	101.06^{c}	50.11^{b}	0.02^{b}
	35	47.45^{c}	122.51	482.20^{b}	102.25^{a}	0.02^{b}
	42	44.18^{c}	120.40	580.34^{b}	132.34^{a}	0.02^{b}
	SEM	4.42	13.81	382.15	5.42	0.05

^{a,b,c} - Means within the same column with different superscripts differ significantly ($P < 0.05$) Cho. – Cholesterol, Trigly - Triglyceride

DISCUSSION

The cholesterol level of the egg yolk which significantly decreased across the treatment as the duration of storage of the egg increased could be due to the changes within the egg which usually occur as the length of storage of the egg is prolonged. Similar decrease in the cholesterol level of eggs (in the control and three other treatments whose feeds consisted of various types of additives) during a four weeks' storage was reported by [9] who found 21, 14, 18 and 16 % decrease in cholesterol concentration in the 4th week of egg storage compared to the level before the eggs were stored. However, [10] found that the cholesterol content of the chicken egg yolk increased from 52.83 mg/g yolk in the first week to 95.00 mg/g yolk in the 5th week. The value of cholesterol obtained in this study, as well as those reported by [10] were higher compared to the 12mg/g reported by [11] in Brazilian chicken egg yolk. According to [12] a value of 243.8 mg/dL was obtained for egg yolk cholesterol obtained from ISA brown laying hens while [13] reported values of 547.28, 691.81 and 796.25 mg/dL for egg cholesterol of Shika brown hens, *Corturnix ypsilophora* and *Gallus domesticus* respectively. The variations in the values obtained by researchers had been attributed to the analytical method used to determine the yolk cholesterol [14,15]. It also depended on the breed, species, the line and the age of the hen whose egg was used and the yolk weight [16,17]. The similar trend in the level of significance found in the yolk cholesterol content of the eggs obtained from the different treatments confirmed that the cholesterol content of eggs is usually the same unless the feed content is altered [18]. It implied that the three commercial feeds had similar nutrient content as reported by [19] who found similar crude protein, high fibre content and similar fat level for these same feeds when their proximate values were analyzed. It also confirms the non-significant effect of the commercial feeds on the parameters (Table 3). The fear of cholesterol consumption had already been clarified by Harvard Researchers who studied 40,000 men and 80,000 women who consumed an egg a day for 14 – 18 years and found that the consumption of an egg a day was not associated with higher risk of coronary heart disease, CHD, [20,21]. This had been confirmed by [22] who reported only a weak relationship between the amount of cholesterol a person consumed and his or her blood cholesterol level. Also, [21,23] also reported that dietary cholesterol had only a small effect on blood cholesterol level and the harmful low-density lipoprotein, LDL than does the mix of fats in diet. Thus [24], concluded that there should be no generalized recommendation regarding the restriction of egg consumption since healthy individuals experience no risk of developing coronary heart attack when eggs consumption was increased, rather they had some beneficial effects. According to [25-27], eating two (2) eggs a day increased the high-density lipoprotein, HDL (good cholesterol) levels by 10%. According to [28,29], higher levels of HDL led to lower risk of heart disease, stroke and health problems. Eating eggs had also been shown to change small and dense LDL cholesterol particles to large LDL particles which is good for health [30] since those who have predominantly large LDL particle sizes have been found to have lower risk of heart disease [31-33]. Several countries had since recognized that dietary cholesterol did not affect blood cholesterol [34]. A report of the style of living in relation to heart disease stated that the evidence obtained was not enough to prove that reduction in the consumption of dietary cholesterol led to the reduction of LDL which is the bad cholesterol [35]. Thus, [36] has removed the upper limit of dietary cholesterol consumption level (300 mg/day) from the new dietary guideline.

The triglyceride levels of the eggs obtained from all the treatments which were not significantly different ($P > 0.05$) suggest that the eggs may have uniform monounsaturated fatty acids (MUFAS) and polyunsaturated fatty acids (PUFAS) which can help to lower the risk of heart disease in those who consume the eggs. It also implied that the commercial feeds were well utilized considering their adequate level of fat/oil inclusion of 4.5 – 6.5 %.

