

## Quality Parameters, Lipids and Antioxidant Profiles of Eggs from Hens Fed Diets with Varied Inclusions of Monosodium Glutamate

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 20/10/2020 Accepted : 24/02/2021</p> <p><b>Keywords:</b> Eggs Lipids Antioxidative Status Monosodium glutamate Laying birds</p>	<p>The study assessed the internal and external egg qualities as well as lipid and antioxidative status of eggs laid by hens fed different inclusion levels of monosodium glutamate was. Three hundred 24-week old Isa Brown pullets were randomly allotted to six experimental diets containing 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25 g/kg MSG respectively in an experiment that lasted sixteen weeks. At the end of the feeding trials, egg samples were collected from each experimental group for laboratory analyses. Egg widths, shape index, and shell weight were significantly reduced among the hens fed diets containing above 0.75 g MSG/kg. Egg yolk length and index from the pullets on diets containing 0.25 and 0.50 g MSG/kg were not significantly influenced when compared with the control but above these levels, the parameters were significantly influenced. The total cholesterol levels of the whole egg across all the treatment groups showed statistical similarities. A significant increase was, however, noted in the malondialdehyde content of the eggs among the hens fed diets containing 0.75 g MSG/kg diet and above while a significant decrease in the superoxide dismutase were noted at the same inclusion levels when compared with the control hens. There was no significant difference in the total antioxidant capacity of the eggs from hens fed diets containing the varying inclusion levels of MSG when compared with those on the control diet.</p>

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### Introduction

The egg is adjudged a complete protein food because it has both all the essential and non-essential amino acids. The qualities of other food proteins are assessed by using egg proteins as a measuring standard (Nimalaratne, 2015). An essential economic yardstick in poultry is egg quality. Poor egg quality will often lead to downgrading while good quality leads to profitable egg production. Egg qualities are broadly classified as internal and external (Adu et al, 2017). Egg grading, pricing, consumer acceptability, and ultimately hatchability is affected by the external qualities (King'ori, 2012).

Apart from the external and internal egg qualities, the biochemical components of eggs also play significant roles in their overall quality and shelf life. The lipid profile of an egg goes a long way in determining its health implication on the consumers. Cholesterol and its esters are also found in chicken eggs. Egg cholesterol is equally influenced by factors such as genetics, diets, age of birds, laying frequency, and veterinary handling (Vorlová et al., 2001). Antioxidant properties are reportedly exhibited by various compounds in both the egg yolk and albumen. Ovotransferrin, ovalbumin, phosvitin, phospholipids,

vitamin E, vitamin A, selenium, and carotenoids contents of the eggs have antioxidant properties (Nimalaratne, 2015). Ovalbumin which constitutes almost 54% of egg white proteins controls the reduction-oxidation reaction thereby inducing antioxidant properties (Alleoni, 2006) and enhanced antioxidant status in the presence of saccharides (Huang et al., 2012). While the metal chelating ability of ovotransferrin enhances its superoxide removing property (Ibrahim et al, 2007), ovomucin hinders oxidative stress in the kidney of humans (Chang et al., 2013). Lysozyme scavenges reactive oxygen species (ROS) and thereby silences oxidative stress genes (Liu et al., 2006). The metal-binding affinity of phosvitin is presumably high which qualifies it as an antioxidant agent against oxidative damage (Samaraweera et al., 2011).

The layers' diet predominantly influences the lipid profiles, antioxidant properties, internal and external egg qualities. In recent times, the use of phyto-genic additives and non-conventional protein sources of plant origin in poultry feeding has gained research attention. Despite the potentials of these feedstuffs, their use in poultry feeding has suffered a serious setback due to reduced palatability

these conferred on the feed (Windisch et al., 2008), and subsequent lack of acceptability. The addition of any flavour enhancing agent to boost the palatability of feed containing these phyto-genic additives will be a welcome development. Monosodium glutamate (MSG) as a flavour enhancer is regarded as an additive that can enhance the palatability of food (Khalil and Khedr, 2016). There have been several opposing views about the safety of MSG as a flavour enhancer in foods. Though it was the excessive dosage of MSG administration that has been implicated in conferring a negative effect on the brain (Eweka and Om'Iniabohs, 2006), there is still a need for assessing the possible effects of this flavour enhancing additive on the quality of the products from the birds fed diets fortified with MSG and to also ensure no deleterious effects would be conferred on the consumers of such products. The study conducted to assess the effects of varied inclusion rates of MSG on the lipid contents, antioxidant profile, external and internal egg qualities of the MSG-treated hens as this would help the farmers and nutritionists to make informed decisions over the use of MSG as flavour enhancing additive to boost the palatability of feeds produced with phyto-genic and non-conventional protein sources.

