# EFFECTS OF DIFFERENT DIETARY LEVELS OF *MORINGA oleifera* LEAF MEAL ON EGG PRODUCTION, QUALITY, SHELF LIFE, FERTLITY AND HATCHABLITY OF DUAL PURPOSE KOEKOEK HENS

**MSc Thesis**


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**June, 2016**

**Bishoftu, Ethiopia**

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**A Thesis Submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirement for Degree of Masters of Science in Tropical Animal Production and Health**

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**June, 2016**

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As MSc research advisors, we hereby certify that we have read and evaluated this Thesis prepared under our guidance by Wubalem Alebachew Amera title: **Effects of Different Dietary Levels of *Moringa oleifera* Leaf Meal on Egg Production, Quality, Shelf Life, Fertility and Hatchability of Dual Purpose Koekoek Hens**, we recommend that it can be submitted as fulfilling the MSc thesis requirement.

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As member of the Board of Examiners of the MSc Open Defense Examination, we certify that we have read, evaluated the Thesis prepared by **Wubalem Alebachew Amera** and examined the candidate. We recommend that it be accepted as fulfilling the Thesis requirement for the degree of Masters in **Tropical Animal Production and Health.**

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**DEDICATION**

This thesis is dedicated to my beloved family and my sister's housband Mr. Birhanu Anjet. Their unreserved financial and moral support for my academic success is highly memorable.

## STATEMENT OF THE AUTHOR

First, I declare that this Thesis is my bonafide work and that all sources of materials used for this Thesis have been duly acknowledged. It has been submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University and is deposited at the University Library to be made available to borrowers under rules of the Library. I solemnly declare that this Thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.



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## BIOGRAPHICAL SKETCH

Wubalem Alebachew was born on April 15/08/1985 E.C, in West Gojjam, Ethiopia. She attended her elementary education in Sebatamit, secondary education in Fasilo and preparatory education in Bahir Dar preparatory school. After completion of her preparatory school education, she joined Aksum University of Agriculture in 2004 E.C and graduated with BSc degree in Animal production technology in 2006 E.C.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ADF | Acid Detergent Fiber |
| AOAC | Association of Official Analytical Chemist |
| BSc | Bachelor of Science |
| CP | Crude Protein |
| CRC | Cassava Root Chip |
| CRD | Completely Randomized Design |
| DM | Dry Matter |
| DZARC | Debre Zeit Agricultural Research Center |
| EE | Ether Extract |
| EIAR | Ethiopia Institute of Agricultural Research |
| FAO | Food and Agriculture Organization |
| FCR | Feed Conversion Ratio |
| GE | Gross Energy |
| HCN | Hydrocyanic Acid |
| HL | Human Leukemia |
| ISA | Institution of Selection Animals |
| LC | Lethal Concentration |
| MOLM | *Moringa oleifera* Leaf Meal |
| NDF | Neutral Detergent Fiber |
| SAS | Statistical Analysis Systems |
| SBM | Soyabean Meal |
| SGS | School of Graduate Studies |

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## *ABSTRACT*

*A study was conducted to evaluate effects of Moringa oleifera leaf meal (MOLM) inclusion in layer rations on egg laying performance, egg quality parameters and egg shelf life. In addition, the study also evaluated the effect of MOLM on fertility, hatchability, embryonic mortality, chick quality and the economic benefits of feeding of MOLM to dual purpose Koekoek hens. Ninety six dual purpose Koekoek hens, aging 41 weeks, and 12 cocks were used and equally divided into four dietary treatments with three replications. Treatment rations contained MOLM [i.e., $T_1$ (0% MOLM), $T_2$ (5% MOLM), $T_3$ (10% MOLM), and $T_4$ (15% MOLM)]. In this study, MOLM was used to substitute for soybean meal (SBM). Hens were weighed at the start and end of the experiment and body weight (BW) change was calculated. Data on feed intake, hen-day egg production, hen-housed egg production and egg weight were recorded. Egg quality parameters were determined at an interval of 15 days on four eggs per replicate for twice. Fertility and hatchability of eggs, chick quality as well as mortality of birds and embryonic mortality of fertile eggs during the incubation period were recorded. The shelf life of eggs was determined by considering albumen and yolk measurements as well as Haugh unit at an interval of 7 days on four eggs per replicate stored for 7, 14, 21 and 28 days. In addition, albumen and yolk pH of eggs stored at 7, 14, 21 and 28 days was determined to evaluate shelf life of eggs. All the above mentioned parameters were improved by the experimental diet except mortality rate and late embryonic mortality especially in T2. Body weight change was 0.32kg in T1, 0.43kg in T2, 0.48kg in T3 and 0.37kg in T4. Feed conversion ratio was 1.73 in T1, 2.10 in T2, 1.52 in T3 and 1.59 in T4. Average egg weight was 48.66g in T1, 54.51g in T2, 49.94g in T3 and 50.31g in T4. Percentage of hen day egg production was 50.69% in T1, 64.60% in T2, 45.23% in T3 and 47.65% in T4. Fertility percentage was 80.00% in T1, 93.33% in T2, 91.11% in T3 and 84.44% in T4. Hatchability percentage was 66.66% in T1, 78.57% in T2, 68.22% in T3 and 70.33% in T4. Higher feed intake and body weight change were recorded for T3 (10% MOLM) while higher yolk color was observed for T3 and T4 (15% MOLM). Generally, substistution of SBM by MOLM at 5% inclusion level showed better result even yhough price of MOLM is higher than SBM. As aresult, Moringa tree production level of should be increase and*

*needs further researches that can give evidence about the nutritional value of Moringa oleifera leaf meal.*

**Keywords:** *Body weight change, feed intake*, *egg production, egg quality, egg shelf life, fertility, hatchability*

# 1. INTRODUCTION

Protein supplementation is often important to improve poultry performance, and this needs to be done with respect to their requirements in addition to the balance of other nutrients available. The expansion of poultry industry depends largely on the availability of good quality feed in sufficient quantities and at prices affordable to both producers and consumers (Odunsi, 2003).This is very important especially for layers which are very sensitive to nutrition such that inadequacies in nutrient supply often lead to fall in egg production and even cessation of lay and deterioration of quality and shelf life of egg (Adenjimi *et al.,* 2011). With the present trend of rising prices of feed ingredients, there has been a search for non-conventional feedstuffs with potentials of improving poultry performance. Of such non-conventional feed sources, leaf protein sources have been reported (Farinu *et al.,* 2008).

One possible source of cheap protein source feed is the leaf meal of some tropical legume browse plants. Leaf meals do not only provide protein source but also some essential vitamins such as vitamins A, C, E (Sanchez-Machado *et al.*, 2006; Moyo *et al.*, 2011) and iron, and the two amino acids generally deficient in other feeds, i.e., methionine and cystine (Makkar and Becker, 1996; Moyo *et al.,* 2011) minerals and oxycarotenoids (Bhatt and Sharma, 2001; Muriu *et al*., 2002). It is also claimed that leaf meals increase poultry productivity as nutritional, therapeutic and prophylactic properties. Among the leaf meals *Moringa oleifera* leaf meal is the one which has the above mentioned nutritional and medicinal values (Fahey, 2005).

The high pepsin soluble nitrogen (82-91%) and the low acid detergent insoluble protein (1-2%) values for the *Moringa* leaf meal suggest that most of the protein in the meal is available to most animals (Makkar and Becker, 1997). *Moringa oleifera* leaves have a negligible content of tannins and have no trypsin and amylase inhibitors or cyanogenic glucosides (Makkar and Becker, 1996; Makkar and Becker, 1997). *M. oleifera* leaves are rich in biologically active carotenoids and tocopherols which have health-promoting potential through preventing free-radical damage that can initiate many illnesses (Smolin

and Grosvenor, 2007). Recently, there has been interest in the utilization of *Moringa* (*M. oleifera*) as a protein source for poultry. *Moringa* leaves have quality attributes that make it a potential replacement for soybean meal or fish meal in non-ruminant diets. *Moringa* can easily be established in the field, has good coppicing ability, as well as good potential for forage production. Furthermore, there is the possibility of obtaining large amounts of high quality forage from *Moringa* without expensive inputs due to favorable soil and climatic conditions for its growth (Makker and Becker, 1999; Sarwatt *et al*., 2002).

*Moringa* foliages are potential inexpensive protein sources for poultry feeding. The advantages of using *Moringa* as a protein resource are numerous, and include the fact that it is a perennial plant that can be harvested several times in one growing season and also has the potential to reduce feed cost (Sarwatt *et al*., 2004). *M. oleifera* leaf meal (MOLM) could replace sunflower seed meal and can be added up to 20% in layers ration (Kakengi *et al*., 2007). *M. oleifera* leaf meal possesses hypocholesterolemic properties and its inclusion in layers diets could facilitate reductions in egg cholesterol content. *M. oleifera* is in the group of high-yielding nutritious browse plants with every part having food value (Olugbemi *et al*., 2010a).

Despite the high nutritional content of *M. oleifera*, there is little information regarding its utilization in poultry feeding as a protein source in the layer ration. As a result, information on effects of feeding *M. oleifera* leaf meal (MOLM) on laying performance or, egg production, egg quality and egg shelf life, fertility and hatchability in chicken is scanty. Such information is needed in designing feeding strategies to improve quality, production and shelf life of egg of layers in resource limited farmers. This is even more crucial for small-scale farmers undertaking farm-based feed formulation, who constantly find it hard to produce with commercial feeds.

Therefore, the objective of this study was to determine:

- ✓ Effects of feeding of varied levels of *M. oleifera* leaf meal (MOLM) on feed intake, body weight change and feed conversion ratio,

- ✓ Effects of feeding MOLM on production, quality, shelf life, fertility and hatchability of eggs and chick quality, and
- ✓ To evaluate the economic feasibility of substituting SBM by MOLM at different levels in dual purpose Koekoek hens.

# 2. LITERATURE REVIEW

## 2.1. Description of *Moringa oleifera*

*Moringa oleifera* commonly referred to as the drumstick tree is a plant from the Moringaceae family and it is the most widely cultivated species of the genus *Moringa*. There are about 13 species in the Moringaceae family which are, *Moringa hildebrandtii* (medicinal), *Moringa drouhardii* (medicinal), *Moringa stenopetala* (edible delicious leaves, *Moringa ovalifolia* "aka Ghost Tree" (medicinal), *Moringa peregrina* (edible), *Moringa oleifera* (most common edible delicious leaves), *Moringa concanensis* (edible leaves), *Moringa rivae* (medicinal, *Moringa ruspoliana* (medicinal), *Moringa arborea* (medicinal), *Moringa borziana* (medicinal), *Moringa pygmaea* (medicinal), *Moringa longituba* (medicinal). Of which *M. oleifera* is the species most widely known. The tree is often called 'multipurpose' due to the fact that all parts including the leaves, pods, seeds, flowers, fruits and roots are edible (Orwa *et al*., 2009).

English common names include: *Moringa*, drumstick tree (from the appearance of the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which resembles horseradish), ben oil tree, or benzoil tree (from the oil which is derived from the seeds). The seeds can be used as a flocculent to clarify water and as a source of non-drying and very stable oil, known as Ben oil (Seewu *et al*., 2010).

*Moringa oleifera* is a fast-growing, deciduous tree. It can reach a height of 10-12 m (32-40 ft) and the trunk can reach a diameter of 45 cm (1.5 ft).The bark has a whitish-grey color and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches and the leaves build up feathery foliage of tripinnate leaves. The flowers are fragrant and bisexual, surrounded by five unequal, thinly veined, yellowish-white petals. The flowers are about 1.0-1.5 cm (1/2) long and 2.0 cm (3/4) broad. They grow on slender, hairy stalks in spreading or drooping later flower clusters which have a length of 10-25 cm. Flowering begins within the first six months after planting. In seasonally cool regions, flowering only occurs once

a year between April and June. In more constant seasonal temperatures and with constant rainfall, flowering can happen twice or even all year-round. The fruit is a hanging, three-sided brown capsule of 20-45 cm size which holds dark brown, globular seeds with a diameter around 1 cm. The seeds have three whitish papery wings and are dispersed by wind and water (Olson and Cariquist, 2001). *Moringa* can be described as a 'much-branched plant', with grey thick bark and a thin crown. The tree requires an annual rainfall of between 250 mm and 3000 mm and survives in a temperature range of 25 to 40°C which makes it suitable for tropical climates. The yield is often low in the first two years and it is discovered that in South India, flowers and fruits appear twice a year, enabling two annual crops (HDRA, 2002).

