Effects of different inclusion levels of *Moringa oleifera* leaf powder on the vitamin profile of fowl eggs

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**ABSTRACT**

The study was carried out to determine the effects of different levels of *Moringa oleifera* leaf powder additive on the vitamin profile of fowl eggs. A completely Randomized Design (CRD) was adopted for the study. The study was carried out in the Agricultural Education poultry farm. The population for the study consisted of 252 Isa Brown day-old chicks. Simple random sampling technique was used to select 240 chicks which were also randomly allotted to 12 pens. On the procedure of the experiment, 240 chicks were randomly selected from 252 Isa Brown day-old chicks bought from day-old chicks distribution centre in Nsukka urban area. The chicks were equally randomly allotted to 12 pens with 20 chicks each. The pens were randomly assigned to 4 different treatment groups with 3 replicates each. T1 was fed with control feed, T2 was fed with feed fortified with 2.5% *M. oleifera* leaf powder, T3 was fed with feed fortified with 5% *M. oleifera* leaf powder while T4 was fed with feed fortified with 7.5% *M. oleifera* leaf powder. Each of these 4 treatment groups had 3 replicates representing T1A, T1B, T1C, T2A, T2B, T2C, T3A, T3B, T3C, T4A, T4B and T4C. The chicks were fed with uniform feed up to week 4. From week 5, experimental feeds were given. The layers were placed on the same treatment conditions such as provision of heat during brooding, provision of clean drinking water, vaccination, deworming, treatment of diseases, regular changing of litters, among others except different experimental feeds given to different groups. The feeds were formulated using feedwin software and produced in Chidera Feed Mill located at Onuiyi, Nsukka. The vitamin profile of eggs was determined by the Central Research and Diagnostic Laboratory located in Ilorin, Nigeria using Atomic Absorption Spectrophotometry (AAS). Data collected were analyzed using mean, bar charts, pie charts and Analysis of Variance. Fowl eggs produced by the inclusion of no *M. oleifera* leaf powder in feeds had the highest amount of vitamins B1, B2, B3, and C. Furthermore, fowl eggs produced by the inclusion of 7.5% *M. oleifera* leaf powder in feeds had the highest amount of vitamins A, D, and E.