## Materials and Methods

The study was undertaken with approval from the Research and Ethics Committee for care and use of animals for research of the Animal Production and Health Department, The Federal University of Technology Akure, Nigeria. Three (300) point-of-lay (POL) Isa Brown pullet of sixteen (Oluyemi and Roberts, 2000) weeks old were purchased from a reliable farm for the study. They were placed on a commercial grower mash until they have reached 20% laying performance (24 weeks of age) before being placed on the treatment rations (Table 1) for sixteen weeks. Throughout the study, the hens underwent the same management practices except for variation in dietary MSG inclusion rates. The already weighed 300 pullets were randomly allotted to the experimental treatments containing 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25 g/kg MSG. Each group contains 50 birds replicated 5 times with 10 birds/replicate. Throughout the experimental period, the birds were fed twice daily (morning and afternoon) and water was also given unrestricted. Recommended vaccination and other medication were administered as at and when due.

### Egg Qualities Assessment

Eggs were collected per treatment on weekly basis for both internal and external qualities assessment. Egg, yolk, and shell weights were assessed using a laboratory scale. The albumen weight was the difference between the weights of the yolk, shell, and whole egg. A micro meter screw gauge was used in determining the shell thickness. Egg weights and linear measurements from each of the treatments were recorded to the nearest 0.01g and 0.01cm, respectively. At about 50% laying performance, eggs collected were analysed weekly. For egg qualities determination, 30 eggs were randomly selected per treatment every week. Internal and external qualities such as egg weight, length, width, index, surface area; yolk weight, height, diameter, ratio, index; albumen weight,

length, height, diameter, index, ratio; shell weight, thickness, ratio and Haugh Unit (HU) were determined (Olumide et al., 2016). Egg surface area (ESA) was determined as reported by (Adu and Olarotimi, 2020) using the formula:

$$ESA/SSA=W^{0.667}\times 4.67$$

W=Average Egg Weight, 0.667 and 4.67 are constants.  
SSA; Shell surface area

Yolk index was calculated using the relationship between yolk height and width:

$$\text{Yolk index} = \frac{\text{Yolk height}}{\text{Yolk width}}$$

Haugh Unit (HU) was estimated as;

$$HU = 100 \log (H+7.57-1.7W^{0.37})$$

H: albumen height, W: egg weight (Oluyemi and Roberts, 2000).

### Egg Mass

The use of egg mass rather than egg numbers is to ensure better comparisons of flocks. It is estimated as;

$$AEM=\% \text{HDP}\times AEM$$

HDP; Hen day production, AEM; Average egg weight in grams (Fikru et al., 2015)

### Egg Specific Gravity

Egg Specific Gravity (ESG) was determined using the formula reported by (Adu and Olarotimi, 2020)

$$ESG = \frac{1.9754EW}{(1.9140EW - ESW)}$$

EW; egg weight, ESW: egg shell weight; 1.9754 and 1.9140 are constants. Shell weight per unit egg surface area

$$(SWUSA)=(3.9782EW)^{0.666}$$

Other egg qualities parameters were estimated using Paganelli's equations as reported by (Hegab and Hanafy, 2019). SA; Surface area of egg

- Area vs. egg volume (V, cm<sup>3</sup>):

$$SA=4.951 EV^{0.666}$$

- Egg density (ED, g cm<sup>-3</sup>) vs. egg weight:

$$ED=1.038 EW^{0.006}$$

- Shell density (ShDgcm<sup>-3</sup>) vs. egg weight:

$$SD=1.945 EW^{0.014}$$

- Shell Volume (SV, cm<sup>3</sup>) vs. egg weight:

$$SV = 2.48 \times 10^{-2} W^{1.118}$$

Table 1: Ingredient composition of the experimental layer diets

Ingredients (kg)	Inclusion Level of MSG (kg/MT)					
	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)
Maize	435	435	435	435	435	435
Soybean meal	141	141	141	141	141	141
Groundnut cake	100	100	100	100	100	100
Wheat Offal	100	100	100	100	100	100
Rice Bran	50	50	50	50	50	50
Corn Bran	50	50	50	50	50	50
Moringa leaf meal	10	10	10	10	10	10
Bone Meal	23	23	23	23	23	23
Limestone	79	78.75	78.50	78.25	78	77.75
Salt	2.50	2.50	2.50	2.50	2.50	2.50
MSG	0.00	0.25	0.50	0.75	1.00	1.25
Layer Premix*	2.50	2.50	2.50	2.50	2.50	2.50
Lysine	4.00	4.00	4.00	4.00	4.00	4.00
Methionine	3.00	3.00	3.00	3.00	3.00	3.00
Total	1000.00	1000.25	1000.50	1000.75	1001.00	1001.25
<b>Analyzed Nutrients</b>						
ME (Kcal/Kg)	2619.52	2619.52	2619.52	2619.52	2619.52	2619.52
Crude Protein (%)	18.04	18.04	18.04	18.04	18.04	18.04
Calcium (%)	3.69	3.68	3.67	3.66	3.65	3.64
Phosphorus (%)	0.51	0.51	0.51	0.51	0.51	0.51
Lysine (%)	1.19	1.19	1.19	1.19	1.19	1.19
Methionine (%)	0.55	0.55	0.55	0.55	0.55	0.55
Crude Fibre (%)	4.32	4.32	4.32	4.32	4.32	4.32
Fat (%)	4.41	4.41	4.41	4.41	4.41	4.41

