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Original Article

Performance of Isa-Brown Layers and the Atomic Absorption Spectrophotometer of the Mineral Content of Eggs Produced by Moringa oleifera Leaf Powder Supplementation

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ABSTRACT

Moringa oleifera leaves richly contain numerous nutrients that can be used to induce performance in animals and when supplemented in layer feeds can improve the feed intake, body weight, egg qualities and mineral content of the eggs. The study was aimed to determine the performance of layers, egg quality and mineral profile of the eggs produced by inclusion of varying percentages of *M. oleifera* leaf powder in the feed of layers. Two hundred and forty Isa-brown layer birds were offered 0%, 2.5%, 5.0% and 7.5% *M. oleifera* leaf powder supplemented feeds respectively, using a completely randomized design. The mineral analysis was done following the procedure of the Association of Official Analytical Chemists using atomic absorption spectrophotometer. Data collected were analysed using analysis of variance at 0.05 level of significance. The results indicated that the body weight, feed intake, FCR, egg weight, egg length and shell thickness were significantly higher in layers fed *M. oleifera* leaf powder. However, the laying percentage was significantly higher in the control. The phosphorous, sodium, zinc, manganese, iron, copper, selenium and chromium contents of the eggs increased as the percentage of moringa inclusion increased. The eggs produced by layers fed with 5% *M. oleifera* leaf powder had significantly highest contents of magnesium, potassium and calcium, but decreased with further supplementation of moringa. The findings indicated that the supplementation of *M. oleifera* leaf powder at various levels improves the mineral contents of eggs but this is significantly achieved at a higher inclusion rate.

INTRODUCTION

Improvement in performance of layers through feeding has been an issue of serious concern among farmers. This is the reason proper attention is given to adequate nutrition because without it, layer performance will decline. Zaghari *et al.* (2011) noted that intake of amino acid greatly influenced the production performance of hens. Layers need a completely balanced feed to sustain laying as inadequate nutrition can make hens stop egg production (Jacob *et al.*, 2017). The quality of feeds offered to layers also determines the production performance. Consequently, different feed qualities were found to significantly affect the Hen Day Production (HDP), number of eggs laid, FCR of laying hens and egg quality parameters at varying ranges (Akinola & Ekine, 2018). Layer diets are commonly supplemented with certain additives to improve the nutritional qualities of the feed, hen performance and egg quality (Bryden *et al.*, 2021). Hence, the feed intake, live weight and egg production are affected by the quality of feed. Performance of layers in terms of feed intake, weight gain, FCR, egg production and egg qualities were significantly improved with adequate feeding (Tamiru *et al.*, 2020). Conversely, weight gain, FCR,

yolk colour and shell thickness were negatively affected by the introduction of sesame hull which reduced the quality of the layer feed (Ferran *et al.*, 2000).

Supplements like amino acids, vitamins and minerals, prebiotics, probiotics and *Moringa oleifera* are included in layer feeds to add value to the eggs (Bryden *et al.*, 2021). Certain supplements increase the mineral content of eggs which invariably helps to improve the mineral content of humans when consumed. Minerals are the inorganic nutrients required for the normal functioning of the body. They are contained in food and can be supplied to animals and humans when the food is consumed and digested. Micronutrients play significant roles in the immune function, oxidation processes and energy metabolism (Overton & Yasui, 2014). Mineral nutrients also help in male reproduction, as an imbalance in the amounts of nutrients may result in deformed spermatogenesis, structural or functional sperm disorder and reduced libido (Tvrdá *et al.*, 2013). People that have attention deficit hyperactivity disorder are known to have deficiencies of zinc, ferritin, and magnesium (Villagomez & Ramtekkar, 2014). Minerals play some structural, physiological, catalytic and regulatory functions in the body and as such, deficiency of any mineral can impair or inhibit metabolic pathways required for normal body functions (Radwińska & Żarczyńska, 2014).

Elevated mineral deficiency in children under the age of five was rampart in developing countries (United Nations Children's Fund [UNICEF], 2011). Nations in developing countries launched food and nutrition policies to reduce micronutrient deficiency by 50 percent before 2011, and also reduce acute malnutrition in children under the age of five by 30% before 2010 (Lambo, 2005). The Copenhagen Agreement in May 2004 prioritized the provision of micronutrients, control of HIV/AIDS, malaria, and provision of quality water and sanitation to the developing nations (UNICEF, 2011). Minerals and other nutrients are richly contained in *M. oleifera* leaves and can, therefore, be supplemented in feed to influence the performance of layers and mineral contents of eggs for the humans.