### 2.2. Uses of *Moringa oleifera*

All parts of *Moringa oleifera* are consumed as food. The plant produces leaves during the dry season and during times of drought, and is an excellent source of green vegetable when little other food is available (FAO, 2014). *M. oleifera* is mainly grown for its leaves in Africa, and much appreciated for its pods in Asia (Bosch, 2004). Leaves, pods, roots and flowers can be cooked as vegetables. The roots have been used as a substitute for horseradish but may be slightly toxic. The leaves are very nutritious and rich in protein, vitamins A, B and C, and minerals. They are highly recommended for pregnant and nursing mothers as well as young children (FAO, 2014).

*Moringa oleifera* leaves are eaten as a salad or dried and ground to make a very nutritious leaf powder. *M. oleifera* leaf powder is used for the re-nutrition of infants suffering from malnutrition. *M. oleifera* flowers are used to make tea, added into sauces or made into a paste and fried. The young pods are prepared and taste like asparagus. Older pods can be added to sauces and curries in which their bitterness is appreciated (Bosch, 2004; Orwa *et al*., 2009; Radovich, 2009; FAO, 2014). The immature seeds can be cooked in many different ways while the mature seeds are roasted and eaten like peanuts. *M. oleifera* seeds contain about 30-40% of edible oil (ben oil) which is used for salad dressing and cooking

and can replace olive oil. Ben oil is resistant to rancidity and provides substantial amounts of oleic acid, sterols and tocopherols (FAO, 2014).

The most important benefit is the medicinal properties of the plant and various studies illustrate a choice of activities, such as antioxidant, anticancer, antiviral, cardio-protective, anti-inflammatory, anti-asthmatic and others. Other applications of the seeds are for the purification of water and for the production of biodiesel. *M. oleifera* seeds contain terygospermin, a potent antibiotic and fungicide effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Due to their high iron content, *M. oleifera* leaves are used in the treatment of anemia in the Philippines. *M. oleifera* roots and bark are used in cardiac and circulatory problems (Orwa *et al*., 2009).

*Moringa oleifera* leaves used as a substitute to conventional concentrate on the *in vitro* gas production and digestibility of groundnut hay and reported that *M. oleifera* leaves appeared to be an alternative source of protein for ruminant production in West African settings and can be used as supplement to diets based on crop residues/poor roughage (Nouala *et al*., 2006). In combination with concentrate, *M. oleifera* leaves further improved the efficiency of concentrate utilization (Ogbe and John, 2012). *M. oleifera* leaves could be utilized as a source of feed supplement to improve growth performance and health status of poultry. However, the high protein content of *Moringa* leaves must be balanced with other energy feeds. *M. oleifera* leaves should be mixed with SBM, wheat middling, corn grain, or whatever else is locally available feed (Martin, 2007).

*Moringa* seed powder has antibacterial properties that make it useful as a natural clarifier for water purification systems and fish ponds (Aruna and Srilatha, 2012). It is considered as a potential natural and inexpensive alternative to toxic alum but further investigation is required since *Moringa* seed powder may have negative effects when combined with chlorine treatment and was found to promote bacteria re growth after first removal (Preston *et al*., 2010; Egbuikwem and Sangodoyin, 2013 ).

*Moringa oleifera* oil has various industrial applications. It is used in the perfume industry as it retains readily fragrance and is not prone to rancidity, and to make paintings or lubricants (Foidl *et al.*, 2001;Bosch, 2004). It has qualities needed to be a biodiesel feedstock (Rashid *et al.*, 2008). The oil cake resulting from seed oil extraction contains about 1% of floculant proteins that bind mineral particles and organics in the purification of drinking water, and are a natural alternative to toxic alumine generally used for water treatments. These proteins are used for sedimenting fibers in the juice and beer industries. *M. oleifera* timber is soft and can only be used for light constructions, but it can produce fibre for ropes and mats as well as pulp for the paper industry. *M. oleifera* bark is a source of dye (Foidl *et al.*, 2001; Bosch, 2004).

*Moringa oleifera* trees are useful for alley cropping as they have a loose canopy, which prevents excessive crop shading. Foliage can be regularly pruned and left in the field to improve soil fertility or fed to livestock in a cut-and-carry system. Phythormones extracted from *Moringa* leaves have been shown to have a growth enhancing effect on various plants, including black gram, peanut, soybean, sugarcane and coffee. Spraying *Moringa* leaf extract on leaves increases plant production by 20-35 % (Foidl *et al.,* 2001).

Other uses of *M. oleifera* include construction (pole and fibre), fuel wood, ornamental (hedge and shade) and medicinal value. Aqueous seed extract of *M. oleifera* has strong antiviral activity against Newcastle disease virus (NDV) using an *in ovo* assay and found that an increase in extract concentration was directly proportional to virus death and inversely proportional to production of antibody against NDV (Maroyi, 2006; Chollom *et al*., 2012).

### 2.3. Nutritional Composition of *Moringa oleifera* Leaves

There is quite a lot of literature on the nutritional value of *Moringa oleifera* leaves with varying nutritional content (Moyo *et al*., 2011). *M. oleifera* has been reported to possess several nutrients (Table 1) , including: Calcium, Magnesium, Potassium, Iron , Vitamin A, and Vitamin C and a crude protein content that varies from 16 to 40% (Foidl *et al.,* 2001*;*

Marcu and Pharm, 2005; Rweyemamu, 2006). *M. oleifera* used as a supplement can improve voluntary intake, digestibility and livestock performance (Aregheore, 2002).

**Table 1:** Mineral contents of dried *Moringa oleifera* leaves

| **Mineral** | **Dry leaf** |
|---|---|
| Calcium (%) | 3.65 |
| Phosphorus (%) | 0.30 |
| Magnesium (%) | 0.50 |
| Potassium (%) | 1.50 |
| Sodium (%) | 0.164 |
| Sulphur (%) | 0.63 |
| Zinc (mg/kg) | 31.03 |
| Copper (mg/kg) | 8.25 |
| Iron (mg/kg) | 490 |
| Manganese (mg/kg) | 86.8 |
| Selenium (mg/kg) | 363.00 |
| Boron (mg/kg) | 49.93 |

**Source:** (Moyo *et al*., 2011)

*Moringa oleifera* leaves could be highly digestible because of its immense nutritional qualities such as its chemical composition (neutral detergent fiber (NDF); acid detergent fiber (ADF); crude protein (CP); gross energy (GE); ether extract (EE)) and amino acids profile (Rubanza *et al*., 2005) (Table 2). *M. oleifera* leaves are rich in carotenoids, ascorbic acid and iron. The leaves are widely recognized as a food source for humans and a dry season feed for animals because of the nutrient contents it contains. Equally important is the fact that some parts of the tree contain toxins and other anti-nutritional factors that might decrease its potential as a source of food for animals or humans. For instance its bark contains tannins, alkaloids, saponin and inhibitors (Makkar and Becker, 1999; Foidl *et al*., 2001).

**Table 2:** Nutritional qualities of *Moringa oleifera* leaf meal

| Nutritive value | Dry leaves | Source |
|---|---|---|
| Crude protein | 25.1-30.29 | Foidl *et al*., 2001; Moyo *et al*., 2011 |
| Neutral detergent fiber | 11.40-21.9 | Moyo *et al*., 2011; Richter *et al*., 2003; Foidl *et al*., 2001 |
| Acid detergent fiber | 8.49-11.4 | Moyo *et al*., 2011; Richter *et al*., 2003; Foidl *et al*., 2001 |
| Gross energy (MJ/kg DM) | 18.7 | Foidl *et al*., 2001 |
| Ether extract | 5.4 | Foidl *et al*., 2001 |
| Lysine | 1.1-1.64 | Richter *et al*., 2003; Moyo *et al*., 2011 |
| Histidine | 0.6-0.72 | Richter *et al*., 2003; Moyo *et al*., 2011 |
| Threonine | 0.8-1.36 | Richter *et al*., 2003; Moyo *et al*., 2011 |
| Arginine | 1.2-1.78 | Richter *et al*., 2003; Moyo *et al*., 2011 |
| Methionine | 0.30 | Moyo *et al*., 2011 |
| Total phenolics | 2.02-2.74 | Moyo *et al*., 2011; Richter *et al*., 2003 |
| Tannins | 0.53 | Richter *et al*., 2003 |
| Condensed tannins (mg/g) | 3.12 | Moyo *et al*., 2011 |

**Source:** Rubanza *et al*. (2005)

### 2.4. Phytochemicals of *Moringa oleifera* Leaf

An examination of the phytochemicals of *Moringa* species affords the opportunity to examine a range of fairly unique compounds (Fahey *et al*., 2001). In particular, this plant family is rich in compounds containing the simple sugar, rahmnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanate (Bennett *et al*., 2003; Fahey *et al*., 2001). Some of the compounds that have been isolated from *Moringa* preparations which are reported to have hypotensive, anticancer and antibacterial activity include benzylsothiocyanate,4-(a-L-rhamnopyranosyloxy) benzyl glucosinolates, 4-(4'-*O*-acetyl-a-L-rhamnopyranosyloxy) benzyl,isothiocyanate, 4-(a-L-Rhamnopyranosyloxy)

and (Daxenbichler *et al*., 1991; Fahey *et al*., 2001; Bennett *et al*., 2003; Mekonnen and Drager, 2003).

Flowers of *Moringa* have been reported to contain flavonoid pigments such as quercetin, kaempferol, rhamnetin, isoquercitrin and kaempferitrin. Extracts of *Moringa* leaves in 80% ethanol contain cytokinine- type hormones (Foidl *et al*., 2001). Extracts of *Moringa* Leaves also appear to have cancer preventive effects, when assayed by the differentiating activity against human promyelocytic leukaemia cells (HL-60) (Siddhuraju and Becker, 2003). Seeds of *Moringa* contain a glucosinolates that on hydrolysis yields 4-(a-L-rhamnosyloxy)-benzyl isothiocyanate, an active bactericide and fungicide. *Moringa* root-bark yields two alkaloids: *Moringine* and *Moringinine* (Grubben and Denton, 2004).

### 2.5. Antioxidant in *Moringa oleifera* Leaf

Concentrations of four natural antioxidants (total phenolics and antioxidant vitamins A, C and E) were measured. The content ranges on a dry weight basis were 74-210 µmol/g for phenolics, 70-100 µmol/g for ascorbate (Vit C), 1.1-2.8 µmol/g for β-carotene and 0.7-1.1 µmol/g for α-tocopherol (Vit E) (Siddhuraju and Becker, 2003). Antioxidant contents of *Moringa* species are high even compared to vegetables and fruits known for high antioxidant contents such as strawberries high in phenolics (330 mg gallic acid (GA)/100g, or ~190 µmol GA/g) (Abbas and Ahmed, 2012); hot pepper high in ascorbate (200 mg/100g, or ~110 µmol/g) (Anwar and Bhanger, 2003) carrot high in β-carotene (10 mg/100g, or ~1.8 µmol/g) and soybean which is high in α-tocopherol (0.85 mg/100g, or ~1.8 µmol/g). *Moringas* are an excellent source of a wide spectrum of dietary antioxidants (Yameogo *et al*., 2011).

The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in *Moringa* leaves. Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in *Moringa* leaves (Siddhuraju and Becker, 2003). *M. oleifera* has been reported to possess some antioxidant properties (Sreelatha and Padma, 2009; Atawodi *et al*., 2010). Although

there are several enzyme systems within the body which scavenge free radicals, the natural (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C (Nair *et al*., 2005). These micronutrient antioxidants may be used as defence system to prevent free radicals from damaging the animal's body. This therefore provides protection to animals against infections and degenerative diseases (Sreelatha and Padma, 2009; Verma *et al.*, 2009).