\*Composition of premix (Nutrivitas®): 2.5 kg of premix contains: Vit. A (10,000,000 iu), Vit. D3 (2,500,000 iu), Vit. E (12,000 iu), Vit. B1 (2000 mg), Niacin (25000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2500 mg), Biotin (75 mg), Folic Acid (2000 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (50%) (200000 mg), Manganese (80000 mg), Iron (40000 mg), Copper (10,000 mg), Zinc (60000 mg), Selenium (200 mg), Iodine (1500 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g), Cobalt (250 mg). ME=metabolizable energy, NFE=nitrogen free extract

### **Egg Lipids and Antioxidant Profiles Determination**

The method employed was as described in earlier research (Elkin et al., 1999). The egg samples were boiled left to cool and their respective weights were recorded. The eggs were weighed again after the shells were removed. The albumen was then separated from the yolk with caution and they were weighed separately. The yolk was pulverized and 1 g was mixed with 15 ml of chloroform-methanol (2:1 v/v) and filtered. The filtrates were used to determine the total cholesterol (TC) high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations respectively as highlighted by (Azeke and Ekpo, 2009). The low-density lipoprotein cholesterol (LDL-C) was calculated as  $LDL-C = (TC - TG - HDL-C) / 5$ .

### **Malondialdehyde (MDA)**

0.1ml of absolute ethanol and reagent (R<sub>4</sub>) was added to the test tubes labeled "blank and standard" respectively while 0.1ml yolk sample was pipetted into the sample and control tubes each. 0.1ml of reagent 1 (R<sub>1</sub>) was added into each of the four tubes followed by 3.0 ml of reagent 2 (R<sub>2</sub>). Then, 1.0ml of reagent 3 (R<sub>3</sub>) was added into each of the tubes labeled "blank, standard and sample" while 0.1ml 50% glacial acetic acid was added into the tube labeled control". The mixtures were thoroughly shaken and incubated in 95°C water for 40 min, and then the tubes were cooled with running water after incubation. They were centrifuged at 3100 rpm for 10 minutes. The supernatant was collected and the absorbance of the sample and standard was read against the blank at 532nm (Alexandra et al, 2019).

The absorbance is determined as:

$$MDA(nmol/mL) = \frac{Abs. S - Abs. C \times COS(nmol/mL)}{Abs. Standard - Abs. Blank}$$

Abs.S; Abs.Sample, Abs.C;Abs.Control, COS; concentration of standard

### **Superoxide Dismutase**

Sample and reagent (R1) were pipetted into a cuvette and mixed well. Reagent (R2) was then added, mixed, and initial absorbance A1 was read after 30 seconds. A timer was started simultaneously. Final absorbance A2 was read after 3 minutes.

$$SOD(nmol/mL) = \frac{A2 - A1}{3}$$

SOD; Superoxide Dismutase

### **Total Antioxidant Concentration**

20µl double deionized water, standard, and sample were pipetted into different cuvettes labeled as reagent blank, standard, and sample respectively. 1ml of chromogen (R2) was pipetted into each of the cuvettes. The mixtures were mixed well and incubated at 37°C to bring to temperature and the initial absorbance (A<sub>1</sub>) was read at 600nm. After this, 200µl of the substrate (R<sub>3</sub>) was added to each of the cuvettes. These were mixed and the timer started immediately. Absorbance (A<sub>2</sub>) was read 3 minutes after.  $A2 - A1 = \Delta A$  of sample/standard/blank

Total Antioxidant Conc. was calculated as:

$\frac{\text{conc of standard}}{\text{Factor}} = (\Delta A \text{ blank} - \Delta A \text{ standard})$

Factor =  $(\Delta A \text{ blank} - \Delta A \text{ standard})$

mmol/l = Factor x  $(\Delta A \text{ Blank} - \Delta A \text{ Sample})$

### Data Analysis

Data collected were subjected to One-Way Analysis of Variance of the GraphPad Prism, software version 6.01 (GraphPad Prism User's Guide. Version 6.01 for Windows 2012). Significant differences among the treatment means were separated using Tukey's Honestly Significant Difference ( $\alpha 0.05$ ) option of the same software.