M. oleifera is a sub-tropical vegetable tree with a high nutritional profile that could be used for feed supplementation, particularly in some poor communities (Mishra *et al.*, 2012). The plant is abundantly found in Nigeria but generally underutilized (Animashaun & Toye, 2013). *M. oleifera* leaves when dried contain crude protein (32.58%), metabolizable energy (295.98 Kcal), calcium (20.003 mg), copper (0.57 mg), iron (28.2 mg), magnesium (368 mg) and phosphorous (204 mg) (Chaudhary & Chaurasia, 2017). The contents of calcium, sodium, magnesium, potassium, and manganese are significantly present in *M. oleifera* leaves (Melesse, 2012). Similarly, moringa leaves are known to contain a good amount of vitamin C, calcium, β-carotene, potassium and protein (Razis *et al.*, 2014). The widespread combination of diuretic along with lipid and blood pressure reducing activities of moringa leaves made it helpful in managing cardiovascular disorder (Okorocha *et al.*, 2015). The flowers, leaves, and roots of *M. oleifera* are good for the treatment of ascites and rheumatism as well as cardiac and circulatory stimulants (Olagbemide & Alikwe, 2014). *M. oleifera* is a good chemopreventive agent in inhibiting several major mechanisms in the cancer process, especially in some less discovered mechanisms (Abd-Karim *et al.*, 2016).

The extract from *M. oleifera* leaf was discovered to increase nutrient intake in lactation Nubian goats (Kholif *et al.*, 2018). The average weight and specific growth rate in fish were found to be the highest at 8.2 g *M. oleifera* supplementation compared to the control (Ayoola *et al.*, 2013; Falowo *et al.*, 2018). Moringa leaves present a high content of digestible nutrients for growing rabbits and it is an alternative resource for feeding rabbits in the tropical region (Caro *et al.*, 2018). However, a study of the weekly spawning events for over nine weeks showed that egg production was highest in zebrafish fed with a control diet and reduced in those fed with control feed supplemented with *M. oleifera* leaf powder, but no egg production was recorded in those fish which consumed only *M. oleifera* leaf powder (Paul *et al.*, 2013). A decrease in egg mass, percentage of egg production and weight of egg were reported at a higher inclusion rate of *M. oleifera* leaf powder (Alebachew *et al.*, 2016). There was no identified study on the mineral contents of Isa Brown eggs fed with *M. oleifera* leaf powder fortified feed. The present study is therefore aimed at determining the performance and mineral contents of eggs produced by the inclusion of varying amount of *M. oleifera* leaf powder in the diet of Isa brown layers.

MATERIALS AND METHODS

Experimental Procedure

The experiment was executed in compliance with the regulatory guidelines of the University of Nigeria Animal Care Ethics Committee (UNN-ACEC). The study adopted a Completely Randomized Design

(CRD) where 240 Isa brown layers were selected by simple random sampling and placed into 12 groups. The twelve groups were also randomly allotted to four different treatment groups of T1, T2, T3 and T4 with each replicating thrice. Layers in T1 were fed with the control feed while T2, T3, and T4 were fed with diets supplemented with 2.5%, 5.0% and 7.5% *M. oleifera* leaf powder supplement respectively (Table 1).

Management of Experimental Birds

The management was carried out based on the procedure described in Thiele and Pottgüter (2008). The 12 pens representing the 12 experimental units were cleaned, washed and disinfected with Vinkokill disinfectant. Litter materials in the form of wood shavings were placed on the floor at 5 - 10 cm thickness. Clean and cool drinking water was provided twice daily while feeds were given every morning. Regular inclusion of multivitamin supplement in the form of Super Mibrovite and Vitalyte in clean drinking water was adopted. Vitalyte and mibrovite are the combination of vitamins, electrolytes and amino acids used in poultry diets to help in the administration of growth and performance. They are used to reduce stress and combat dehydration. Vitalyte was administered at 30 g to 40 litres of water for 7 days while mibrovite was administered at 150 g to 400 litres of water for 8 days during the period of illness.

The layer birds were kept in deep litter system. The dimensions of the experimental units were the same, having the length of 276 cm and width of 271 cm. Each unit had two plastic feeders with the capacity of 2 kg each and two plastic drinkers of 15 l capacity each. One wooden laying box of 95.2 cm length, 35 cm width and 25 cm deep was installed in the dark corner of each of the units where egg collection was done twice daily. There were some windows on the side walls of the poultry house, chimneys fixed on the ceiling and electrical fan serving the purpose of negative pressure. Each unit was provided with a white 25 w electric bulb for lighting. The lighting time of 17 h of light and 7 h of darkness throughout the experimental laying period was maintained.