**Key words:** *Moringa oleifera*, vitamin profile, eggs, different inclusion levels.

**INTRODUCTION**

*Moringa oleifera* leaf is a good source of antioxidant compounds such as ascorbic acid, Flavonoid, Phenolics and Carotenoids (Anwar et al., 2007). The authors stated that the presence of β-Carotene, Vitamins A, C and E in *M. oleifera* leaves further explain their reducing potentials albeit the mode of action is yet to be elucidated. Vitamin C is known to act as a scavenger of free radicals, while it indirectly regenerates Vitamin E. The leaves of *M. oleifera* are excellent source of Vitamin A (four times the amount in Carrots), rich in Vitamin C (seven times the amount in...
Oranges), good source of Vitamin B and other minerals, outstanding source of calcium (four times the amount in milk), protein (twice the amount in milk) and potassium (three times the amount in bananas) (El-Awady, 2003). *M. oleifera* leaves with high content of phenolics and flavonoids show greater antioxidant activity, anti-radical power, reducing power, inhibition of lipid peroxidation, protein oxidation and hydroxide-induced deoxyribose degradation, and scavenging power of superoxide anions and nitric acid radicals, than do its fruits and seeds (Wangcharoen and Gomolmanee, 2011). The antioxidant activity of Moringa leaf extract was found to be higher than that of standard Vitamin E and remains unaffected at pH 4 and 9 in the dark at 5 and 25°C, respectively for 15 days, although the activity significantly decreases when heated to 100°C for 15 min (Arabshahi-D et al., 2007). *M. oleifera* provides an excellent source of feed ingredient for supplementation in livestock feed production. *M. oleifera* was included at the ratio of 0.2, 0.4, and 6% to determine the productive performance of Japanese quail and was found to have positive effect on the live body weight and body weight gain as compared with the quails placed on basal feed (Moustafe et al., 2015). Similarly, Gakuya et al. (2014) supplemented *M. oleifera* leaf meal at the rate of 1.25, 2.5, 5, and 7.5% in layer chicken feed and found that there was a slight decrease in live weight in all groups during the first week but later appreciated. Supplementation of *M. oleifera* to determine the growth performance and health status on young post-weaning rabbit showed that rabbit fed with *M. oleifera* supplemented feed had the best average weight and growth compared to rabbits fed with mixed and standard feed (Djakalia et al., 2011). *M. oleifera* leaves, sunflower cake, and grass hay were supplemented in goat feed to determine the chemical composition, fatty acid contents, and antioxidant potentials of goat meat and it was found that the total unsaturated fatty acids on goat meat from goats supplemented with grass hay had the lowest value, followed by the meat obtained by the supplementation of *M. oleifera*, and finally those supplemented with sunflower cake which recorded the highest value (Qwele et al., 2013). Supplementation of *M. oleifera* leaf meal at the rate of 0.5, 1.0, 1.5, and 2.0% was done to determine the performance of Vanaraja hens and it was discovered that significant improvement in egg production and feed conversion ratio were recorded in Vanaraja laying hens fed diet supplemented with 0.5% *M. oleifera* leaf meal compared to control but, laying hens fed diet supplemented with 1.0 and 1.5% *M. oleifera* leaf meal had similar egg production and feed conversion ratio compared to the group fed with 0.5% supplemented diet (Swain et al., 2017). Egg is one of the most complete and versatile foods currently available, being an excellent source of high quality and easily digestible protein as well as good source of certain Vitamins and Minerals (Pérez-Vendrell et al., 2004). The authors noted that the levels of Vitamins in eggs are directly linked to the levels in the diet of the hen. Narahari (2003) stated that table egg is the most nutritious, unadulterated, yet relatively inexpensive natural food, with a high digestibility coefficient. Because eggs are inexpensive, middle class population can afford to buy eggs for consumption across major Asian nations for protein supply, and this led to constant increase in the per capita consumption of eggs (Zaheer, 2015). Fowl egg protein is the best protein available in nature for human consumption, with a well balanced amino acids profile, having the highest biological value, protein efficiency ratio, net protein value, net protein utilization and chemical score (Narahari, 2003). Eggs provide almost 18 vitamins and minerals, the composition of which can be affected by several factors such as hen diet, age, strains, as well as environment (Fraeye et al., 2012). They contain other biologically active compounds that may have roles in the therapy and prevention of chronic and infectious diseases (Miranda et al., 2015).

The levels of nutrients in fowl eggs are directly linked to the levels in the feed of hens. Similarly, feed quality can be measured in terms of the quality of the raw materials used in producing the feed. In line with this, inclusion of *M. oleifera* leaf meal was found to reduce the total lipid, triglycerides, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and atherogenic index (AI) in both serum and yolk while induced a significant increase in high density lipoprotein (HDL) in serum from all treated groups (El-Sheikh et al., 2015). Similarly, inclusion of *M. oleifera* leaf meal in layer diet was found to yield better egg production and external egg quality characteristics compared to the control (Ebelebe et al, 2013). Hence, the study was aimed to determine the effects of different inclusion levels of *M. oleifera* leaf powder on the vitamin profile of fowl eggs.

**MATERIALS AND METHODS**

The study adopted a Completely Randomized Design (CRD). Completely randomized design in the view of Obi (2002) is an experimental design used when the experimental units are homogeneous such as same animal breed, where environmental effects are homogeneous such as animal house and treatments are assigned to experimental units at random. The study was carried out in Agricultural Education poultry farm, University of Nigeria, Nsukka.