## Result and Discussion

### External Egg Qualities

There is little or no information regarding the effects of MSG on the qualities of chicken eggs, and there has been insufficient study regarding the effects of dietary MSG in hens. Egg quality is crucial in the consumer acceptability of the product. Therefore, to maintain egg quality, genuine attention must be paid to the issues of its preservation and marketing (Tabeeh, 2011). Results of this experiment indicated that external egg quality parameters (Table 2) such as shell thickness, egg weight, egg length, egg volume, shell index, shell volume, egg surface area, and shell weight per unit of surface area were not significantly ( $p > 0.05$ ) influenced by MSG inclusion at any inclusion level when compared with the control diet though significant difference ( $p < 0.05$ ) occurred within treatment groups. On the other hand, egg specific gravity, egg density, shell ratio, and shell density were not significantly ( $p > 0.05$ ) influenced at all by the inclusion of MSG at any rate. Egg widths, shape index, and shell weight were significantly ( $p < 0.05$ ) reduced above 0.75 g MSG/kg diet when compared with the control diet.

Dietary protein goes a long way in influencing eggs. It is required to synthesize egg albumen, and a decrease in dietary protein may reduce the amount of albumen, leading to a smaller egg size (Bezerra et al., 2015). The reduction in egg weight and volume among the hens on diets containing 1.00 and 1.25 g MSG/kg in this experiment could be attributed to the effect of MSG in lowering the dietary protein of these diets. Since the diets were iso-nitrogenous, it could be that MSG at an inclusion rate of 1.00 g/kg diet was capable of interfering with dietary protein availability. More researches to ascertain this claim will be necessary since there is very scanty information in the literature on the effect of monosodium glutamate supplementation in diets of commercial layers. The insignificant difference recorded in egg specific density, shell density and shell ratio among the hens across all treatments agreed with previous the finding (Bezerra et al., 2015). Eggs with thick and strong shells usually have a better appeal to consumers (Oke, 2014) and this is a characteristic mark of economic sell point. The statistically similar eggshell thickness among the pullets indicated that MSG did not adversely affect calcium absorption and utilization in the hens. Egg weight, specific density, and SWUSA as well as shell density, thickness, volume, index, and ratio which did not differ significantly among the MSG-treated hens when compared with those on the control diet agreed with another author (Dong, 2010).

### Internal Egg Qualities

While external egg quality deals with the physical appeal of an egg to the consumer, the internal egg quality relates to the functional characteristics of it. From the results of the internal egg qualities (Table 3), the egg yolk length and ratio from the pullets on diets containing 0.25 and 0.50 g MSG/kg were not influenced significantly ( $p > 0.05$ ) when compared with the control but above this level, these parameters were significantly ( $p < 0.05$ ) influenced. The yolk width and weight were statistically similar ( $p > 0.05$ ) across the treatment groups and the control. Yolk index and height, however, were significantly ( $p < 0.05$ ) influenced in birds on diets above 0.50 g MSG/kg.

The yolk index is a useful indicator that defines the spherical characteristic of the yolk and this reflects the freshness of the egg (Torricco et al., 2014). Therefore, the significant reduction in the yolk index observed on diets containing 0.75 g MSG/kg and above is indicative of a progressive vitelline membrane weakness resulting from water absorption by the yolk from the albumen (Hidalgo et al, 2008). The significant reduction in egg weights observed in diets fed 1.00 and 1.25 g/kg MSG could not be said to be responsible for lower yolk weights since there was no significant difference between yolk weights and this was in concordance with another finding (Mohiti-Asli et al., 2010). Albumen and Haugh unit are major determinants of internal egg quality. The higher albumen height and Haugh unit of the eggs from the treatment diets indicated that increasing the MSG level in the present study did not affect the superiority of the egg qualities adversely. Furthermore, higher albumen heights of the eggs from the treatment diets compared to the control further validated that MSG did not influence albumen quality negatively at any of the inclusion levels. The Haugh units observed in the eggs from the hens fed MSG diets being higher than those on the control diet suggests that the nutrients in the treatment diets are available and not affected by MSG inclusion. However, there is a dearth of information on the influence of MSG on these internal egg parameters. The outcome of this study agreed with (Dong, 2010) in that there was no significant impact of MSG treatment on yolk weight but disagreed with their claim that yolk index and Haugh unit were statistically similar among hens fed glutamate treated diets as a significant decrease in yolk index was observed among hens on diets containing 0.75 g/kg MSG and above while a dose-dependent improvement in Haugh units was observed.

### Egg Biochemical Profiles

Nutrition, as well as management, plays important role in the cholesterol contents of eggs (Oke et al., 2014). Cholesterol is useful in the body as a precursor for hormones and vitamin D synthesis (Adeniyi et al, 2016). Egg cholesterol content is an interesting quality factor for consumers. Research efforts have been geared towards the production of eggs with lower cholesterol content. Manipulation of protein and energy contents of the layers' diets through the use of hypocholesterolemic feed additives is currently gaining more attention (Idowu et al., 2002). The effects of MSG on blood serum lipid metabolism have been well documented unlike in the whole egg and egg yolk where the effects on lipid profiles have not gained research attention. The results of the lipid profiles of the eggs are as shown in Table 4.