Vaccination was not administered during the experimental laying periods. Treatment of diseases was done using NCO Mix. NCO mix contains florfenicol 150 mg, neomycin 180 mg and colistin 1,200000 IU. It is effective for the prevention and treatment of infections from E-coli, salmonella, clostridium and non-specific enteritis. NCO mix was administered at 199 g to 300 litres of water for 5 days. Deworming was achieved using levadex while ectoparasite control was done using Rambo insecticide powder.

Harvesting and Processing of *M. oleifera* Leaf into Powder

Harvesting and processing were done according to the procedure described in Mishra *et al.* (2012). The fresh ends of moringa plants were cut and the leaves removed from the rachises. The leaves were then washed with clean water and air-dried inside rooms for 3-5 days. The dried leaves were ground using a grinding machine and the powder kept in a black polythene bag where there was no direct sunlight prior to the use. The powder was subjected to laboratory analysis to determine the nutritional composition (Table 3) using the procedure in the Association of Official Analytical Chemists [AOAC] (2005).

Feed Formulation

The feeds were formulated using feed formulation software called FeedWin, developed by the PTC+ (Barneveld, The Netherlands). T1 feed (Control) was produced with conventional feed ingredients such as maize, rice bran, soybean meal, wheat bran, bone meal, groundnut cake, methionine, lysine, limestone, and salt. The T2 feed was produced with feed ingredients used in T1 but was supplemented with 2.5% *M. oleifera* leaf powder. Similarly, T3 feed was produced with feed ingredients used in T1 but was supplemented with 5.0% *M. oleifera* leaf powder, while T4 feed was produced with feed ingredients used in T1 but was supplemented with 7.5% *M. oleifera* leaf powder (Table 1). Dosing, grinding and mixing were done in Chidera Feed Mill located in Nsukka, Enugu State, Nigeria. The nutritional composition of the experimental feeds is presented in Table 1.

Sampling of Eggs

Ten eggs were randomly sampled from each of the replicates (30 eggs/treatment) daily to determine the egg weight while four eggs were also randomly sampled from each of the replicates (12 eggs/treatment) fortnightly to determine the other external egg qualities. At the end of the experiment, four eggs were randomly sampled (12 eggs/treatment) from each of the replicates to determine the mineral contents of the eggs. The sampled eggs were labelled and taken to the Central Research and Diagnostic Laboratory, Ilorin, Nigeria where they were boiled. The boiled eggs were cracked and the content (albumen and yolk) homogenized for mineral content determination.

Table 1 – Composition of the Experimental Diet (%).

Ingredients	Control	2.5% *Moringa oleifera*	5% *Moringa oleifera*	7.5% *Moringa oleifera*
Maize	53.70	60.00	60.00	60.20
Groundnut Cake	10.00	2.00	5.00	2.00
Wheat Bran	3.00	2.87	2.08	3.70
Bone Meal	4.60	-	-	-
Soybean Meal	10.00	18.80	14.20	15.00
Rice Bran	9.00	10.00	9.80	7.73
Moringa Leaf Powder	-	2.50	5.00	7.50
Salt	1.00	1.50	1.50	1.50
Limestone	7.54	-	-	-
DL-Methionine 99%	0.02	0.09	0.08	0.08
L-Lysine HCl 78.5%	0.02	0.05	0.15	0.10
Soybean Oil	1.06	2.10	2.10	2.10
Toxin Binder	0.06	0.09	0.09	0.09
Total	100	100	100	100
Laboratory Analysis of the Nutritional Composition of the Experimental Diets (%)				
Metabolizable Energy (Kcal/kg)	2681	2810	2793.86	2744.19
Crude Protein	16.44	16.08	16.05	15.98
Crude Fibre	4.12	5.61	5.70	5.44
Ether Extract	5.19	5.69	5.96	5.78
Digestible Methionine	0.28	0.37	0.34	0.35
Digestible Lysine	0.70	0.80	0.80	0.80
Digestible Methionine + Cysteine	0.60	0.63	0.58	0.60
Calcium	3.68	6.10	12.09	18.08
Phosphorous	0.80	1.52	2.51	3.50

Determination of the External Qualities of the Eggs

Egg weight was measured using a digital scale (Model: SF-400). The weights of the eggs measured in each replicate were pooled and the average taken. Egg length was measured as the distance between the two ends using Vernier callipers. Egg width was taken as the diameter of the egg at the broadest cross-sectional region using Vernier callipers. Thickness of the egg shell was measured using a micrometre screw gauge after emptying the egg content and air drying the shell for 24 h.