On the procedure of experiment, 240 chicks were randomly selected from the population of 252 chicks and placed in 12 treatment groups. The 12 groups were also
randomly allotted to four different treatment groups on the 5th week of rearing. Uniform feed was given to them on the 1st, 2nd, 3rd and 4th weeks to place them at the same condition to ensure uniformity. The four main groups were: T1 – Control group (No M. oleifera leaf powder supplement given), T2 – 1st experimental group (2.5% M. oleifera leaf powder supplement given), T3 – 2nd experimental group (5% M. oleifera leaf powder supplement given), and T4 – 3rd experimental group (7.5% M. oleifera leaf powder supplement given). The four treatment groups were in three replicates which gave rise to 12 groups. Hence, there were T1 A, T1 B, T1 C, T2 A, T2 B, T2 C, T3 A, T3 B, T3 C, T4 A, T4 B, and T4 C groups.

Before the arrival of the day-old chicks, the brooding pen was properly prepared by keeping litter materials on the floor. The pen was heated up to 34°C. Minimum and maximum thermometer was placed on the floor to check temperature fluctuations. The hens were regularly vaccinated against coccidiosis, Newcastle disease, gumbo pox, and fowl pox. Prior to the transfer of pullets to the rearing pens, the pens were cleaned, washed and disinfected with Vinkokill disinfectant. Litter material was placed on the floor after drying. Feed, clean water and antibiotics were provided regularly. The birds were kept on the same rearing condition except different feed given to different treatment groups and they started egg dropping at the age of 20 weeks. The birds were kept in the rearing house during the laying periods to avoid the stress of transferring to the laying pen. Laying boxes were provided in the same pens for their eggs dropping.

Harvesting and processing of Moringa oleifera leaves into powder

Moringa leaves were harvested in the researchers’ Moringa farm and Moringa plants within the vicinity of the study area. Harvesting was done by removing the bipinnate leaves from the branches of Moringa plants. The leaves were stripes off from the pinnae, washed and drained of water. The drained leaves were spread in a clean room for 3-5 days. Windows and doors were opened to facilitate indoor drying. The dried M. oleifera leaves were ground using grinding machine to get the powder.

Feed formulation and production

Starter feeds were formulated to feed the birds from weeks 5-6, growers feeds were formulated to feed them from weeks 7-20, and layer feeds from weeks 21-30. In each of the feeds, T1 was formulated with 0% inclusion of M. oleifera leaf powder, T2 was formulated with inclusion of 2.5% M. oleifera leaf powder, T3 was as well formulated with inclusion of 5% M. oleifera leaf powder while T4 was formulated with inclusion of 7.5% M. oleifera leaf powder. The feeds were formulated using feed formulation software called Feedwin developed by the former PTC+ now known as Aeres Training Centre International located in Barneveld, the Netherlands. Production of the feeds which involved dosing, grinding and mixing was done in Chidera Feed Mill located in Onuiyi, Nsukka.

Determination of vitamins

Vitamin C

Reagents:

1). Phosphotungstic acid: colour developing solution: Solution A – 20gms of sodium Tungstate Na₂WO₄; 2H₂O, Disodium Hydrogen Phosphate Na₂HPO₄;2H₂O -10gm taken in 300ml distilled water and warmed to dissolve. Solution B – 15ml Distilled water and 5ml of H₂SO₄. Solution B was poured in warm solution A and the content was boiled gently for minimum of 2 hours under reflux. The resulting solution was then cooled to room temperature by allowing it to stand on the table and mixture was diluted to 500ml with distilled water.

2). 0.5% Oxalic acid solution- 0.5gms of Oxalic acid dissolved in distilled water and volume made up to 100ml.

3). Stock Std. Ascorbic acid – this solution was prepared by dissolving 50mg of L-ascorbic acid in 100ml of 0.5% (w/v) Oxalic acid solution.

4). Working standard solution (1mg/100ml) - Dilute stock standard solution 50 times 0.5% Oxalic acid.

Procedure - 1ml (Ascorbate was extracted from 1g of the sample using 4%TCA) of sample was taken in a test tube marked test ‘T’ and 2 ml of distilled water in a test tube marked blank ‘B’.