### 2.6. Potential Toxicity of *Moringa oleifera*

No adverse effects were reported in any of the human studies that have been conducted and continued to be used around the world as foods and as medicine without the report of ill effects. The safety of an aqueous leaf extract given orally to rats at doses of 400, 800, 1600, and 2000 mg/kg body weight was examined (Jabeen *et al*., 2008). The treatment was either an acute single dose or given daily for 21 days except the highest dose. Various parameters were assessed including blood cell counts and serum enzyme levels. Consumption of *M. oleifera* leaves at doses of up to 2000 mg/kg was safe. A dose-dependent decrease in body weights of the rats occurred over the 21 days of the study (Adedapo *et al.,* 2009). *Moringa* seed powder can be toxic to animals and particularly to fish. This toxicity may be used in pond management to control predators of cultured fish (Adeniji and Lawa, 2012).

The median lethal concentration ($LC_{50}$) of *Moringa* seed powder in common carps was 124.0 mg/L after 96 h and the sub lethal concentration was 12.4 mg/L. Common carps exposed during 35 days at the latter concentration had all their blood parameters altered. In one set of experiment, human peripheral blood mononuclear cells were exposed *in vitro* to graded doses of the extract and cytotoxicity was assessed. Cytotoxicity occurred at 20 mg/kg, a concentration not achievable by oral ingestion. In another set of experiments, rats were given 1000 and 3000 mg/kg of the extract, and the animals were assessed for up to 14 days (Kavitha *et al.*, 2012).

The *M. oleifera* leaf extract was shown to be genotoxic based on blood cell analysis at the 3000 mg/kg dose, a dose that greatly exceeds commonly used doses. A dose of 1000 mg/kg was deemed safe and did not produce genotoxicity when given to rats, a dose still in excess of commonly used doses (Asare *et al*., 2012). In the sub-acute toxicity study, the 400 mg/kg dose of the extract caused significant increase in the level of packed cell volume (PCV) while the other 2 doses caused significant decrease. The 800 mg/kg dose on the other hand caused significant decrease in the levels of hemoglobin and red blood cell counts while the other 2 doses caused insignificant changes. The study therefore showed that the plant could precipitate some level of anemia if the animals are exposed to this plant for a long period of time. The varied changes of the effects of this plant extract on the haematological parameters may be attributable to the presence of isothiocyanate producing glycosides (Fahey, 2005).

Acute poisoning by hydrocyanic acid (HCN) or prussic acid causes a histotoxic anoxia with a syndrome of dyspnoea, tremor, convulsions and sudden death. Toxicity of hydrogen cyanide (HCN) occurs after ingestion and absorption. Once they are in the bloodstream, there is little difference between toxic and lethal levels of cyanide. HCN has a high affinity for iron and reacts with the trivalent iron of mitochondrial cytochrome oxidase, the terminal respiratory catalyst linking oxygen with metabolic respiration. The reduction of serum levels of protein due to *Moringa* is an indication that toxicants such as isothiocyanate and glycoside cyanides may cause stress-mediated mobilization of protein to cope with the detrimental condition so imposed (Das and Mukherjee, 2000).

Treatment of this poisoning is aimed at fixing the highly lethal cyanide ion in a harmless form, and then converting it into thiocyanate, which is readily excreted by the kidneys. Sodium nitrite can also be administered intravenously to convert some hemoglobin into methaemoglobin. Cyanide combines readily with methaemoglobin to form the non-toxic cyanomethaemoglobin. Sodium thiosulphate is then administered to act as a sulphur-donor for the conversion of the cyanide moiety of cyanomethaemoglobin to thiocyanate under the action of the enzyme rhodanase (Adedapo, 2002).

## 2.7. Inclusion of *Moringa oleifera* in Chicken Diets

The leaves are highly nutritious and contain significant quantities of vitamins (A, B and C), calcium, iron, phosphorus and protein (Murro *et al*., 2003). Furthermore, heavy metals such as mercury, arsenic and cadmium which are potentially toxic are absent from the leaves of *M. oleifera*, thus making their incorporation into poultry diet safe (Donkor *et al*., 2013). Chickens will not voluntarily consume *Moringa* leaves or *Moringa* leaf powder. However, about half the protein content can be extracted from the leaves in the form of a concentrate that can be added to chicken feed (Price, 2007).

The nutrient value of *Moringa* leaves can be increased for chickens through the addition of phytase to break down phytate leading to increased absorption of phosphorus. Phytase should be simply mixed with the leaves without heating (Fuglie, 2009). If uncontrolled, raw *M. oleifera* in poultry diets can be dangerous because of high bio-availability of protein; therefore particular care must be taken to avoid excessive protein intake (Gaia, 2005). *M. oleifera* seems to reduce the activity of pathogenic bacteria and moulds and improves the digestibility of other foods, thus helping chickens to express their natural genetic potential assessed the effects of MOLM inclusion in poultry diets on growth performances, carcass and organs' characteristics and production performance (Gaia, 2005 and Ayssiwede *et al*., 2011).

MOLM can be safely included in cassava-based layer diets up to 10% without negatively affecting productivity. The inclusion level of MOLM is lower for broilers compared to layers (Olugbemi *et al*., 2010b). MOLM has potential of a hypocholesterolemic agent using layers fed cassava-based diets and that *M. oleifera* possesses hypocholesterolemic properties and that it can be included in layers diets to facilitate reductions in egg cholesterol content (Olugbemi *et al*., 2010c).

## 2.8. Effect of *Moringa oleifera* Leaf Meal on Egg Production

Addition of 10% and 20% *Moringa oleifera* leaf meal to the laying hen diet as a substitute for sunflower seed meal in Isa brown breed significantly decreased total egg weight (Kakengi *et al*., 2007). Egg production percentage decreased with an increase of *M. oleifera* leaf meal level (Austic and Neisheim, 2004). Decrease in total egg weight production, egg production percentage, and average egg weight at a higher level of *Moringa oleifera* leaf meal, are mainly due to low digestibility of energy and protein (Olugbemi *et al.,* 2010a).

Supplementation of *Moringa oleifera* leaf meal at levels of up to 10% to laying hens had no significant effect on laying percentage (Abou-Elezz *et al.,* 2011). Average egg weight significantly increased as a result of the supplementation of *M. oleifera* leaf meal when compared to a control (Ebenebe *et al*., 2013). Addition of MOLM on Dominant CZ layers up to 10% had no effect on HDEP (hen day egg production), egg weight. Inclusion of different levels of *M. oleifera* leaf meal (0%, 5%, 10%, and 15%) in the laying hens' diets linearly decreased egg-laying percentage and egg mass, while egg weight showed a quadratic trend with the increased levels of *M. oleifera* leaf meal (Olugbemi *et al*., 2010a).

Use of *Moringa oleifera* leaf meal at levels above that (15% and 20%), are expected to produce adverse effects (Kakengi *et al*., 2007 and Abou-Elezz *et al*., 2011). The 5 % MOLM could have a beneficial nutritional impact for hens, while using higher levels (10 and 15 %) adversely affected the egg laying rate and egg mass production (Mutayoba *et al*., 2003). Also levels of 5% MOLM and below had no difference on the number of eggs laid with that 0% MOLM (Gakuya *et al*., 2014).

## 2.9. Effect of *Moringa oleifera* Leaf on Feed Intake, Body Weight Change and Feed Conversion Ratio

Addition of 10% and 20% *Moringa oleifera* leaf meal to the laying hen diet, as a substitute for sunflower seed meal in Isa brown breed, significantly increased feed intake and dry matter intake and Feed conversion ratio (kg egg/kg feed) increased when 20% *M. oleifera* leaf meal was added to the laying hen diet (Kakengi *et al*., 2007). Increase in feed intake and feed conversion ratio, and at a higher level of *M. oleifera* leaf meal, are mainly due to low digestibility of energy and protein (Olugbemi *et al.,* 2010a).

Inclusion of *Moringa oleifera* leaf meal at levels of up to 10% in a cassava chip-based diet offered to laying hens had no significant effect on feed intake and feed conversion ratio. Addition of MOLM in cassava based diets on Dominant CZ layers up to 10% had no effect on average DM intake, final BW, FE, and mortality rate of hens. Change in BW was higher as level of MOLM increases. Inclusion of different levels of *M. oleifera* leaf meal (0%, 5%, 10%, and 15%) in the laying hens' diets and feed intake showed a quadratic trend with the increased levels of *M. oleifera* leaf meal with the absence of a significant effect on feed conversion ratio (Kakengi *et al*., 2007; Olugbemi *et al*., 2010a and Abou-Elezz *et al*., 2011).

Use of *Moringa oleifera* leaf meal in the diet of Rhode Island Red chicks produced significant increase in feed intake, average weight change and feed conversion ratios when compared to a control diet (Melesse *et al*., 2011). Addition of 5% *M. oleifera* leaf meal to cassava-based broilers' diet (20% and30%) had no significant effect on feed conversion ratio, final body weight, and feed cost per kilogram of weight gain when compared to a diet free of cassava and free of *M. oleifera* leaf meal, a diet containing 20% cassava and 0% *M. oleifera* leaf meal, and a diet containing 30% cassava and 0% *M. oleifera* leaf meal. However, levels above 5% of *M. oleifera* leaf meal decreased broilers' performance (Olugbemi *et al*., 2010b).

The inclusion of *Moringa oleifera* leaf meal at amounts up to10% did not produce significant effects on feed consumption, body weight and feed conversion ratio (Juniar *et al.,* 2008). Feed intake and feed conversion ratio were improved significantly with the inclusion of MOLM in the broiler's diet. The diet supplemented with 5% MOLM showed significantly highest total feed intake with better feed conversion ratio as compared to the 0%, 3% and 7% MOLM containing experimental diets. The experimental treatments had no significant effect on the mortality rate. Only one bird from each treatment died, which cannot be related in any way to the experimental treatments (Safa and Tazi, 2014).

Feeds with MOLM included were well tolerated by the birds (Gakuya *et al*., 2014; Ashong and Brown, 2011; Djakalia *et al*., 2011 and Nuhu, 2010). Feed intake was low in the first week and this could be attributed to stress associated with change in environment and also to introduction of new feed in 0% to 10% MOLM for layers (Gakuya *et al*., 2014). Although there was a decreasing trend of feed intake with increase of MOLM in the layers diet, the decrease was not statistically significant (Nuhu, 2010 and Gakuya *et al*., 2014)). Various diets formulated with MOLM in weaner rabbits did not affect feed intake (Nuhu, 2010). Inclusion of MOLM in broiler feed did not affect feed intake up to 7.5% (Gakuya *et al*., 2014).

Inclusion of MOLM at the level of 0%, 25%, 50%, 75% and 100% did not affect feed intake but it affects feed conversion ratio of broilers. Feed intake increased as MOLM inclusion increased probably due to increased bulk and metabolizable concentration (Gadzirayi *et al.,* 2012). Significant progressive increase in feed intake was on birds fed 10% and 20% MOLM levels. The results show that there was no significant difference in mean feed intake between 0% MOLM) and 25% MOLM where dietary treatments did not show any significant effect on feed intake and dry matter intake up to 5% MOLM (Kakengi *et al*., 2007).

Final weight declined as MOLM level increased. In the study of supplementing soyabean meal with MOLM, mean weight of broilers was significantly different for 50% MOLM, 75% MOLM and 100% MOLM. However, there was no significant difference in the

mean weight of broilers between 0% MOLM and 25% MOLM (Gadzirayi *et al.,* 2012). Significant weight gain differences were noted between on100 and 0% MOLM and between 25% and 100% MOLM. The difference could be due to high fibre levels that were in treatment five with 100% MOLM in the diet as protein source. Monogastrics cannot utilize high crude fiber diets efficiently (Gadzirayi *et al.,* 2012).