Table 2. External egg qualities of layers fed diets with different levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	p
<b>Egg</b>							
EWt (g)	55.60±0.60 <sup>ab</sup>	55.90±0.41 <sup>ab</sup>	54.20±0.42 <sup>b</sup>	56.50±0.57 <sup>a</sup>	54.00±0.75 <sup>b</sup>	53.90±0.24 <sup>b</sup>	0.0024*
EL (cm)	5.38±0.03 <sup>ab</sup>	5.41±0.02 <sup>a</sup>	5.31±0.02 <sup>b</sup>	5.42±0.02 <sup>a</sup>	5.42±0.02 <sup>a</sup>	5.40±0.01 <sup>a</sup>	0.0010*
EW (cm)	4.30±0.02 <sup>a</sup>	4.30±0.08 <sup>ab</sup>	4.29±0.01 <sup>ac</sup>	4.31±0.01 <sup>a</sup>	4.24±0.02 <sup>c</sup>	4.24±0.01 <sup>bc</sup>	0.0006*
SI	79.90±0.07 <sup>ab</sup>	79.40±0.19 <sup>bc</sup>	80.70±0.39 <sup>a</sup>	79.50±0.17 <sup>b</sup>	78.10±0.25 <sup>d</sup>	78.50±0.07 <sup>cd</sup>	<0.0001*
ESA (cm <sup>2</sup> )	69.10±0.50 <sup>ab</sup>	69.30±0.34 <sup>ab</sup>	68.00±0.35 <sup>b</sup>	69.80±0.47 <sup>a</sup>	67.80±0.63 <sup>b</sup>	67.70±0.20 <sup>b</sup>	0.0024*
EM(g/hen/day)	38.40±2.04 <sup>b</sup>	40.50±0.67 <sup>ab</sup>	39.00±0.33 <sup>ab</sup>	43.20±1.39 <sup>a</sup>	37.30±0.87 <sup>b</sup>	36.50±0.52 <sup>b</sup>	0.0016*
ESG	1.10±0.01	1.10±0.01	1.10±0.00	1.10±0.01	1.09±0.00	1.10±0.01	0.0667 <sup>ns</sup>
ED (gcm <sup>-3</sup> )	1.06±0.00	1.06±0.00	1.06±0.00	1.06±0.00	1.06±0.00	1.06±0.00	0.0625 <sup>ns</sup>
EV (cm <sup>3</sup> )	52.40±0.56 <sup>ab</sup>	52.60±0.38 <sup>ab</sup>	51.10±0.39 <sup>b</sup>	53.20±0.53 <sup>a</sup>	50.90±0.70 <sup>b</sup>	50.80±0.22 <sup>b</sup>	0.0024*
<b>Shell</b>							
ShWt (g)	6.24±0.11 <sup>ab</sup>	6.42±0.01 <sup>a</sup>	6.06±0.00 <sup>bc</sup>	6.28±0.05 <sup>ab</sup>	5.92±0.07 <sup>c</sup>	5.96±0.05 <sup>c</sup>	<0.0001*
ST (mm)	0.42±0.01 <sup>ab</sup>	0.41±0.01 <sup>ab</sup>	0.39±0.00 <sup>b</sup>	0.43±0.01 <sup>a</sup>	0.40±0.01 <sup>ab</sup>	0.40±0.01 <sup>ab</sup>	0.0067*
ShR (%)	11.20±0.20	11.50±0.11	11.20±0.08	11.10±0.09	11.00±0.03	11.10±0.14	0.0637 <sup>ns</sup>
ShI	9.03±0.15 <sup>ab</sup>	9.26±0.07 <sup>a</sup>	8.92±0.04 <sup>ab</sup>	8.99±0.06 <sup>ab</sup>	8.73±0.02 <sup>b</sup>	8.81±0.10 <sup>b</sup>	0.0011*
ShD (gcm <sup>-3</sup> )	2.06±0.00	2.06±0.00	2.06±0.00	2.06±0.00	2.06±0.00	2.06±0.00	0.0525 <sup>ns</sup>
SWUSA (gcm <sup>-2</sup> )	57.80±0.42 <sup>ab</sup>	58.00±0.28 <sup>ab</sup>	56.90±0.29 <sup>b</sup>	58.40±0.39 <sup>a</sup>	56.70±0.53 <sup>b</sup>	56.60±0.17 <sup>b</sup>	0.0024*
ShV (cm <sup>3</sup> )	2.22±0.03 <sup>ab</sup>	2.23±0.02 <sup>ab</sup>	2.16±0.02 <sup>b</sup>	2.26±0.03 <sup>a</sup>	2.14±0.03 <sup>b</sup>	2.14±0.01 <sup>b</sup>	0.0022*

Values are means ± SEM; Means in a row without common superscripts are significantly (P<0.05) different. Level of significance = ns (not significant) = P>0.05; \* = P< 0.05; Egg Weight (EWt), Egg Length (EL), Egg Mass (EM), Egg Width (EW), Shape Index (SI), Shell Index (ShI), Egg Surface Area (ESA), Egg Mass (EM), Egg Specific Gravity (ESG), Egg Density (ED), Egg Volume (EV), shell weight (ShWt), Shell Volume (ShV), Shell Thickness (ST), Shell Ratio (ShR), Shell Density (ShD), Shell Weight per unit of Surface Area (SWUSA), MSG levels in g/kg diet.