Determination of Minerals Using Atomic Absorption Spectrophotometer

Preparation of the Sample

0.2 g of homogenized eggs was weighed in a crucible and ignited in a muffle furnace for 6-8 hours at a temperature. After cooling, 5 ml $NHNO_3$ solution was included and evaporated to dryness on a steam bath. After drying, it was heated at 400^0C for 10-15 min in a furnace until a perfect greyish white was obtained. 10 ml 1NHCl was added after cooling and filtered into 50 ml volumetric flask. The crucible and filter paper were washed with 10 ml portion 0.1NHCl solution. The filtrate was used for mineral determination.

Determination of Calcium by EDTA Titration Method

Stock solution was used. 25 ml ash solution of eggs was diluted with 50 ml of water and 2 ml buffer solution was dissolved (16.9 g NHCl in 143 ml NH4OH), 1.25 EDTA was added and diluted to 250 ml with water. Then, 250 NACN (pH 10.0) was added and this was followed by 200 mg indicator erichrome black T (0.5 erichrome black T and 100 g NaCl). One litre of water was used to dilute 0.01M EDTA by titration. End point was seen when the solution turned reddish.

Calculation:

$$Ca = \frac{A \times Tca \times V1 \times 100}{W \times V2}$$

A = ml EDTA

Tca = Titration factor of EDTA

V1 = Total volume

V2 = Aliquot for determination

W = weight of sample

Determination of Magnesium

This was done following the recommendation of AOAC (2010). 10 ml of the ash solution of egg was pipetted into a 250 ml beaker. 25 ml pH 10 buffer was added before 25 ml distilled water. 0.1 g EBT indicator was added and the solution swirled to get

a wine-coloured solution which was titrated against 0.01N EDTA to a clear blue end point. Magnesium was calculated by: Titre value × 24 × 0.01 × 10 × 100

Determination of Potassium

Potassium was determined using the procedure of AOAC (2010). The flame photometer was set up according to the prescription of the manufacturer. Several range of standards to cover the expected range of the samples were produced. Such standards include 2, 4, 6, 8, 10 ml/l. The highest standard was aspirated into the equipment and the sensitivity control adjusted to get the reading of 100. Furthermore, deionised water was aspirated into the equipment and the zero-control adjusted to the reading on zero. The other standards were aspirated in ascending order and the readings were taken. The readings were used to plot a potassium calibration. 10 ml of the ash solution of eggs was aspirated into the equipment and the flame emission taken. Potassium concentration was then extrapolated from the standard calibration curve.

Determination of Manganese

Manganese was determined using the procedure of AOAC (2010). 2 g of the ash solution of eggs was placed in a 250 ml beaker and 10 ml nitric acid solution added. Then, 100 ml 1.25% potassium periodate solution was added. The entire mixture was heated for about 10 min. The solution was placed on ice to cool to room temperature, after which the cuvette was filled with the content of the beaker and absorbance taken at 530 nm.

Determination of Selenium

This was done according to the method of Li *et al.* (2006). 10 ml of the ash solution of eggs was pipetted in a 30 ml test tube followed by the addition of 1 ml 2% potassium iodide solution. Then, 1 ml 1M HCl was added and the mixture gently shaken until a yellowish colour appeared. Then, 0.5 ml of 0.025 Safranine O solution and 2 ml acetate buffer solution of pH 4 were added. The absorbance of the resultant solution was taken at 532 nm against a blank.

Calculation:

$$Se(\mu/100g) = \frac{|sample| \times conc.\ Standard}{|of|\ standar \times weight\ of\ sample}$$

Determination of Zinc

The dithizone method in AOAC (2010) was adopted. 10 ml ash solution of eggs was pipetted into a 30 ml test tube. 5 ml acetate buffer was added, followed by 1 ml 2% sodium thiosulphate solution. Then, 5 ml 0.05% dithizone solution was added. The mixture was left to react for about 5 min before 3 ml carbon tetrachloride solution was added. The mixture was separated into two layers. The upper layer was taken for spec reading at 540 nm.

Calculation:

$$Zn(mg/100g) = \frac{|sample| \times conc.\ Standard}{|of|\ standard \times weight\ of\ sample}$$

Determination of Iron

The ortho-phenanthroline procedure described in AOAC (2010) was used. 10 ml of the ash solution of eggs was placed in a 30 ml test tube. 1 ml hydroxylamine hydrochloride was added. The mixture was allowed for 5 min before the addition of 5 ml acetate buffer and 1 ml ortho-phenanthroline. The pink mixture was read in a spectrophotometer at 510 nm.