### 2.10. Effects of *Moringa oleifera* Leaf Meal on Egg Quality

The yolk color values increased significantly and linearly with the inclusion level of MOLM and LLM (*Leucena* Leaf Meal) in RIR (Rhod Island Red) hens. All yolks obtained from the control group (0% leaf meal) in both experiments were whiter than the lowest degree of the yolk color fan. It is well documented that leaf meals are a good source for yolk pigments MOLM treatments had no adverse effects on any of shell proportion in the egg; shell thickness and egg shape index (Abou-Elezz *et al.,* 2011 and Kaijage *et al*., 2004).

*Moringa oleifera* leaf meal has high carotene content (Abou-Elezz *et al.,* 2011), (Etalem *et al*., 2013) which ranges from 15.25 to 16.30 (mg/100 g). The albumen height increased in inclusion of MOLM while the yolk index decreased as accompanying the increase in MOLM levels in the diet (Price, 2000 and Kaijage *et al*., 2004). Albumen height increased, with the inclusion of MOLM levels (Berry and D'Mello, 2000). Interestingly, having eggs with higher albumens and lower yolk index is implying relatively lower concentrations of cholesterol which is a good quality attribute for egg consumers (Kaijage *et al*., 2004).

In addition, using LLM or MOLM in the laying hens' diets increased significantly the yolk index, which is a good quality trait (Odunsi *et al*., 2002). Similar results have been observed when hens were fed on different levels of leaf meal of *Gliricidia sepium*), Siam weed (Fasuyi *et al*., 2005), *Mangrof* and *Tephrosia bractereolata* (Al-Harith, 2006). Meanwhile, no adverse effects were found on the shell weight (Akande *et al*., 2008).

Inclusion of *Moringa* at lower levels in Isa brown breed improved egg quality but higher levels of inclusion resulted in lower productivity and poorer egg quality indices (Abou-Elezz *et al.,* 2011). Chickens being monogastrics cannot handle appreciable quantity of vegetative material. The results were however in contrasts with results from other leaf meal. An increase in egg weight values with increase in MOLM inclusion level up to 20% (Mellau, 1999). Bhatnagar *et al.* (1996) however found non-significant effect on egg weights at 0%, 5% and 10% inclusion levels but egg weight was lowest at 20% inclusion level.

The substitution of sunflower with MOLM at 5 % levels in the diet showed a positive effect on egg weight but the reason of this could not be explained although probably might be associated with higher sulphur containing amino acids reported in *Moringa* leaves. It has a positive influence of sulphur containing amino acids on egg weight (Kakengi *et al.,* 2007). However, the substitution of sunflower seed meal with MOLM at 10 and 20% levels in the diet showed a moderate progressive depression of egg weight (North, 1990).

The decrease in weight at higher levels of MOLM was also not clear but probably was due lower energy and crud protein (CP) availability and also associated with lower digestibility of crud fiber (CF) component reported in various other leaf meals. However, variability of egg weights and weeks of age in birds fed different levels of MOLM observed was not clear but differences in the initial egg weights probably attributed to this trend (North, 1990). *M. oleifera* leaves have been extensively used as animal feeds. It has as a hypocholesterolemic agent in layers fed cassava based diets over a 90 day period and showed that it possesses hypocholesterolemic properties and its inclusion in layers diets facilitate reductions in egg cholesterol content (Olugbemi *et al.,* 2010b).

Weight of sampled eggs and albumen weight, egg length, Albumen weight, yolk index, and yolk color, Shell weight and yolk height, yolk weight in CZ layers are increased with 5% MOLM cassava based supplementation of MOLM. But, egg width, egg shape index, shell thickness, Haugh unit, and yolk length are not affected by supplementation of MOLM (Etalem *et al.,* 2014). *M. oleifera* leaf meal had no influence on egg weight

(Olugbemi *et al*., 2010b). No big differences in egg weights from layers fed on *M. oleifera* leaf and twig meals at different levels ranging from 0.2 to 0.8%. There was no a consistent trend in yolk color as levels of *M. oleifera* leaf and twig powder were increased from 0.2 to 0.8% (Paguia *et al*., 2012). The result revealed that there was no significant difference in the value obtained for albumen index. Yolk index values showed significance. The result obtained for Haugh unit showed that there was no significant difference among the treatment group (Olabode and Okelola, 2014).

### 2.11. Effects of *Moringa oleifera* Leaf Meal on Shelf Life of Eggs

*Moringa oleifera* is among the most promising species based on their high antioxidant activity, high contents of micro-nutrients and phytochemicals, processing properties, ease of growing, and also on palatability, stability and shelf life of poultry product (Yang *et al.,* 2006 and Jung *et al*., 2010). The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in *Moringa* leaves (Foidl *et al*., 2001). Their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in *Moringa* leaves and used to prolong shelf life of poultry products (Siddhuraju and Becker, 2003).

The total antioxidant capacity of fruit and vegetable extracts reflects concentrations of ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), beta-carotene (vitamin A precursor), various flavonoids, and other phenolic compounds (Pennington and Fisher, 2009). Some authors have demonstrated the high antioxidant activity of a-tocopherol, ascorbic acid, and their high radical scavenging abilities (Kulisic *et al*., 2004). Furthermore, some synergistic effects among ascorbic acid, a-tocopherol, and ß-carotene have been reported against oxidation (Yeum *et al*., 2009). In addition, phenolic compounds have a high antioxidant activity through three mechanisms: free-radical scavenging activity (Zheng *et al*., 2009), transition-metal-chelating activity (Andjelkovic *et al*., 2006), and/or singlet-oxygen quenching capacity (Mukai *et al*., 2005).

## 2.12. Effects of *Moringa oleifera* Leaf Meal on Fertility and Hatchability of Eggs

Fertility, hatchability, and chick quality parameters appeared to be not negatively affected by the dietary inclusion of MOLM. Hatchability of fertile eggs was actually improved in the diets containing MOLM as compared with the control diet in dominant CZ layers (Etalem *et al.,* 2014). *M. oleifera* leaf contains higher levels of zinc and vitamin E, which can play a beneficial role in hatchability of eggs (Moyo *et al.,* 2011; Park *et al*., 2004 and Mahmood and Al-Daraji, 2011). With increasing zinc concentration in the diets, increased hatchability of Brown parent stock layers (Durmus *et al.,* 2004).

Zinc helps in protecting the structure of the genetic material or the DNA chromatin in the sperm nucleus, a structure important for successful fertilization (Brown and Pentland, 2007). *M. oleifera* contains significant amount of iron, phosphorus, calcium, and is relatively rich in vitamin C (Agbaje *et al*., 2007). As a result of ascorbic acid supplementation to diets hatchability of indigenous Venda hens were improved (Adesola *et al.,* 2012). However, the relatively poor hatchability and higher embryonic mortality observed in the control group might be happening due to a deficiency in critical nutrients, such as zinc, vitamin E, and so on, which are important for better hatchability (Park *et al.,* 2004 and Mahmood and Al-Daraji, 2011).

## 2.13. Effect of *Moringa oleifera* Leaf Meal on Chick Quality and Embryonic Mortality

Chick weight was higher for 5% MOLM and 50% CRC with 5% MOLM as compared with 50% CRC, whereas the value for 0% MOLM was not different from other treatments (Etalem *et al*., 2014). Chick weight becomes increasing as protein level increases in the diet of chicken (Coon *et al.,* 2006). Chick length and yield percentage were not significantly affected by treatment. Supplementation with seleno-methionine has been shown to improve the anti-oxidative status of eggs, embryos and chicks (Hubbard, 2011).

Early embryonic mortality was higher for treatment containing 0% MOLM treatment containing 5% MOLM. Mid-embryonic mortality was lower for 5% MOLM than feed having 0% MOLM. Late embryonic mortality was not affected by treatment (Etalem *et al*., 2014). Inclusion of Major minerals (Calcium, phosphorus, sodium, potassium and magnesium) in chicken diets generally improves embryo and chick quality (Hubbard, 2011).

# 3. MATERIALS AND METHODS

## 3.1. Study Area

The experiment was conducted at Debre Zeit Agricultural Research Center (DZARC), located 47 km South East of Addis Ababa at an altitude of 1900 meters above sea level and at $8^{o}44'$ N latitude and $38^{o}$, 38' E longitude. The average annual rainfall and average minimum and maximum temperatures for the area are 1100 mm, and 8.9 $^{o}$C and 28.3 $^{o}$C, respectively (DZARC, 2003).

## 3.2. Experimental Rations and Treatments

Feed ingredients used in the formulation of the experimental rations for the study were corn grain, wheat middling, Noug seedcake, SBM, MOLM, meat and bone meal, vitamin premix, salt limestone, lysine and methionine (Table 3). Leaf was harvested from young *Moringa oleifera* trees of about four years of age from an orchard found in DZARC poultry farm. The harvested leaves from the tree were spread out on a concrete floor and allowed to dry for a period of three days under shade and aerated conditions then run through a hammer mill sieve with a size of five mm to produce the leaf meal. All the ingredients, except wheat middling, SBM, vitamin premix, lysine and methionine were also milled in sieve size of five mm and stored until required for the formulation of experimental rations.

Based on the chemical analysis result, 4 treatment rations (i.e., $T_1$= diet containing 0% MOLM; $T_2$= diet containing 5% MOLM; $T_3$ = diet containing 10% MOLM; and $T_4$= diet containing 15% MOLM) was formulated to which MOLM substitutes SBM. Treatment rations were formulated to be nearly isocaloric and isonitrogenous (Table 3), to meet the minimum ME of 2,750 kcal/kg of DM and 16.5% of CP requirement of laying hens (Leeson and Summers, 2005).

**Table 3:** Proportion **(%)** of ingredients used for formulating experimental diets

| Ingredient (%) | Treatment | | | |
|---|---|---|---|---|
| | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** |
| Corn grain | 63 | 61.8 | 58 | 53.3 |
| Wheat middling | 6.3 | 2 | 2.8 | 6 |
| Noug seedcake | 4.5 | 10 | 12 | 12.5 |
| SBM | 18.0 | 13.0 | 8.0 | 3.0 |
| MOLM | 0.0 | 5.0 | 10.0 | 15.0 |
| Meat and bone meal | 1 | 1 | 2 | 3 |
| Vitamin premix | 0.5 | 0.5 | 0.5 | 0.5 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 |
| Limestone | 6 | 6 | 6 | 6 |
| Methionine | 0.1 | 0.1 | 0.1 | 0.1 |
| Lysine | 0.3 | 0.3 | 0.3 | 0.3 |
| Total % | 100 | 100 | 100 | 100 |
| CP% | 16.56 | 16.60 | 16.62 | 16.63 |
| ME (kcal/kg DM) | 2759 | 2765 | 2767 | 2769 |
| DM % | 89.48 | 89.56 | 89.60 | 89.68 |
| Ash (% DM) | 8.93 | 9.20 | 10.90 | 10.97 |
| EE (% DM) | 6.55 | 7.14 | 7.26 | 7.34 |
| CF (% DM) | 6.14 | 7.05 | 7.20 | 7.26 |

MOLM: *Moringa oleifera* leaf meal; SBM: soybean meal; $T_1$: No MOLM inclusion; $T_2$: 5%; $T_3$: 10%; $T_4$: 15% MOLM of the total ration substituting SBM; CP: Crude Protien; DM: Dry Matter.

### 3.3. Experimental Design

Completely randomized design (CRD) was employed in this experiment. Ninety six dual purpose Koekeok laying hens and 12 cocks at the age of 41 weeks were equally divided into four dietary treatments with three replications. Birds were sourced from DZARC poultry farm. Twenty four hens and 3 cocks were used in each dietary treatment, which

were further divided into three groups of eight hens and one cock. Nine birds were randomly assigned to one of the 12 pens.

### 3.4. Management of Experimental Birds

A wire mesh partitioned deep litter floor house covered with disinfected Teff straw litter material was used. Before the commencement of the actual experiment, the experimental pens, watering and feeding troughs, and laying nests were thoroughly cleaned, disinfected, and sprayed against external parasites. Hens were vaccinated against Newcastle, Gumboro (infectious bursal disease), fowl typhoid, and fowl pox diseases.