Table 3. Internal Egg qualities of layers fed diets with different levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	p
<b>Yolk</b>							
Length (cm)	4.20±0.01 <sup>a</sup>	4.19±0.01 <sup>a</sup>	4.17±0.01 <sup>ab</sup>	4.12±0.02 <sup>b</sup>	4.17±0.02 <sup>ab</sup>	4.12±0.01 <sup>b</sup>	<0.0001*
Width (cm)	3.85±0.02	3.83±0.01	3.84±0.01	3.83±0.01	3.85±0.02	3.83±0.00	0.7852 <sup>ns</sup>
Height (cm)	1.77±0.03 <sup>a</sup>	1.76±0.01 <sup>a</sup>	1.74±0.01 <sup>ab</sup>	1.67±0.01 <sup>bc</sup>	1.65±0.01 <sup>c</sup>	1.67±0.00 <sup>c</sup>	<0.0001*
Weight (g)	13.70±0.16	13.60±0.03	13.60±0.11	13.50±0.05	13.50±0.09	13.30±0.03	0.2651 <sup>ns</sup>
E: Y (%)	24.60±0.02 <sup>ab</sup>	24.30±0.16 <sup>ab</sup>	24.90±0.08 <sup>ab</sup>	23.90±0.15 <sup>b</sup>	25.20±0.15 <sup>a</sup>	24.80±0.07 <sup>ab</sup>	<0.0001*
Index	45.90±0.11 <sup>a</sup>	45.80±0.07 <sup>ab</sup>	45.79±0.18 <sup>ab</sup>	43.60±0.22 <sup>c</sup>	42.90±0.23 <sup>c</sup>	43.50±0.09 <sup>c</sup>	<0.0001*
<b>Albumen</b>							
Length (mm)	58.80±0.23 <sup>c</sup>	59.30±0.27 <sup>bc</sup>	59.30±0.05 <sup>bc</sup>	60.00±0.22 <sup>ab</sup>	60.00±0.42 <sup>ab</sup>	60.60±0.33 <sup>a</sup>	0.0005*
Height(mm)	7.33±0.04 <sup>c</sup>	7.50±0.05 <sup>b</sup>	7.48±0.04 <sup>b</sup>	7.58±0.03 <sup>ab</sup>	7.60±0.02 <sup>ab</sup>	7.69±0.02 <sup>a</sup>	<0.0001*
Weight (g)	33.80±0.24 <sup>ab</sup>	34.00±0.31 <sup>ab</sup>	33.20±0.31 <sup>ab</sup>	34.60±0.47 <sup>a</sup>	32.80±0.58 <sup>b</sup>	32.70±0.19 <sup>b</sup>	0.0034*
Width (mm)	12.80±0.08 <sup>ab</sup>	12.60±0.06 <sup>ab</sup>	12.30±0.07 <sup>ab</sup>	12.40±0.07 <sup>ab</sup>	12.20±0.13 <sup>b</sup>	13.40±0.64 <sup>a</sup>	0.0311*
E: A (%)	60.80±0.30	60.90±0.30	61.20±0.10	61.30±0.21	60.70±0.28	60.60±0.15	0.2534 <sup>ns</sup>
Index	20.50±0.07 <sup>b</sup>	20.90±0.07 <sup>a</sup>	20.90±0.07 <sup>a</sup>	21.00±0.04 <sup>a</sup>	21.10±0.09 <sup>a</sup>	20.80±0.16 <sup>ab</sup>	0.0014*
Y: A	0.41±0.02 <sup>ab</sup>	0.40±0.03 <sup>bc</sup>	0.41±0.00 <sup>ab</sup>	0.39±0.04 <sup>c</sup>	0.414±0.04 <sup>a</sup>	0.41±0.02 <sup>ab</sup>	<0.0001*
Haugh Unit	87.00±0.22 <sup>c</sup>	87.90±0.20 <sup>b</sup>	88.2±0.24 <sup>b</sup>	88.20±0.13 <sup>b</sup>	89.00±0.12 <sup>a</sup>	89.50±0.08 <sup>a</sup>	<0.0001*

Values are means ± SEM; Means in a row without common superscripts are significantly (P<0.05) different. Level of significance=ns (not significant) =P>0.05; \* =P< 0.05, E: Y=Egg: Yolk Ratio, E: A=Egg: Albumen Ratio, Y: A=Yolk: Albumen Ratio, MSG levels in g/kg diet.