Calculation:

$$Fe(mg/100g) = \frac{|sample| \times conc.\ Standard}{|of|\ standard \times weight\ of\ sample}$$

Determination of Phosphorous

In a 10 ml ash solution of egg pipetted into a 30 ml test tube, 2 ml vanado-molybdate solution was added and stood for 10 min before it was read off in a Jenway 6305 spectrophotometer at 400 nm wavelength against a reagent blank. Phosphorous concentration was extrapolated from a phosphorous standard curve prepared from different concentrations of phosphorous.

Determination of Copper

The optimized resorcinol method of copper determination described in Shabir (2011) was adopted in this experiment. 0.6 ml of 0.2M ammonia was added to 10 ml ash solution of eggs. Then, 0.2 ml of 0.1% resorcinol reagent was added, well mixed and allowed standing at room temperature. The colour was monitored at 450 nm (UV-Visible Spectrophotometer SL – 150) following one hour standing at room temperature. The reaction mixture was incubated in a boiling water bath for 3 min and the reading of the sample was done within 5 min following the cooling to room temperature.

Statistical Analysis

The data collected were analysed using one-way analysis of variance (ANOVA) and later subjected to the Duncan's multiple range test to separate the differences between the mean. Level of significance

was set at 0.05. The statistical analysis was done using SPSS version 20.0 software (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Table 2 – The Nutrient Composition of *M. oleifera* Leaf Powder used in Feed Formulation.

Nutrients	Composition
Metabolizable energy	299.33 Kcal
Crude protein	30.21%
Ether extract	5.77%
Crude fibre	10.67%
Ash	12.00%
Phosphorous	40.53mg/100g
Calcium	240mg/100g

The nutrient composition of the experimental *M. oleifera* leaf powder used in the feed supplementation shows a metabolizable energy content of 299.33 Kcal, crude protein content of 30.21%, ether extract content of 5.77%, crude fibre content of 10.67%, ash content of 12.00%, carbohydrate content of 31.64%, phosphorous content of 40.53 mg/100g, and calcium content of 240 mg/100g (Table 2). The ash and carbohydrate constituents of the *M. oleifera* leaf powder used in the feed formulation were higher than that of Aja *et al.* (2013) who obtained the ash (10.0%) and carbohydrate (23.6%) contents, but lower contents of crude fibre (35.0%) and ether extract (20%). Ogbe & Affiku (2011) found a lower content of crude protein (17.0%), crude fibre (7.09%), ash (7.93%), and phosphorous (30.15) and a higher content of carbohydrate (63.11%), and a metabolizable energy (440.11 Kcal). However, Witt (2013) reported a higher content of energy (304 Kcal), carbohydrate (36%), phosphorous (297 mg/100g), ether extract (6%), and a lower content of protein (24%) and crude fibre (9%) in dried *M. oleifera* leaves. The differences in the nutrient contents of *M. oleifera* leaves may be due to the variabilities in the physical and chemical characteristics of soils. The nutrient constituents of plants are affected by the plant nutrients present in the soil which are dependent on the soil physical, chemical and biological characteristics (Abdul Khalil *et al.*, 2015). Variabilities in the temperature of the soil influence the amount of moisture in the soil, available air, and plant nutrients which are essential for the growth of plants (Onwuka & Mang, 2018).

The body weight of the layers showed a significant difference (p<0.05=0.04) among the treatment groups. The layers in the control group had the least body weight compared to the groups that fed *M. oleifera* leaf powder. The layers that received 2.5% *M. oleifera* leaf powder had the highest body weight and decreased as the moringa supplementation increased. Significant difference (p<0.05=0.05) was also found to exist in the feed intake of the layers. Similarly, the group that consumed the control feed had the lowest feed intake (93.25 g/b/d). Layers fed with 2.5% *M. oleifera* diet had the highest feed intake and feed intake decreased as the moringa inclusion increased. The results further indicated that there was a significant difference (p<0.05=0.002) in the FCR of the experimental birds. The layers fed the control feed had the lowest FCR while the group that received 7.5% *M. oleifera* feed had the highest FCR which decreased progressively as moringa inclusion decreased. The result was in line with Sy and Thu (2015), who state that *M. oleifera* leaf meal achieved higher daily weight gain in broilers than the control while the lowest FCR was recorded in the control group. However, FCR was found to decrease progressively with the increased inclusion of *M. oleifera* leaf meal in pullet diet (Ugwuoke *et al.*, 2020). The inclusion of *M. oleifera* leaf meal was found to improve body weight, feed conversion efficiency, feed intake and protein efficiency of layer birds (Ojha *et al.*, 2018). *M. oleifera* leaf meal improvement in the layer's performance might be attributed to its immunomodulatory effects against certain diseases (Akram *et al.*, 2020). It was known that moringa has antibiotic, antimicrobial, and antifungal properties that make it an outstanding additive in layer production (Ojha *et al.*, 2018).