Other health precautions and sanitary measures were also taken throughout the study period. Birds were fed the experimental ration at 130 g/bird per day but this amount was adjusted with regard to the level of their production throughout the experimental periods (NRC, 1994). Clean water was available at all times. Diets were offered in a round feeder and water in a plastic fountain. Fluorescent lamp was placed for the lighting system to increase the lighting period to 16 h per day in order to increase feed intake and laying (Yasmeen *et al*., 2008). Birds were adapted to respective treatment diet for a week before the commencement of the actual data collection.

### 3.5. Feed Intake, Body Weight Change and Feed Conversion Ratio

The experimental period lasted for 12 weeks from November-January during which the amount of feed offered to and refused from birds per pen was recorded daily. The amount of feed consumed per bird was determined as the difference between the feed offered and refused. Feed offered and refused was sampled per day and pen, and pooled per treatment for the entire experimental period for chemical analysis. Hens were weighed at the start and end of the experiment and body weight (BW) change was calculated as the difference between the final and initial BW. Feed conversion ratio was determined as a unit egg weight per unit feed consumed (Abou-Elezz *et al*., 2011). Mortality was registered as it occurred.

### 3.6. Egg Weight, Hen Day Egg Production and Hen Housed Egg Production

Eggs were collected three times a day from each pen at 0800, 1300 and 1700 hours. The sum of the three collections along with the number of birds alive on each day was recorded and summarized at the end of the period. Eggs collected daily were weighed immediately after collection for each pen and average egg weight was computed by dividing the total egg weight to the number of eggs. Hen-day egg production (HDEP) and hen housed egg production as percentage were determined following the method of Hunton (1995).

%HDEP = total number of eggs produced/total number of hens present on that day x100

%HHEP = total number of eggs produced/number of hens originally housed x100

### 3.7. Measurement of Egg Quality and Shelf Life

Egg quality parameters were assessed at the middle and end of the experiment in terms of egg weight and shape index externally and the internal egg quality parameters were assessed by breaking eggs on a flat glass and separating each of the components such as shell weight, shell thickness, yolk color, yolk weight, yolk length, yolk height, yolk index, albumen weight, albumen height and Haugh unit.

The shell, albumen and yolk were carefully separated and weighed individually using a sensitive balance of 0.01 g precision. Shell weight and thickness were taken by removing the internal membrane. Shell thickness was measured as the average of the blunt, middle and sharp points of the egg by using a digital micrometer. Albumen and yolk height were measured by tripod micrometer (Safaa *et al*., 2008b). Haugh unit (HU) was calculated using the formula (100 log10 (h + 7.57 − 1.7w0.37, (Keener *et al*., 2006): where; h = observed albumen height (mm), w = weight of egg (g).

Yolk color was determined by comparing the color of properly mixed yolk sample with the color strips of Roche color fan measurement, which consists of 1-15 strips ranging

from pale to orange yellow in color. Length and width of the egg and the length of yolk were measured by using digital caliper and the egg and yolk shape indexes were computed according to (Safaa *et al*., 2008a) and (Ebrahimi *et al*., 2012) respectively.

Egg shape index = width of egg/length of egg x100

Yolk index = yolk height/yolk length

For the measurement of egg shelf life, eggs were stored for 7, 14, 21 and 28 days at room temperature, 12 eggs per treatment were randomly taken and albumen and yolk measurements were taken and their pH by using pH meter twice during the experimental period at the middle and end of the experiment (Olugbenga *et al.,* 2015).

### 3.8. Fertility and Hatchability of Eggs

Eggs for incubation were collected towards the end of the study ($11^{th}$ weeks of lay) and stored for seven days at a temperature of 10-14 $^{o}$C. Medium or average sized eggs (30 eggs for each replication) were selected and used for incubation. Fertility was checked by candling the incubated eggs on the $9^{th}$ day of incubation in the dark room with egg Candler. Average percentage fertility was determined by dividing the total number of eggs found fertile at candling by total number of eggs set times 100.

Average percentage hatchability of the fertile eggs were computed by dividing the number of chicks hatched by the number of fertile eggs times 100. Embryonic mortality of the incubated eggs at different stages was determined by breaking of eggs at the end of the incubation that seemed to be mortal to determine early, mid and late embryonic mortalities, and all unhatched eggs were broken and opened to determine the age at death (Bonnier and Kasper, 1990).

### 3.9. Chick Quality

Chick quality assessment was performed by employing chick weight, chick length and yield percentage at hatching as well as by considering visual scoring. Chick length was determined by stretching the chick along a ruler and measuring the length from beak to the end of the middle toe. Chick weight was measured by weighing the chick at hatching. Yield percentage that evaluates the weight loss during incubation was calculated as the percentage of average chick weight to average initial weight of eggs set for incubation (Molenaar, 2009). Visual scoring was determined by considering whether the chick is clean, dry, free of deformities or lesions, had bright eyes or not (Reijrink *et al*., 2010b).

### 3.10. Laboratory Analysis

Samples of feed offered to and refusal from birds and the formulated diets from the respective treatments were analyzed for DM, CF, total ash, EE and Kjeldahl nitrogen (N). The CP content was determined as N x 6.25 (AOAC, 1998).

### 3.11. Economic Analysis

To estimate the profitability of feeding MOLM, the partial budget was calculated as the difference between the feed costs incurred during the experimental period per bird and sale of eggs and birds. The net income (NI) was calculated by subtracting total cost (TC) from the total return (TR).

$$NI = TR - TC$$

$$TC = FC/bird = (TFI \times cost/100Kg)/100,$$

Where, TFI= Total feed intake and FC = feed cost

The marginal rate of return (MRR) which measures the increase in net income (ΔNI (NI from diets with MOLM minus NI from control diets)) associated with each additional unit

of expenditure (ΔTC (TC from diets with MOLM minus TC from control diets)) was computed as:

MRR = ΔNI/ΔTC (Miles and Jacob, 2000).

### 3.12. Statistical Analysis

Data were analyzed using the general linear model procedures of Statistical Analysis Systems software with the model containing treatments. Differences between treatment means were separated using Tukey Kramer test (SAS, 2009). Significant differences were declared at ($p<0.05$). The following model was used for the analysis:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where, $Y_{ij}$ = individual measurement on each bird
$\mu$ = overall mean effect
$T_i$ = effect due to the $i^{th}$ dietary treatment
$e_{ij}$ = error term

# 4. RESULTS

## 4.1. Chemical Composition of Experimental Feeds

The chemical composition of experimental diets for dual purpose Koekoek hens is shown in Table 3. The CP and ME contents were similar across treatments, since the diets were formulated to be iso-nitrogenous and iso-caloric. The DM and EE contents were also similar across the treatments even if they show an increasing trend. The ash and CF contents were a bit higher for T3 and T4, although all are within the recommended levels for feeding chickens.

## 4.2. Feed Intake, Body Weight Change and Feed Conversion Ratio

The effect of replacing soybean meals (SBM) by *Moringa oleifera* leaf meal (MOLM) at different levels on feed intake, body weight change and feed conversion ratio of dual purpose Koekeok hens presented in Table 4. Total feed intake was higher ($P<0.05$) for hens in $T_1$ and $T_3$ as compared to the others with the lowest intake recorded for $T_2$. Average initial body weight was similar along treatments. Final average weight was higher ($P<0.05$) for hens in $T_2$ and $T_3$ than hens in $T_4$ and $T_1$. Hens in $T_2$ had higher ($P<0.05$) body weight change than hens in $T_3$ and $T_4$ but not significantly differ ($p>0.05$) with $T_1$. Also, hens in $T_1$ had no significant difference ($P>0.05$) in weight change with hens in $T_3$. The feed conversion ratio (kg egg/kg feed) was higher ($P<0.05$) for $T_2$ than the rest of the treatments, with no differences ($P>0.05$) between the other treatments. Mortality rate was not affected by the dietary treatment only one bird in each treatment was died.

**Table 4:** Feed intake, body weight change and conversion efficiency of dual purpose Koekoek hens fed different levels of MOLM for 12 weeks

| Parameters | Treatments | | | | |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | Sig. |
| TFI (kg) | 10.73±0.01$^{a}$ | 10.16±0.04$^{c}$ | 10.73±0.07$^{a}$ | 10.37±0.01$^{b}$ | * |
| IABW (kg) | 1.54± 0.02$^{a}$ | 1.54±0.02$^{a}$ | 1.54±0.01$^{a}$ | 1.54±0.01$^{a}$ | NS |
| FABW (kg) | 1.86±0.01$^{b}$ | 1.93±0.02$^{a}$ | 1.90±0.01$^{ab}$ | 1.77±0.01$^{c}$ | * |
| BWC (kg) | 0.32±0.02$^{ab}$ | 0.38±0.01$^{a}$ | 0.35±0.01$^{b}$ | 0.22±0.02$^{c}$ | * |
| FCR (kg egg/kg feed) | 1.73±0.04$^{b}$ | 2.10±0.03$^{a}$ | 1.52±0.01$^{b}$ | 1.59±0.10$^{b}$ | * |

*: P<0.05; Means followed by the same letter in rows do not differ statistically from one another by the Tukey test at 5% probability; MOLM: *Moringa oleifera* leaf meal; SBM: Soybean meal; $T_1$: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM; NS: Non-significant; TFI: Total feed intake; IABW: Initial average body weight; FABW: Final average body weight; BWC: Body weight change; FCR: Feed conversion ratio.

### 4.3. Egg Production

Substitution of hens with 5% MOLM resulted in higher (P<0.05) total egg weight (TEW) and hen day egg production (HDEP) than the rest of the treatments. Supplementation at 10% and 15% did not result in statistically significant differences (P>0.05) in TEW and HDEP% than the control. Hen housed egg production (HHEP %) was higher (P<0.05) for hens in $T_2$ than the rests. But, there was no statistical differences (P>0.05) between$T_1$, $T_3$ and $T_4$ in their HHEP (Table 5).

**Table 5**: Egg production of dual purpose Koekoek hen in different dietary levels of MOLM

| Parameters | Treatments | | | | Sig. |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | |
| TEW (kg) | 18.49±0.46[b] | 21.37±0.38[a] | 16.28±0.15[b] | 16.45±1.08[b] | * |
| HDEP (%) | 50.69±0.18[b] | 64.60±0.17[a] | 45.23±2.89[b] | 47.65±1.67[b] | * |
| HHEP (%) | 47.02±1.26[b] | 61.91±0.46[a] | 44.57±2.91[b] | 46.90±6.33[b] | * |

*: P<0.05; Means followed by same letter in rows do not differ statistically from one another by the Tukey test at 5% probability; MOLM: *Moringa olifera* leaf meal; SBM: Soybean meal; $T_1$: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM; TEW: Total egg weight; HDEP: Hen day egg production; HHEP: Hen housed egg production.

### 4.4. Egg Quality

Egg quality parameters of 41 weeks aged dual purpose Koekoek hens fed varied dietary MOLM levels is presented in Table 6. Egg length was higher for $T_2$ ($P<0.05$) than the others. It was in the order of $T_2>T_3>T_4>T_1$ with significant difference between treatments. Egg width was also higher ($P<0.05$) in $T_2$ than $T_1$, $T_3$ and $T_4$. Hens in $T_3$ and $T_4$ had higher egg width than hens in $T_1$ with no differences ($P>0.05$) between $T_3$ and $T_4$. Significantly higher ($P<0.05$) egg shape index was observed in $T_1$ than in $T_2$ and $T_3$ with no differences between $T_2$ and $T_3$, and $T_3$ and $T_4$, but hens in $T_2$ had less egg shape index than $T_4$.

Egg weight was higher ($P<0.05$) in $T_2$ than $T_1$, $T_3$ and $T_4$. $T_2$ also had higher ($P<0.05$) egg shell thickness than others. $T_1$, $T_3$ and $T_4$ had no significant difference between each other in their shell thickness. Shell weight was higher ($P<0.05$) in $T_2$than other treatments. Haugh unit was higher ($P<0.05$) in $T_2$ than the rest of the treatments. Similarly, Yolk weight was significantly higher ($P<0.05$) for $T_2$ than others. Albumen height was higher ($P<0.05$) for $T_2$ than $T_1$, but was same ($P>0.05$) with that for the rest of the treatments. Albumen weight was higher ($P<0.05$) for $T_2$ than all the remaining treatments. Similarly, $T_3$ had higher ($P<0.05$) albumen weight than $T_1$, but was similar ($P<0.05$) with that of $T_4$, which had statistically similar albumen weight with T1.