Table 4. Egg biochemical profiles of the layers fed with different levels of MSG

Parameters	A (0.00)	B (0.25)	C (0.50)	D (0.75)	E (1.00)	F (1.25)	P-Value
<b>Whole Egg mg/egg</b>							
TC (mg/dl)	192.51±1.22	193.85±0.50	196.15±0.42	198.54±1.66	200.85±2.95	205.99±1.72	0.2182 <sup>ns</sup>
HDL-C (mg/dl)	80.06±0.16 <sup>d</sup>	81.42±0.18 <sup>c</sup>	83.28±0.10 <sup>b</sup>	85.91±0.03 <sup>ab</sup>	86.60±0.20 <sup>ab</sup>	91.08±0.18 <sup>a</sup>	<0.0001*
TG (mg/dl)	435.14±0.06 <sup>a</sup>	434.38±0.06 <sup>ab</sup>	432.12±0.04 <sup>ab</sup>	425.34±0.08 <sup>b</sup>	409.50±0.03 <sup>c</sup>	402.71±0.01 <sup>d</sup>	<0.0001*
VLDL-C (mg/dl)	87.03±0.01 <sup>a</sup>	86.87±0.06 <sup>ab</sup>	86.42±0.02 <sup>ab</sup>	85.07±0.01 <sup>ab</sup>	81.90±0.03 <sup>b</sup>	80.54±0.01 <sup>bc</sup>	<0.001*
LDL-C (mg/dl)	25.42±0.15 <sup>c</sup>	25.55±0.09 <sup>c</sup>	26.45±0.11 <sup>bc</sup>	27.56±0.23 <sup>b</sup>	32.35±0.27 <sup>ab</sup>	34.37±0.26 <sup>a</sup>	0.0108*
<b>Egg Yolk mg/g of yolk</b>							
TC (mg/dl)	12.93±0.06 <sup>c</sup>	13.46±0.12 <sup>bc</sup>	13.96±0.09 <sup>bc</sup>	16.71±0.04 <sup>b</sup>	17.11±0.08 <sup>ab</sup>	18.82±0.08 <sup>a</sup>	<0.0001*
HDL-C (mg/dl)	5.82±0.02 <sup>c</sup>	6.09±0.03 <sup>bc</sup>	6.12±0.00 <sup>bc</sup>	7.87±0.00 <sup>b</sup>	8.07±0.01 <sup>ab</sup>	8.99±0.01 <sup>a</sup>	<0.0001*
TG (mg/dl)	23.77±0.01 <sup>a</sup>	22.92±0.01 <sup>ab</sup>	21.51±0.08 <sup>b</sup>	19.77±0.08 <sup>c</sup>	19.31±0.04 <sup>c</sup>	16.19±0.01 <sup>d</sup>	0.0003*
VLDL-C (mg/dl)	4.75±0.03 <sup>a</sup>	4.58±0.02 <sup>ab</sup>	4.30±0.03 <sup>ab</sup>	3.95±0.03 <sup>b</sup>	3.86±0.01 <sup>b</sup>	3.24±0.01 <sup>c</sup>	0.0012*
LDL-C (mg/dl)	2.36±0.01 <sup>c</sup>	2.79±0.01 <sup>c</sup>	3.54±0.01 <sup>bc</sup>	4.89±0.01 <sup>b</sup>	5.19±0.01 <sup>ab</sup>	6.59±0.01 <sup>a</sup>	0.0005*
Atherogenic index	0.40±0.00 <sup>c</sup>	0.46±0.00 <sup>c</sup>	0.58±0.01 <sup>bc</sup>	0.62±0.01 <sup>b</sup>	0.64±0.01 <sup>b</sup>	0.73±0.04 <sup>a</sup>	<0.0001*

Values are means ± SEM, means in a row without common superscripts are significantly (P<0.05) different. Level of significance = ns (not significant) = P>0.05; \* = P<0.05, Total Cholesterol (TC); High Density Lipoprotein Cholesterol (HDL-C); Triglyceride (TG); Very Low-Density Lipoprotein Cholesterol (VLDL-C); Low Density Lipoprotein Cholesterol (LDL-C); Atherogenic index = LDL: HDL ratio