Layers fed control feed and those that received 2.5% *M. oleifera* diets started laying eggs one week before the layers fed 5% *M. oleifera* leaf diet and

Table 3 – Growth Performance of Isa Brown Layers Fed *M. oleifera* leaf powder.

	Control	2.5% *Moringa oleifera*	5% *Moringa oleifera*	7.5% *Moringa oleifera*	*p*-value
Initial Body Weight (g/bird)	1516.34±2.16	1509.64±2.11	1522.00±3.25	1525.00±4.23	0.92
Final Body Weight (g/bird)	1624.85 ± 3.23^{d}	1843.05 ± 1.54^{a}	1758.22 ± 1.93^{b}	1698.18 ± 2.33^{c}	0.04*
Feed Intake (g/bird/day)	93.25 ± 0.28^{d}	103.11 ± 0.92^{a}	93.71 ± 0.64^{b}	89.24 ± 0.75^{c}	0.01*
FCR	3.25 ± 0.01^{d}	3.50 ± 0.03^{c}	3.84 ± 0.05^{b}	4.31 ± 0.01^{a}	0.002*

Values allotted with different alphabetic superscripts differ significantly (p<0.05).

Table 4 – Laying percentage of layers fed different *M. oleifera* leaf powder supplement.

Age (Weeks)	Control	2.5% *Moringa oleifera*	5% *Moringa oleifera*	7.5% *Moringa oleifera*	Mean ($\overline{X}$)
20	15.40	10.05	-	-	12.56±0.04
21	20.00	17.58	14.57	11.33	15.87±0.01
22	30.67	24.83	22.01	21.32	24.71±0.04
23	41.50	37.11	34.57	32.04	36.31±0.09
24	48.17	44.05	40.84	37.73	42.70±0.10
25	55.95	49.74	44.54	43.92	48.54±0.06
26	61.55	56.16	53.18	51.82	55.68±0.11
27	73.77	67.53	65.29	63.74	67.59±0.06
28	75.65	73.17	68.38	65.90	70.78±0.01
29	76.44	76.42	70.39	67.10	72.59±0.15
30	76.50	77.55	73.03	72.76	74.96±0.04
Mean ($\overline{X}$)	52.33±0.03[a]	48.56±0.08[b]	48.68±0.01[b]	46.77±0.03[c]	

Values allotted with different alphabetic superscripts differ significantly (p<0.05).

those that received 7.5% *M. oleifera* leaf diets. Layers fed control feed significantly had the highest laying percentage which decreased progressively as the *M. oleifera* supplementation increased (Table 4). It was found in another study that *M. oleifera* leaf meal decreased linearly the egg laying rate in Rhode Island Red hens (Abou-Elezz *et al.*, 2011). The laying birds that received restricted commercial feed and *M. oleifera* leaves were found to produce lower egg percentage than the control (Mohammed *et al.*, 2012). Similarly, Mabusela *et al.* (2018) reported that the inclusion of *M. oleifera* decreased the feed intake and laying percentage. The decrease in the laying percentage might be attributed to the negative effect of *M. oleifera* on the follicle stimulating hormone of laying hens (Ajuogu *et al.*, 2019) which resulted to decreasing the laying potential. There was a reported decrease in FSH of female Wistar rats administered graded levels of *M. oleifera* leaf extract, where the control produced significantly higher amount of FSH compared to the experimental groups (Nwamarah *et al.*, 2015). Ladokun *et al.* (2015) also reported a significant decrease in the concentration of FSH in WAD bucks fed moringa leaves on the 4th and 8th weeks of the experiment. FSH which is produced in response to slow-frequency pulsatile GnRH is responsible for the growth and maturation of immature oocytes into mature secondary follicle prior to ovulation (Holesh *et al.*, 2021). When the growth and maturation of oocytes are affected due to the feeding of *M. oleifera* based diet, ovulation will be affected and egg production will consequently be affected.