Yolk color was higher ($P<0.05$) with the same value for $T_3$ and $T_4$ than that of $T_1$ and $T_2$. Hens in control had the poorest yolk color. It was in the order of $T_1<T_2<T_3=T_4$. Yolk length was highest ($P<0.05$) for $T_2$ than that of for hens in the rest of the treatments. Hens in $T_1$ had the lowest yolk length as compared to that for $T_3$ and $T_4$, which had similar yolk length. Yolk height was the least for $T_1$ than the rest of the treatments, which had similar yolk height. Hens in $T_2$ had higher yolk index ($P<0.05$) than hens in $T_1$, but yolk index did not vary between $T_3$ and $T_4$. Similarly, hens in $T_1$ had similar yolk index with hens in $T_3$ and $T_4$. Fig. 1 shows yolk color determination by Roche color fan and measuring egg length by digital micrometer.

**Table 6:** Effects of feeding different levels of MOLM a substitute of SBM on egg quality parameters of dual purpose Koekoek hens

| **Parameters** | **Treatments** | | | | **Sig.** |
|---|---|---|---|---|---|
| | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | |
| Egg length(mm) | $46.40\pm0.02^{d}$ | $55.02\pm0.35^{a}$ | $51.83\pm0.33^{b}$ | $50.46\pm0.01^{c}$ | * |
| Egg width (mm) | $37.55\pm0.24^{c}$ | $41.71\pm0.21^{a}$ | $40.02\pm0.52^{b}$ | $39.76\pm0.12^{b}$ | * |
| Egg shape index | $80.92\pm0.56^{a}$ | $75.80\pm0.10^{c}$ | $77.23\pm0.76^{bc}$ | $78.79\pm0.25^{ab}$ | * |
| Egg weight (g) | $48.66\pm0.36^{b}$ | $54.51\pm0.47^{a}$ | $49.94\pm0.91^{b}$ | $50.31\pm0.33^{b}$ | * |
| AST (mm) | $0.29\pm0.003^{b}$ | $0.38\pm0.003^{a}$ | $0.31\pm0.01^{b}$ | $0.30\pm0.01^{b}$ | * |
| Shell weight (g) | $5.67\pm0.33^{b}$ | $8.66\pm0.33^{a}$ | $6.33\pm0.66^{b}$ | $6.00\pm0.57^{b}$ | * |
| AH(mm) | $5.33\pm0.33^{b}$ | $7.33\pm0.33^{a}$ | $5.66\pm0.33^{ab}$ | $7.00\pm0.57^{ab}$ | * |
| Haugh unit | $75.33\pm0.33^{b}$ | $87.33\pm0.33^{a}$ | $79.00\pm0.57^{b}$ | $77.67\pm1.45^{b}$ | * |
| AW (g) | $21.67\pm0.33^{c}$ | $29.66\pm1.20^{a}$ | $25.33\pm0.33^{b}$ | $23.33\pm0.88^{cb}$ | * |
| Yolk weight (g) | $15.33\pm0.33^{b}$ | $20.66\pm0.33^{a}$ | $17.00\pm1.00^{b}$ | $17.33\pm0.33^{b}$ | * |
| Yolk color | $1.00\pm0.00^{c}$ | $8.66\pm0.33^{b}$ | 11.33±0.33a | 11.33±0.33a | * |
| YL(mm) | $40.37\pm0.22^{c}$ | $45.50\pm0.30^{a}$ | $42.96\pm0.29^{b}$ | $42.19\pm0.19^{b}$ | * |
| YH(mm) | $11.00\pm 0.57^{b}$ | $15.66\pm 0.33^{a}$ | $14.00\pm 0.57^{a}$ | $13.66\pm 0.33^{a}$ | * |
| Yolk index | 0.27±0.57b | $0.34\pm0.33^{a}$ | $0.33\pm0.57^{ab}$ | $0.32\pm0.33^{ab}$ | * |

*: $P<0.05$; Means followed by same letter in rows do not differ statistically from one another by the Tukey test at 5% probability. MOLM: *M. oleifera* leaf meal; SBM: Soybean meal; T: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM; AST: Average Shell Thickness; AH: Albumen Height; AW: Albumen Weight; YL: Yolk Length; YH: Yolk Height.


**Fig 1**: Yolk color determination by Roche color fan (left) and measuring egg length by digital micrometer (right)

### 4.5. Egg Shelf Life

*4.5.1. Yolk and albumen pH*

The effect of feeding different levels of dietary MOLM on yolk and albumen pH of eggs of dual purpose Koekoek hens is shown Table 7. The results revealed that the yolk pH of eggs stored for 7 days was the lowest ($P<0.05$) for $T_2$ than the rest of the treatments. Although $T_1$ and $T_3$ had similar yolk pH at 7 days of storage, but their yolk pH was lower than $T_4$. Also, the yolk pH of eggs stored for 14 days was the lowest ($P<0.05$) for $T_2$ than the rest of the treatments, but all other treatments had similar yolk pH. In the same way, the yolk pH of eggs stored for 21 days was lower ($P<0.05$) for $T_2$ than for $T_4$.But $T_1$ and $T_3$ were not significantly differing with $T_4$. The pH of eggs stored for 28 days was the lowest for $T_2$ than eggs in the rest of the treatments, but eggs in $T_1$ had higher ($P<0.05$) pH than eggs in $T_3$, which had no significant difference with pH of eggs in $T_4$.

At 7 days of storage, $T_2$ had lower mean albumen pH but, was not statistically different ($P<0.05$) with $T_1$ and $T_3$. Hens in $T_4$ had higher mean value of than $T_2$ but were not significantly different with $T_1$ and $T_3$. At 14 days of storage, $T_1$ had higher ($P<0.05$)

albumen pH than $T_2$ and $T_3$ but it was not statistically different ($P>0.05$) with $T_4$. Lower pH was recorded for eggs from $T_2$ than the others. Hens in $T_1$ showed higher ($P<0.05$) albumen pH than $T_2$ and $T_3$ but was not significantly different ($P>0.05$) with $T_4$ at 21 days of storage. Eggs from $T_2$ had lower pH than other treatments. At 28 days of storage, lower mean value ($P<0.05$) was recorded in $T_2$ than others. There was no significant difference ($P>0.05$) in albumen pH between $T_1$, $T_3$ and $T_4$. Fig. 2 shows a pH meter being used by the researcher in laboratory for measuring the pH of albumen and yolk.

**Table 7:** Effects of MOLM on egg shelf life of dual purpose Koekeok hens in terms of yolk and albumen pH at 7, 14, 21 and 28 days of storage time.

| **Parameters** | **Treatments** | | | | **Sig.** |
|---|---|---|---|---|---|
| | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | |
| Yolk pH, EST(d) | | | | | |
| 7 | $6.32\pm0.05^{b}$ | $6.15\pm0.01^{c}$ | $6.39\pm0.03^{b}$ | $6.55\pm0.02^{a}$ | * |
| 14 | $6.52\pm0.09^{a}$ | $6.23\pm0.01^{b}$ | $6.51\pm0.01^{a}$ | $6.66\pm0.008^{a}$ | * |
| 21 | $6.61\pm0.13^{ab}$ | $6.29\pm0.02^{b}$ | $6.61\pm0.05^{ab}$ | $6.76\pm0.01^{a}$ | * |
| 28 | 7.05±0.10a | 6.51±0.01c | 6.81±0.02b | 6.97±0.02ab | * |
| Albumen pH, EST(d) | | | | | |
| 7 | $8.34\pm0.08^{ab}$ | $8.18\pm0.02^{b}$ | $8.32\pm0.03^{ab}$ | $8.66\pm0.18^{a}$ | * |
| 14 | $8.78\pm0.06^{a}$ | $8.21\pm0.02^{c}$ | $8.44\pm0.01^{b}$ | $8.75\pm0.003^{a}$ | * |
| 21 | $8.92\pm0.01^{a}$ | $8.24\pm0.008^{c}$ | $8.58\pm0.02^{b}$ | $8.86\pm0.03^{a}$ | * |
| 28 | $8.97\pm0.05^{a}$ | $8.30\pm0.04^{b}$ | $8.83\pm0.01^{a}$ | $8.94\pm0.008^{a}$ | * |

*: $P<0.05$; Means followed by same letter in rows do not differ statistically from one another by the Tukey test at 5% probability. MOLM: *Moringa oleifera* leaf meal; SBM: Soybean meal. $T_1$: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM; EST: egg storage time; d: days.

**Fig 2:** Measuring pH of albumen and yolk by using pH meter

*4.5.2. Yolk and albumen measurements*

The effect of MOLM on yolk and albumen measurements at different storage times is summarized in Table 8. The result indicated that different levels of dietary treatment of MOLM as substitution of SBM had statistically significant effect on those parameters regarding with storage time. At 7 days of storage, higher ($P<0.05$) albumen height was recorded in $T_2$ than $T_1$ and $T_3$ but was not significantly different ($P>0.05$) with $T_4$. At 14 days of storage, $T_2$ showed similar value with that of 7 days of storage than the others.

Hens in $T_1$ had lower mean value of albumen height than the rest but not was statistically different ($P>0.05$) with $T_3$. Also there was no significant difference ($P>0.05$) between $T_3$ and $T_4$. At 21 days of storage, higher value ($P<0.05$) of albumen height was recorded in $T_2$ than the others. There was no significant difference between $T_1$, $T_3$ and $T_4$. At 28 days of storage, lower value ($P<0.05$) was observed in $T_1$ of albumen height than others but it did not vary with $T_4$. At 28 days of storage, eggs of hens in $T_2$ had significantly ($P<0.05$) higher albumen height than the rest of the treatments. Albumen height of hens in $T_3$ was not statistically different ($P>0.05$) with that of in $T_4$.

Haugh unit was affected by higher mean value obtained in $T_2$ and it was significantly different ($P<0.05$) with others at 7 days of storage. There was no significant difference between $T_1$, T3 and $T_4$. At 14 days of storage, lower value was obtained in $T_1$ than the rest, while higher value was measured in $T_2$. There was no significant difference ($P<0.05$) between $T_3$ and T4. At 21 days of storage, higher mean value for Haugh unit was recorded

in $T_2$, while lower value was recorded in $T_1$. There was no significant difference ($P<0.05$) in Haugh unit between $T_3$ and $T_4$. At 28 days of storage, $T_1$ had lower mean value of Haugh unit than others and $T_2$ had higher Haugh unit than the rest. Eggs of hens in $T_3$ and $T_4$ were not statistically different ($P>0.05$) in their Haugh unit with each other.

Higher albumen weight was obtained from eggs of birds fed in $T_2$ than others. The albumen weight in $T_1$, T3 and $T_4$ at 7 days of storage was not significantly ($P>0.05$) different. At 14 days of storage, higher ($P<0.05$) albumen weight was obtained in $T_2$ than the rest, while lower one was recorded in $T_1$ but it did not significantly differ with $T_3$. There was no also significant difference ($P>0.05$) in albumen weight between $T_3$ and $T_4$. At 21 days of storage, $T_1$ had lower value whereas $T_2$ had higher value than others. But, there was no significant difference ($P>0.05$) between $T_3$ and $T_4$. Similarly, lower and higher mean value of albumen weight was recorded in T1 and T2 respectively than the rest of treatments. There was statistical difference ($P>0.05$) between $T_3$ and T4 in albumen weight at 28 days of storage.