The present study recorded no significant ( $p>0.05$ ) influence in the total cholesterol content of the whole egg irrespective of the increase in MSG inclusion levels though a corresponding non-significant ( $p>0.05$ ) increase resulted. On the other hand, a significant ( $p<0.05$ ) increase, dose-dependently, was observed in the yolk total cholesterol content. The significant ( $p<0.05$ ) elevation in egg yolk cholesterol content observed with increasing MSG inclusion level may be explained by the observation of a previous researcher (Faitarone et al., 2013). Since the pullets feed intake increased (108, 109, 111, 115, 120, and 123 g/day for Groups A, B, C, D, E, and F, respectively) as MSG inclusion rate increased, an increase in energy intake above maintenance and production requirements necessitated excessive cholesterol synthesis. Therefore, the excessive energy was converted to cholesterol, and the subsequent excessive cholesterol was transferred to the egg yolk. The highest whole egg total cholesterol recorded is, however, within the reference range of 190-213 mg/egg (Naviglio et al., 2014). This showed that the inclusion levels of MSG did not adversely affect the egg quality of the treated hens but there is a tendency that prolonged feeding above 0.50 g MSG/kg diet may be hypercholesterolemic and could negatively affect egg quality. Furthermore, there was a significant ( $p<0.05$ ) increase in LDL cholesterol as well as concentrations of HDL cholesterol. It has been reported that HDL cholesterol reduces the chance of coronary heart disease (Weggemans et al., 2001). The increase in both whole egg and egg yolk TC, HDL-C, and LDL-C agreed with the other reports (El Malik and Sabahelkhier 2019; Alwaleedi, 2016) on the potential of MSG in increasing cholesterol concentrations. For the atherogenic index, eggs from pullets on diets containing MSG inclusion levels 0.00 to 0.50 g/kg diet were preferable for consumers because of their low cholesterol, LDL/HDL, and lower atherogenic index which supported the findings of (Attia et al., 2015). It should therefore be noted that eggs with low atherogenic and hypocholesterolemic indices are good for retarding atherosclerosis and thus the risk of cardiovascular disorders (EL-Wakf et al., 2010).

For the antioxidant status of the eggs from the pullets on diets with varied MSG inclusion levels, a significant increase was noted in the malondialdehyde (MDA) content (Figure 1). MDA is a lipid peroxidation biomarker. However, a decrease in the total antioxidant capacity (T-AOC) (Figure 2) and superoxide dismutase (SOD) (Figure 3) were noted. This showed that increasing the level of MSG in the diets of the pullets favoured MDA increase and lower T-AOC and SOD activity in eggs. However, the reduced antioxidants status of egg yolk by MSG supplementation is attributable to the oxidative stress capacity of MSG, especially, at high inclusion levels. Thus, feeding pullets with diets containing MSG above 0.50 g/kg could lead to deterioration of eggs keeping quality during storage (King et al., 2012).

### Conclusion

The present study established that the inclusion of dietary MSG up to 0.50 g/kg diet did not negatively influence the quality of the eggs produced by the hens fed the MSG-fortified diets within the tolerable limit.

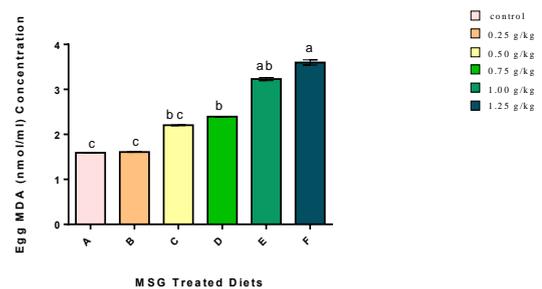
However, above this tolerable inclusion level, the egg qualities were compromised and such eggs were not safe for human consumption due to increased atherogenic index, cholesterol, and increased level of malondialdehyde content of the eggs.

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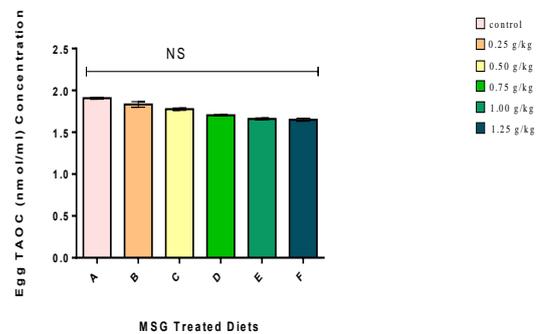
### Conflict of Interest

There is no known conflict of interest in any form in this study.



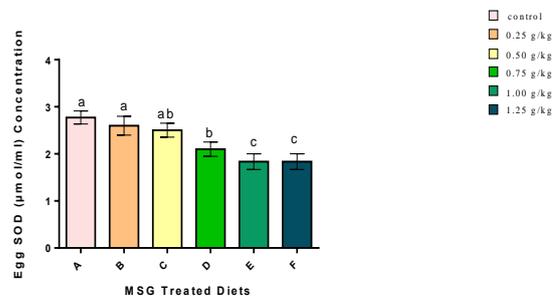
MDA: malondialdehyde

Figure 1: Effects of MSG on egg MDA concentration



TAOC: Total Antioxidant Capacity

Figure 2: Effects of MSG on egg TAOC concentration



SOD: Superoxide Dismutase

Figure 3: Effects of MSG on egg SOD concentration

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