There was a significant difference (p<0.05 = 0.01) in the average weight of eggs produced by layers fed *M. oleifera* leaf powder. The treatment group fed 2.5% *M. oleifera* leaf diet had significantly higher average egg weight compared to the others. The average egg weight decreased progressively as the moringa supplementation rate increased. The average egg weight of layers fed T1 feed was the lowest, but not significantly different from the average egg weight of the layers fed T4 feed (Table 5). Similarly, significant difference existed in the length of the eggs produced by the inclusion of *M. oleifera* leaf powder. Treatment that received 2.5% *M. oleifera* leaf powder had significantly higher egg length than others and decreased progressively as the moringa inclusion level increased. The result showed that no significant difference existed in the width of the eggs produced by the inclusion of *M. oleifera* leaf powder. There was a significant difference (p<0.05 = 0.03) in the thickness of the egg shells produced by the inclusion of *M. oleifera* leaf powder in feed where the group that received 2.5% *M. oleifera* leaf diet significantly produced the thickest shell. Kouatcho *et al.* (2020) noted that relatively heavier eggs were observed in the treatment groups supplemented with *M. oleifera.* The experimental layers that received *M. oleifera* leaf meal produced heavier eggs than the control (Teteh *et al.*, 2016).

Table 5 – External Qualities of Eggs Produced by Layers Fed with *M. oleifera* Leaf Powder.

	$\overline{X}$Control	$\overline{X}$2.5% *Moringa oleifera*	$\overline{X}$5% *Moringa oleifera*	$\overline{X}$7.5% *Moringa oleifera*	Mean	*p*-value
Average Egg Weight (g)	50.26[c]	54.21[a]	52.08[b]	50.61[c]	51.79	0.01
Egg Length (mm)	48.63[c]	53.85[a]	51.74[b]	49.29[c]	50.88	0.01
Egg Width (mm)	43.17	43.94	43.58	43.46	43.54	017
Shell Thickness (mm)	0.41[c]	0.49[a]	0.45[b]	0.44[b]	0.45	0.03

Table 6 – Mineral contents of eggs produced by inclusion of varying amounts of M. *oleifera* leaf powder in feeds of layers.

Mineral Contents (mg/100g)	Control	2.5% *Moringa oleifera*	5% *Moringa oleifera*	7.5% *Moringa oleifera*	Pooled SEM	*p*-value
Phosphorous	76.69^{d}	167.03^{c}	204.9^{b}	301.95^{a}	24.34	< 0.001
Sodium	99.74^{d}	107.17^{c}	151.69^{b}	283.43^{a}	22.36	< 0.001
Magnesium	8.38^{d}	18.96^{b}	37.02^{a}	16.72^{c}	3.50	0.001
Zinc	0.85	0.97	1.01	1.12	0.07	0.559
Manganese	0.001^{d}	0.02^{c}	0.11^{b}	0.34^{a}	0.05	<0.001
Iron	0.40^{b}	1.13^{a}	1.43^{a}	1.81^{a}	0.18	0.001
Potassium	132.01^{c}	210.19^{b}	222.76^{a}	215.63^{b}	11.43	<0.001
Calcium	58.47^{d}	81.79^{b}	98.32^{a}	75.85^{c}	10.75	<0.001
Copper	0.06^{d}	0.76^{c}	1.17^{b}	1.28^{a}	0.15	<0.001
Selenium (µg/100g)	7.66^{d}	8.82^{c}	28.23^{b}	31.11^{a}	3.97	<0.001
Chromium	0.001^{d}	0.003^{c}	0.01^{b}	0.03^{a}	0.003	<0.001

Values allotted with different alphabetic superscripts differ significantly (p<0.05).

The mineral constituents of eggs produced by the inclusion of varying amount of *M. oleifera* leaf powder in feed of layers shows that eggs produced by feeding layers with 7.5% *M. oleifera* leaf powder fortified feed had significantly higher content of phosphorous (301.95), sodium (283.43), manganese (0.34), iron (1.81), copper (1.28), selenium (31.11) and chromium (0.03) than the other treatment groups (Table 6). The above minerals were found to increase as the addition of *M. oleifera* leaf powder increases showing significantly lower content of minerals in the control group. The findings are similar to that of Moyo *et al.* (2011) who discovered that *M. oleifera* leaf powder is a significant source of iron, selenium, and copper to layers when included in the feed. The results are equally similar to Amabye (2016) who reported a high presence of magnesium, potassium, phosphorous, iron, and sodium with the inclusion of *M. oleifera* leaf powder in the feed of layers. A significant increase in iron and copper with the inclusion of *M. oleifera* leaf powder in feed was found and it therefore proves that it is an excellent additive in layer feeds (Abioye & Aka, 2015).