**Table 8:** Effects of MOLM on egg shelf life of dual purpose Koekoek hens in terms of albumen and Haugh measurements at different storage times (7, 14, 21 and 28 days of storage).

| Parameters | Treatments | | | | |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | Sig. |
| Albumen height, EST(d) | | | | | |
| 7 | 6.00±0.00$^{b}$ | 8.00±0.00$^{a}$ | 6.00±0.00$^{b}$ | 7.00±1.00$^{a}$ | * |
| 14 | 4.66±0.33$^{c}$ | 8.00±0.00$^{a}$ | 5.33±0.33$^{cb}$ | 6.00±0.00$^{b}$ | * |
| 21 | 4.33±0.33$^{b}$ | 7.00±0.00$^{a}$ | 5.00±0.00$^{b}$ | 5.00±0.00$^{b}$ | * |
| 28 | 4.00±0.00$^{c}$ | 6.66±0.33$^{a}$ | 5.33±0.33$^{b}$ | 4.33±0.33$^{cb}$ | * |
| Haugh unit, EST(d) | | | | | |
| 7 | 80.00±0.00$^{b}$ | 87.33±0.33$^{a}$ | 80.33±0.33$^{b}$ | 79.67±0.33$^{b}$ | * |
| 14 | 74.33±0.33$^{c}$ | 87.33±0.33$^{a}$ | 80.00±0.57$^{b}$ | 78.66±0.33$^{b}$ | * |
| 21 | 71.66±0.33$^{c}$ | 85.66±0.33$^{a}$ | 78.66±0.33$^{b}$ | 77.00±0.57$^{b}$ | * |
| 28 | 69.00±0.57$^{c}$ | 85.00±0.57$^{a}$ | 77.00±0.57$^{b}$ | 74.66±0.66$^{b}$ | * |
| Albumen weight, EST(d) | | | | | |
| 7 | 22.00±0.00$^{b}$ | 30.00±1.15$^{a}$ | 23.00±1.00$^{b}$ | 23.67±0.88$^{b}$ | * |
| 14 | 18.66±0.33$^{c}$ | 30.00±1.15$^{a}$ | 21.00±0.57$^{cb}$ | 22.33±0.33$^{b}$ | * |
| 21 | 16.00±0.57$^{c}$ | 28.00±0.57$^{a}$ | 20.33±0.33$^{b}$ | 20.33±0.33$^{b}$ | * |
| 28 | 14.33±0.33$^{c}$ | 26.00±0.57$^{a}$ | 20.33±0.33$^{b}$ | 19.66±0.33$^{b}$ | * |

*: P<0.05; Means followed by same letter in rows do not differ statistically from one another by the Tukey test at 5% probability; MOLM:*Moringa oleifera* leaf meal; SBM: Soybean meal; $T_1$: Ration containing 0% MOLM;$T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM; EST: egg storage time; d: days.

Table 9 presents effect of MOLM on egg shelf life of dual purpose Koekoek hens in terms of yolk measurements at different storage times. Yolk weight was higher in $T_2$ and lower in $T_1$, but no statistical difference was detected between $T_3$ and $T_4$. There was significant difference (P<0.05) between $T_3$ and others at 7 days of storage. At 14 days of storage, $T_2$ resulted the same value as that of at 7 days of storage and lower value obtained in $T_1$ but it did not significantly differ (P>0.05) with $T_4$. Hens in $T_3$ had significantly higher (P<0.05)

yolk weight than other treatments at 14 days of storage. At 21 days of storage, higher value was obtained in $T_2$ and lower value in $T_1$, but there was not significantly differences ($P>0.05$) between $T_1$ and $T_4$. Yolk weight of hens in $T_3$ was significantly higher ($P<0.05$) than other treatments. At 28 days of storage, lower value was recorded in $T_1$ with non-significant difference ($P>0.05$) with $T_4$. Higher value was obtained in $T_2$ than others. The yolk weight of hens in $T_3$ was statistically differ ($P<0.05$) than other treatments.

At 7 days of storage, higher yolk length was observed in $T_2$ while lower in $T_1$ than $T_3$ and $T_4$. There was significant difference ($P<0.05$) between each treatment. At 14 days of storage, higher mean value was recorded in $T_2$ and lower in $T_1$ than others. There was no statistical difference ($P>0.05$) between $T_3$ and $T_4$. Likewise, at 21 days of storage, higher value was obtained in $T_2$ and lower in $T_1$ than others. The yolk length in $T_3$ and $T_4$ was not different ($P>0.05$) between each other. At 28 days storage, lower yolk length was recorded in T1 and higher in $T_2$ and was higher ($P<0.05$) than $T_3$ and $T_4$.

Lower yolk height was obtained in $T_1$ than the others but it had no significant difference with $T_4$. Eggs in $T_2$ had higher mean yolk height ($P<0.05$) than $T_1$ but was not statistically different between $T_3$ and $T_4$ at 7 days of storage. There was also no significant difference ($P>0.05$) between $T_3$ and $T_4$. At 14 days of storage, higher yolk height was obtained in $T_2$ and lower one was in $T_1$ which was lower ($P<0.05$) than $T_3$ and $T_4$ with non-significant difference between $T_3$ and $T_4$. Similarly, at 21 days of storage, $T_2$ had higher value and $T_1$ had lower mean value ($P<0.05$) than $T_3$ and $T_4$. There was no statistical difference ($P>0.05$) between $T_3$ and $T_4$. At 28 days of storage, $T_2$ had higher yolk height ($P<0.05$) than the others and lower value was obtained in $T_1$ than others but was not variable ($P>0.05$) with $T_4$. There was no significant difference ($P>0.05$) between $T_3$and $T_4$.

**Table 9:** Effects of MOLM on egg shelf life of dual purpose Koekoek hens in terms of yolk measurements at different storage times (7, 14, 21 and 28 days of storage).

| Parameters | Treatments | | | | Sig. |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | |
| Yolk weight EST(d) | | | | | |
| 7 | 16.67±0.33$^{c}$ | 21.66±0.33$^{a}$ | 19.33±0.33$^{b}$ | 17.33±0.33$^{c}$ | * |
| 14 | 14.33±0.33$^{c}$ | 21.66±0.33$^{a}$ | 18.00±0.57$^{b}$ | 15.66±0.33$^{c}$ | * |
| 21 | 13.00±0.57$^{c}$ | 20.00±0.57$^{a}$ | 17.00±0.57$^{b}$ | 14.33±0.33$^{c}$ | * |
| 28 | 12.33±0.33$^{c}$ | 20.00±0.57$^{a}$ | 15.33±0.33$^{b}$ | 13.33±0.33$^{c}$ | * |
| Yolk length EST(d) | | | | | |
| 7 | 37.38±0.22$^{d}$ | 47.50±0.30$^{a}$ | 42.63±0.26$^{b}$ | 41.19±0.40$^{c}$ | * |
| 14 | 36.39±0.80$^{c}$ | 47.50±0.30$^{a}$ | 41.02±0.31$^{b}$ | 40.15±0.68$^{b}$ | * |
| 21 | 33.13±0.30$^{c}$ | 45.42±0.35$^{a}$ | 40.14±0.33$^{b}$ | 38.61±0.71$^{b}$ | * |
| 28 | 31.62±0.47$^{d}$ | 45.42±0.35$^{a}$ | 38.99±0.36$^{b}$ | 36.75±0.50$^{c}$ | * |
| Yolk height EST(d) | | | | | |
| 7 | 11.33±0.33$^{b}$ | 15.66±0.33$^{a}$ | 14.00±0.57$^{a}$ | 13.67±0.88$^{ab}$ | * |
| 14 | 10.33±0.33$^{c}$ | 15.66±0.33$^{a}$ | 13.33±0.33$^{b}$ | 13.33±0.33b | * |
| 21 | 9.33±0.33$^{c}$ | 15.00±0.57$^{a}$ | 12.33±0.33$^{b}$ | 11.33±0.33$^{b}$ | * |
| 28 | 9.00±0.57$^{c}$ | 15.00±0.57$^{a}$ | 11.33±0.33$^{b}$ | 10.33±0.33$^{cb}$ | * |

*: P<0.05; Means followed by same letter in rows do not differ statistically from one another by the Tukey test at 5% probability; MOLM: *Moringa oleifera* leaf meal; SBM: Soybean meal; $T_1$: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10% MOLM; $T_4$: Ration containing 15% MOLM; EST: egg storage time; d: days.

### 4.6. Fertility, Hatchability and Embryonic Mortality

Table 10 shows the effect of feeding different dietary levels of MOLM on fertility, hatchability and embryonic mortality of dual purpose Koekoek hens. The result revealed that, eggs from those hens fed on $T_2$ had higher fertility percentage (P<0.05) but it did not statistically different (P>0.05) with $T_3$. Hens in $T_1$ had lower value (P<0.05) than the others. Hatchability percentage was higher (P<0.05) in $T_2$ than the others. Hens in $T_1$ had

lower percent (P<0.05) than the others but it was not significantly differ (P>0.05) with $T_3$. There was no also significant difference (P>0.05) between $T_3$ and $T_4$.

High (P<0.05) early embryonic mortality was observed in $T_4$ but it was not statistically different (P>0.05) with $T_3$. Hens in $T_2$ had lower early embryonic mortality (P<0.05) than the others. Mid embryonic mortality was higher (P<0.05) in $T_1$ but it was not significantly different (P>0.05) between $T_2$ and $T_3$. There was no mid embryonic mortality observed in $T_4$. There was significant difference (P<0.05) between $T_1$ and $T_4$. Late embryonic mortality was not affected by the experimental diet. Fig. 3 shows the embryonic mortality of an incubated egg.

**Table 10:** Fertility, hatchability and embryonic mortality of dual purpose Koekoek hens fed different dietary levels of MOLM

| **Parameters** | **Treatments** | | | | **Sig.** |
|---|---|---|---|---|---|
| | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | |
| Fertility (%) | 80.00±0.57$^{c}$ | 93.33±0.57$^{a}$ | 91.11±0.57$^{a}$ | 84.44±0.57$^{b}$ | * |
| Hatchability (%) | 66.66±0.88$^{c}$ | 78.57±0.57$^{a}$ | 68.22±0.57$^{bc}$ | 70.33±0.33$^{b}$ | * |
| Embryonic mortality (%) | | | | | |
| Early | 5.00±0.57$^{b}$ | 4.00±0.57$^{c}$ | 6.66±0.66$^{a}$ | 7.00±0.57$^{a}$ | * |
| Mid | 1.33±0.57$^{a}$ | 0.66±0.57$^{ab}$ | 0.33±0.57$^{ab}$ | 0.00±0.00$^{b}$ | * |
| Late | 0.33±0.03$^{a}$ | 1.33±0.33$^{a}$ | 1.33±0.33$^{a}$ | 0.33±0.03$^{a}$ | NS |

*: P<0.05; Means followed by same letter in rows do not differ statistically from one another by the Tukey test at 5% probability; MOLM: *Moringa oleifera* leaf meal; SBM: Soybean meal; NS: non-significant; $T_1$: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM.

**Fig 3:** Embryonic mortality

### 4.7. Chick Quality

Table 11 presents chick quality of dual purpose Koekoek hens under different dietary levels of MOLM. Hens in $T_2$ had higher mean value for average chick weight ($P<0.05$) than the others. There was no significant difference ($P>0.05$) between $T_1$, $T_3$ and $T_4$. Average chick length was also higher ($P<0.05$) in $T_2$ than $T_1$but there was no difference ($P>0.05$) between $T_3$ and $T_4$. Hens in $T_1$ had lower value ($P<0.05$) of chick length than the rest of treatments. Yield percentage was higher ($P<0.05$) in $T_3$ than $T_4$ but it did not statistically differ ($P>0.05$) with $T_1$ and $T_2$. Hens in $T_4$ also had no significant difference ($P>0.05$) with $T_1$ and $T_2$. Percentage of visual scoring was higher ($P<0.05$) in $T_1$ than others but there was no significant difference ($P>0.05$) between the rest of treatments. Fig. 4 shows a chick with legs deformed.