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Mix thoroughly and allow standing for 30 min at room temperature. Then tubes were centrifuged at 3000 rpm for 10 min. The clear supernatant was taken in cuvette without disturbing precipitate and absorbance was measured at 700 nm against blank.

Calculation: (Abs of sample/Abs of Standard) × concentration of Standard × (Volume of homogenizing
solution/weight of sample)

**Vitamin B1**

5 g of sample was homogenised with 50 ml of ethanolic NaOH solution. This was filtered into a 100 ml flask. 10 ml of the filtrate was pipetted into a beaker and colour developed by the addition of 10 ml K$_2$MnO$_4$. The absorbance is read at 360 nm. A blank sample was also prepared and read at the same wavelength. The values are extrapolated from a standard.

Calculation: \((\text{Abs of sample}/\text{Abs of Standard}) \times \text{concentration of Standard} \times (\text{Volume of homogenizing solution/weight of sample})\)

**Vitamin B2**

Five grams (5 g) of sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 100 ml of 30% hydrogen peroxide (H$_2$O$_2$) and allowed to stand over hot water bath for 30 min. Two millilitres (2 ml) of 40% sodium sulphate added to make up to 50 ml mark and absorbance read at 470 nm in a spectrophotometer.

Calculation: \((\text{Abs of sample}/\text{Abs of Standard}) \times \text{concentration of Standard} \times (\text{Volume of Solvent/weight of sample})\)

**Vitamin B3**

5 g of the sample was treated with 50 ml of 1N H$_2$SO$_4$ and shaken with magnetic shaker for 30 min. Three drops of ammonia solution added to the mixture and filtered. 10 ml of the filtrate was pipetted into a 50 ml volumetric flask, and 1 ml KCN added.

The mixture acidified with 0.02 M H$_2$SO$_4$ and the absorbance read at 470 nm

Calculation: \((\text{Abs of sample}/\text{Abs of Standard}) \times \text{concentration of Standard} \times (\text{Volume of homogenizing solution/weight of sample})\)

**Vitamin E**

Into three stopper centrifuge was measured 1.5 ml homogenized sample, 1.5 ml standard and 1.5 ml water (blank), respectively. Then in test and blank 1.5 ml xylene was added to all the tubes, stopper mixed well, and centrifuged. 1 ml of the xylene layers was transferred into other stopper tubes while measures were taken not to include any ethanol or protein. 1 ml Dipyridyl reagents was added to each tube was stopper and mixed. 1.5 ml of the mixture was pipetted into colorimeter cuvettes and extinction of test and standard was read against the blank at 460 nm. Then in turn beginning with the blank 0.33 ml ferric chloride was added and the absorbance was read at 520 nm.

Calculation= \([[\text{Abs of Sample at 520 nm} - \text{Abs of Sample at 460 nm}] / \text{Abs of Standard at 460 nm}] \times \text{concentration of Standard} \times (\text{Volume of Solvent/weight of sample})\)

**RESULTS**

The results of the vitamin profile of fowl eggs produced by inclusion of varying levels of *M. oleifera* leaf powder in feeds show that vitamin B1, vitamin B2, vitamin B3, and vitamin C were slightly higher in eggs produced by the layers fed with control diet than other treatment groups (Figures 2, 3, 4 and 6). These are the water soluble vitamins. The vitamin B5 content of eggs produced by inclusion of 2.5% *M. oleifera* leaf powder in diet was higher than the vitamin B5 content of eggs from other treatment groups (Figure 5). Similarly, the fat soluble vitamins which are the vitamin A, vitamin D and vitamin E were slightly higher in eggs produced by inclusion of 7.5% *M. oleifera* leaf powder in diet than the control diet.
Figure 2: Bar chart analysis of the vitamin B1 content of eggs produced by layers fed with *Moringa oleifera* leaf powder fortified feeds.