Furthermore, the addition of *M. oleifera* leaf powder at 5% was found to produce eggs with the highest amount of magnesium (37.02), potassium (222.76) and calcium (98.32). Moringa improved the content of the above minerals to the point of 5%, where further addition decreased the contents (Table 1). The decrease in calcium, potassium and magnesium with the further increase in Moringa may be due to the availability of some anti-nutrient factors that bind calcium, potassium and magnesium thereby reducing their bio-availability. Phytates present in *M. oleifera* leaves chelate minerals like calcium, potassium and magnesium hence rendering them bio-unavailable (Soetan & Oyewole, 2009). Complex calcium ion caused by phytates in the digestive tract is capable of causing calcium deficiency in birds (Bora, 2014). Deficiency of calcium results in osteoporosis (Borje & Nordin, 2010). Magnesium is one of the macro elements which function as a cofactor in many enzymes. It acts as a counter ion for ATP; structural functions of proteins and nucleic acids required for DNA and RNA synthesis (Gröber *et al.*, 2015). Magnesium is required for the production of energy, and accelerates oxidative phosphorylation, and glycolysis processes (Al-Fartusie & Mohssan, 2017) as well as muscle relaxation and protein synthesis (Villagomez & Ramtekkar, 2014).

Potassium regulates osmotic pressure of the body tissues; affects contractility and tension; and maintains the physiological blood pH (Radwińska & Żarczyńska, 2014). The production of eggs high in varying amount of minerals by the inclusion of 7.5% *M. oleifera* in feeds could be due to the significant amount of minerals in *M. oleifera* leaves. Nutrients contained in *M. oleifera* can enrich the mineral contents of eggs by its transformation in the body of the layers (Abbas, 2013). Macro or trace elements found in eggs are very important in the oxidation and immune system of the body. Copper is an important component of many enzymes including cytochrome oxidase, monoamine oxidase, catalase, peroxides, ascorbic acid oxidase, lactase, tyrosinase, and superoxide dismutase (Al-Fartusie & Mohssan, 2017). Depression in the immune system of the body has been associated with the deficiencies of zinc and selenium (Vanholder *et al.*, 2002). Metal ions like copper, iron, and selenium play significant roles in determining the antioxidant enzyme activities of the body (Nenkova *et al.*, 2017). Supplementation of *M. oleifera* leaf powder was reported to increase the levels of all the micronutrients in animals and man (Glover-Amengor *et al.*, 2016; Falowo *et al.*, 2018).

Varying inclusion of *M. oleifera* leaf powder had no statistically significant effect on the zinc content of the eggs (Table 6). Though variations in the mean zinc

contents of the eggs in different treatment groups were recorded, the variations were not statistically significant. This might be attributed to the negligible requirement of zinc as those available in the feed were sufficient to provide the daily zinc dietary need of the birds. Zinc is a micronutrient required in minute quantity but very significant to the growth and production of layers. This is in line with Jeroch (2011) who recommended a low-level supply of zinc in layer feeds but a higher level in the feeds of breeders. However, deficiency of zinc in the feed of layers presents several malnutritional abnormalities. Insufficient supply of zinc according to Tomaszewska *et al.* (2017) leads to inadequate mineralization of bone, poor skeletal formation and decrease in body weight of birds. Zinc is a constituent of many enzymes, DNA and RNA (Bahakaim *et al.*, 2014). Zinc increases the egg laying rate, egg mass, fertility and hatchability of eggs in layers (Li *et al.*, 2019). Non-significant difference in the zinc content of eggs can also be due to the poor utilization of zinc content of *M. oleifera*. This is in agreement with Naz *et al.* (2016) who discovered that the potency of zinc was dependent on its absorption in the intestine and bioavailability in the blood of layers.

CONCLUSION

M. oleifera is a good supplement in layer feed as it promotes average weight, feed intake, FCR, egg weight, egg length and shell thickness. However, the inclusion of *M. oleifera* in layer feed affects their laying potentials. *M. oleifera* at the rate of 7.5% is recommended to be included in layer feed to induce the mineral content of eggs which will help in reducing mineral deficiency in children when consumed. Further research is needed in finding the comparative effects of *M. oleifera* leaf and seed powders on the mineral contents of Isa brown eggs. The findings of this study can be used by the feed manufacturers to produce improved layer feed for increased minerals in eggs.

DISCLOSURE STATEMENT

The authors have no competing interests to declare.

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