The pattern of microbial load on the shell (outer surface) which was significantly ($P < 0.05$) higher on day 0 - 7, followed by day 14 – 21 and least on day 28 showed that the microbes accumulated on the eggs due to contact with the cages (which were old, about 10 years) and possibly the initial collection materials. This confirmed the report by [37] who stated that contact of eggs with contaminated surfaces (nesting material), dust, storage containers, handlers and the rearing environment can affect the microbial load of the eggs. It also supported the report by [38] who stated that the initial microbial count was low on eggshell but significantly higher on the eggs that were laid in old boxes than those laid in new boxes. According to [39] freshly laid eggs have immature cuticle which is not able to resist bacterial penetration on the shell. The gradual reduction and the lowest level recorded on day 28 suggested that the accumulation gradually decreased when the eggs were removed from the poultry house and stored at room temperature, away from the initial source of microbial contact. The eventual increase on day 35 confirmed the report by [40] who attributed such to the poor functioning of the eggshell under humid condition. It also supported the report by [41] who stated that there is usually an increase of psychrophilic bacteria, staphylococci, coliform moulds and yeast on the shell surface of the egg and in the inner content of the egg during storage

The inner microbial load which showed no invasion of the micro-organisms within the first 7 days and even very low levels up to day 21 of storage despite the outer invasion across the treatment groups indicated

that the cuticles on maturation exhibited high resistance to the penetration of micro-organisms. This confirmed the earlier finding by [39] who stated that the chemical composition of the mature cuticle determines the risk of trans-shell contamination by salmonella and the cuticle that is rich in protein exhibit decreased shell permeability, thus, usually have greater resistance against the penetration of salmonella. The gradual accumulation of micro-organisms which was highest significantly ($P < 0.05$) on day 35 of storage across the treatments however, supported the deteriorative changes noticed after day 35, such that the eggs could not be analyzed beyond day 35 in FT1 and FT2.

The specific gravity of the eggs which was significantly ($P < 0.05$) higher within the first 7 days across all the treatments supported the finding of [42] who stated that higher specific gravity indicated that the eggs were of good quality and will have the ability to resist forces that cause cracks. The specific gravity of an egg according to [43] usually projects the quantity of the eggshell in comparison to the other components of the egg. The eggs that were 7 – 35 days old which had significantly lower ($P < 0.05$) specific gravity across the treatment groups showed why the inner microbial load had an increasing trend from day 14. This result was in line with the report of [43] who stated that specific gravity of eggs decreased by about 0.001 units each day the egg was stored. This supported the report by [44] who found that microbial penetration was common in eggs that had lower specific gravity. This is true since specific gravity of eggs is used to assess the shell thickness of the egg which relates to the porosity of the eggs [45]. Thus, when specific gravity of an egg decreases, more cracks will be expected when the eggs are handled or transported [43,46].

CONCLUSION

The higher levels of cholesterol up to day 28 in all the treatments proved that all the three commercial layers' feeds were good for egg production. It showed that eating egg up to when they are 28 days old could support high density lipoproteins, HDL, which is the good cholesterol, (already found by other researchers that it led to reduction in the risk of heart disease, stroke and other health problems). The uniformity in the level of triglyceride in this study points to the presence of monounsaturated fatty acids (MUFAS) and polyunsaturated fatty acids (PUFAS) which are usually involved in lowering the risk of heart disease. Thus, eating eggs within the first 28 days during the dry season in the humid tropics can provide lipids which are beneficial to the individual. Although the microbial load on the outer shell depicted the effect of the use of old battery cages, the inner content showed that the eggs were very healthy up to day 28. While the farmers are assured of continuous sales of good quality eggs till day 28 when the low and high temperature is within 21 – 22 ^{0}C and 26.5 – 33 ^{0}C, old cages should be avoided in the collection of the egg to minimize the outer microbial load.

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