Figure 3: Bar chart analysis of the vitamin B2 content of eggs produced by layers fed with *Moringa oleifera* leaf powder fortified feeds.

Figure 4: Bar chart analysis of the vitamin B3 content of eggs produced by layers fed with *Moringa oleifera* leaf powder fortified feeds.
powder than other treatment groups (Figures 1, 7 and 8). However, there was no statistically significant difference (p>0.05) in the mean vitamin profile of fowl eggs produced by inclusion of different levels of M. oleifera leaf powder in feeds (Table 1).

DISCUSSION

The findings of the study were in line with the findings of Abioye and Aka (2015) who reported that there was a significant increase in vitamin A (beta carotene) as a result of increase in the inclusion level of M. oleifera leaf powder in feed. The findings were also in line with Anwar et al. (2007) who found that the presence of β-carotene, vitamin A, C and E in M. oleifera leaf powder explains the reason people recommend it for organic feed additive. Beta-carotene from M. oleifera leaves was found to be effective in overcoming vitamin A deficiency in male albino rats though, serum vitamin A levels remained lower as compared with the group fed with vitamin A acetate (Bu Nambiar and Seshadri, 2004). Similarly, the result of nutritional characterization of M. oleifera leaves indicated that dried leaves powder of Moringa had a high level of beta-carotene and vitamin A (Moyo et al., 2011). This was equally in line with the study on the nutrient value of M. oleifera lam leaves which discovered Moringa as a rich source of beta-carotene, vitamin C, vitamin E, vitamin A, vitamin B2, vitamin B1, and vitamin K (Broin, 2006). Akhouri et al. (2013) found that M. oleifera is actually a good source of provitamin A, Vitamin B and C but the bioavailability of the nutrients are hindered by the presence of anti-nutrient.
Figure 1: Bar chart analysis of the vitamin A content of eggs produced by layers fed with *Moringa oleifera* leaf powder fortified feeds.

Figure 7: Pie chart analysis of the vitamin D content of eggs produced by layers fed with *Moringa oleifera* leaf powder fortified feeds.

Figure 8: Bar chart analysis of the Vitamin E content of eggs produced by layers fed with *Moringa oleifera* leaf powder fortified feeds.
The presence of anti-nutrient factors in *M. oleifera* leaves was accounted for the slight decrease in the amount of water soluble vitamins B1, B2, B3, and C contained in eggs produced by layers fed with inclusion of *M. oleifera* leaf powder. The anti-nutrient factors made it possible that the rich nutrients contained in *M. oleifera* leaves were unavailable to the layers for egg production. There were reported presence of tannin, oxalate, phytate, and saponin (Ndubuaku et al., 2015), tannin, phytate, phenolics, alkanoids, flavonoids, saponon, and terpenoids (Ijarotimi et al., 2013) in *M. oleifera* leaves which inhibit the bioavailability of the nutrients contained in them when fed to layers. The metabolism and utilization of numerous vitamins in *M. oleifera* leaves are affected by these anti-nutrient factors. The abundance of anti-nutritional factors and toxic influences in plants used as animal feed calls for concern (Soetan and Oyewole, 2009). Tannins have the ability to precipitate proteins and render them unavailable (Aganga and Tshwenyane, 2003). Dietary tannins form complexes with metal ions and macro-molecules such as proteins and polysaccharide and reduce the efficiency (Dei et al., 2007) thereby causing conditional deficiency of some nutrients.

**Conclusion**

Inclusion of *M. oleifera* leaf powder in layer feed at the rate of 7.5% had slight better result in the fat soluble vitamin A, vitamin D and vitamin E contents of fowl eggs than other treatment groups. Similarly, water soluble vitamin B1, vitamin B2, vitamin B3, and vitamin C were slightly higher in eggs produced by the layers fed with control diet than other treatment groups. However, there was no statistically significant difference (p>0.05) in the mean vitamin profile of fowl eggs produced by inclusion of different levels of *M. oleifera* leaf powder in layer feed.

**REFERENCES**