**Table 11:** Effects of substituting SBM by MOLM on chick quality of dual purpose Koekoek hens

| **Parameters** | **Treatments** | | | | **Sig.** |
|---|---|---|---|---|---|
| | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | |
| ACW (g) | $32.63\pm0.27^{b}$ | $35.03\pm0.34^{a}$ | $32.29\pm0.38^{b}$ | $32.95\pm0.26^{b}$ | * |
| ACL (cm) | $15.83\pm0.12^{b}$ | $17.12\pm0.17^{a}$ | $16.69\pm0.13^{a}$ | $16.60\pm0.12^{a}$ | * |
| YP (%) | $61.48\pm0.11^{ab}$ | $60.62\pm0.44^{ab}$ | $61.96\pm0.61^{a}$ | $60.18\pm0.04^{b}$ | * |
| VS (%) | $2.33\pm0.33^{a}$ | $0.00\pm0.00^{b}$ | $0.00\pm0.00^{b}$ | $0.33\pm0.03^{b}$ | * |

*: $P<0.05$; Means followed by same letter in rows do not differ statistically from one another by the Tukey test at 5% probability; MOLM: *Moringa oleifera* leaf meal; SBM: Soybean meal; $T_1$: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM; ACW: Average Chick Weight; ACL: Average Chick Length; YP: Yield Percentage; VS: Visual Scoring.

**Fig. 4:** Chick with leg deformity

### 4.8. Partial Budget

The partial budget analysis is presented in Table 12. It indicated that, there was significant difference regarding those parameters which used to determine the profitability of substituting MOLM instead of SBM in the present study. Higher feed cost was calculated for $T_4$ while, lower one was for $T_1$. There was significant difference ($P<0.05$) among all treatments in feed cost. Higher number of eggs was recorded hens in $T_2$ and lower value for $T_3$. Hens in $T_1$ and $T_2$ significantly differed ($P<0.05$) with each other and also with $T_3$ and $T_4$. But there was no difference ($P>0.05$) between $T_3$ and $T_4$ in their number of eggs which was laid.

Returns obtained from egg sale was higher ($P<0.05$) for $T_2$ but it was lower for $T_3$. There was no significant difference ($P>0.05$) between $T_3$ and $T_4$. Revenue obtained from sale of birds was similar along with all treatments with the same mean value. As a result, there was no statistical difference ($P>0.05$) among treatments. Total return was higher for $T_2$ while it was lower for $T_3$.

$T_4$ had higher feed cost per bird whereas $T_1$ had lower value. There was a significant difference ($P<0.05$) along with all the treatments. Higher net return was calculated for $T_1$ while lower one was for $T_4$. Change in net return was determined on the base of the control group ($T_1$). For this, higher value was obtained for $T_2$ but lower value observed for $T_4$ with intermediate value for $T_3$. The indication of negative signs was the amount of

loss in relation to the control group. As a result, $T_2$ had a chance of losing 26.84 Birr when comparing with $T_1$. Similarly, $T_3$ and $T_4$ lost 341.91 and 403.91 Birr than the control group. There was a statistical difference ($P<0.05$) among all the treatments.

In addition, change in total return was also determined by using $T_1$ as reference point. Higher value was deliberated from $T_2$whereas lower one was for $T_3$. These values could give an understanding by how many Birr was the treatments were greater or lesser when compared with $T_1$ on the base of their total return. According to this, $T_2$ obtained additional income of 42 Birr when compared to $T_1$. Conversely, $T_3$ and $T_4$ lost 189 and 185 Birr than $T_1$. $T_2$ had significant difference with $T_3$ and $T_4$ but there was no significant difference between $T_3$ and $T_4$.

Change in total cost was also higher for $T_4$ and lower for $T_2$ followed by $T_3$ when compared with $T_1$. This showed that by how many Birr do these treatments used up extra cost than $T_1$. On the base of this, $T_2$ had an additional cost of 68.84 Birr than $T_1$. Likewise, $T_3$ used up further 152.95 Birr than $T_1$. $T_4$ also spend more 218.41 Birr than $T_1$.

The marginal rate of return measures the increase in net income associated with each additional unit of expenditure. It is also determined relating to $T_1$ (control group). Negative sign indicated that, the decreasing of net income as increasing of unit of cost when compared with $T_1$. Thus, $T_2$ decreased its net income by 0.39 Birr every additional unit of cost than $T_1$. As well, $T_3$ and $T_4$ decreased their net income by 2.24 and 1.85 Birr than $T_1$.

**Table 12:** Effects of inclusion of different proportion of MOLM in dual purpose Koekoek hens ration on net income and marginal rate of return

| **Parameters** | **Treatments** | | | | **Sig.** |
|---|---|---|---|---|---|
| | **T1** | **T2** | **T3** | **T4** | |
| Cost/100Kg diet | 676.67±1.00$^{d}$ | 1387.57±1.00$^{c}$ | 2097.32±1.5$^{b}$ | 2801.74±1.0$^{a}$ | * |
| TFI(Kg)/bird | 10.66±0.01$^{a}$ | 10.16±0.04$^{c}$ | 10.73±0.07$^{a}$ | 10.37±0.019$^{b}$ | * |
| TEP | 380.00±1.15$^{b}$ | 392.00±1.00$^{a}$ | 326.00±1.52$^{c}$ | 327.00±1.52$^{c}$ | * |
| Egg sell | 1330.00±1.5$^{b}$ | 1372.00±1.00$^{a}$ | 1141.00±1.5$^{c}$ | 1144.50±1.0$^{c}$ | * |
| Hen sell | 840±1.00 | 840±2.00 | 840±1.00 | 840±1.15 | NS |
| Total return | 2170.00±2.5$^{b}$ | 2212.00±3.00$^{a}$ | 1981.00±2.5$^{c}$ | 1984.50±1.5$^{c}$ | * |
| TFC /bird | 72.13±1.52$^{d}$ | 140.97±1.73$^{c}$ | 225.04±2.51$^{b}$ | 290.54±1.52$^{a}$ | * |
| Net return | 2097.86±1.7$^{a}$ | 2071.02±3.4$^{b}$ | 1755.95±2.0$^{c}$ | 1693.96±1.0$^{d}$ | * |
| CNR | - | -26.84±1.73$^{b}$ | 341.91±3.60$^{c}$ | -403.91±2.64$^{d}$ | * |
| CTC | - | 68.84±1.15$^{c}$ | 152.91±1.73$^{b}$ | 218.41±1.00$^{a}$ | * |
| CTR | - | 42.00±0.57$^{a}$ | 189.00±2.00$^{c}$ | -185.50±1.73$^{c}$ | * |
| MRR | - | -0.39±0.01$^{b}$ | -2.24±0.01$^{d}$ | -1.85±0.004$^{c}$ | * |

*: P<0.05; Means followed by the same letter in rows do not differ statistically from one another by the Tukey test at 5% probability. MOLM: *Moringa oleifera* leaf meal; SBM: Soybean meal; TFI: total feed intake; TFC: Total feed cost; TEP: total egg produced; CNR: Change in net return; CTC: change in total cost; CTR: Change in total return; MRR: marginal rate of return; $T_1$: Ration containing 0% MOLM; $T_2$: Ration containing 5% MOLM; $T_3$: Ration containing 10%MOLM; $T_4$: Ration containing 15% MOLM.

# 5. DISCUSSION

## 5.1. Nutrient Composition of Experimental Diets

In the current study CF shows an increasing trend as increasing of inclusion level of MOLM. This was parallel with the finding of Tesfaye *et al*. (2012) and Gadzirayi *et al*. (2012). Conversely, Kakengi *et al*. (2007) reported a decreasing trend of CF with increasing of MOLM. CP and ash contents obtained in the present study were increased as increasing of MOLM inclusion level which is consistent with the finding of Gadzirayi *et al*. (2012). But, Tesfaye *et al*. (2012) reported that the CP content of the control diet was higher than levels in the 5% MOLM diet, and the ash content decreased as the level of MOLM increased in the diet.

## 5.2. Feed intake, Body Weight Change and Conversion Ratio

Substitution of (SBM) by *Moringa oleifera* leaf meal (MOLM) at different levels (5%, 10% and 15%) in dual purpose Koekoek hens showed a significant effect on their feed intake, BW change and feed conversion ratio (FCR) in the current study. This was similar with the finding of Olugbemi *et al.* (2010a) who noted that addition of 10% and 20% *M. oleifera* leaf meal to the laying hen diet increases these parameters. Similarly, Kakengi *et al*. (2007) reported that substitute for sunflower seed meal in Isa brown breed significantly increased feed intake and FCR. The result of the current study was also consistent with those reported for broilers, were supplementation with 5% MOLM showed significantly better feed conversion ratio as compared to the 0%, 3% and 7% MOLM containing experimental diets (Safa and Tazi, 2014).

According to Melesse *et al*. (2011) the use of *Moringa stenopetala* leaf meal in the diet of Rhode Island Red chicks produced significant increase in feed and FCR when compared to a control diet. In the same way, Gadzirayi *et al. (*2012) observed that significant differences in feed conversion ratio as evidenced by the variation in weight change in 0%, 25%, 50%, 75% and 100% MOLM. But the present study did not agree with the

finding of Etalem *et al*. (2014) who noted that addition of MOLM on Dominant CZ layers up to 10% had no effect on average feed intake, and final BW, FCR of hens. Olugbemi *et al*. (2010b) also found that addition of 5% *M. oleifera* leaf meal to broilers' diet had no significant effect on FCR and final BW when compared to a diet free of *M. oleifera* leaf meal.

Similar to this finding and conversely to the current study, Juniar *et al.* (2008) observed that inclusion of *M. oleifera* leaf meal at amounts up to10% did not produce significant effects on feed consumption, BW and FCR. Nuhu (2010) and Gakuya *et al.* (2014) also reported non-significant effect of MOLM addition in poultry diets. The reduction of feed intake in birds fed 5% MOLM may be due to reduced palatability of the diet (Kakengi *et al.,* 2003*).* The improvement in BW and FCR in the present study may be attributed to rich content of nutrients in MOLM (Sarwatt *et al*., 2004; Kakengi *et al*., 2003) and antimicrobial properties of *Moringa* (Fahey *et al*., 2001). Since egg production and egg weight are higher in a special diet containing 5% MOLM, egg weight is higher; as a result FCR is improved in the current study.

### 5.3. Egg Production Parameters

Egg production parameters (total egg weight and HDEP) were significantly higher for birds fed diets containing 5% MOLM whereas HHEP was showed a lower value for birds at 10% MOLM added diets than 5% MOLM and similar with the rest treatments (0% and 15% MOLM added diets. on the contrary, Olugbemi *et al.* (2010b)showed a non-significant effect on HDEP for hens fed a diet containing MOLM at 0, 5, and 10% of the diet. In addition Etalem *et al*. (2014) observed a non-significant effect of a diet containing *M. oleifera* leaf meal (MOLM) in layer rations at 5% on HDEP.

The current study was in line with Zanu *et al*., (2011) who reported similar results on response to various levels of MOLM in diets in laying chickens. In contrast, Kwari *et al.* (2011) and Olabode and Okelola (2014) noted non-significant results on egg weight and egg production when fed *M. oleifera* leaf and twig meals at different levels ranging from

0.2 to 0.8%. The significant effect of MOLM on egg weight and egg production in the present study might be due to the presence of lysine and methionine in *Moringa* as reported by (Bunchasak and Silapasort, 2005). Wu *et al*. (2007) and Fakhraei *et al*. (2010) also showed that increased methionine and lysine in the feed improves egg production and increases egg weight.

The higher egg production in layers fed the diet containing MOLM could be due to the improvement in balanced nutrient supply by MOLM in the diet. *M. oleifera* leaf meal contains lysine, methionine and a combination of other amino acids, which might supply the required amount of essential nutrients for better production (Sohail *et al*., 2003). In accordance with the present finding, Uma (2000) reported that methionine and lysine levels in poultry diets have positive correlation with egg production. Egg production increased significantly as dietary levels of lysine increased from 0.50 to 0.64 % (Fakhraei *et al*., 2010).

Decrease in egg mass production, egg production percentage and egg weight at higher levels of *M. oleifera* leaf meal was attributed to low digestibility of energy and protein (Kakengi *et al*., 2007). Jacob *et al. (*2014) reported that there are positive correlation between feed conversion ratio, egg weight and egg production. So, increase of egg weight in $T_2$ might lead to increasing in egg production and improving FCR in the current study.

### 5.4. Egg Quality Parameters

Both internal and external egg quality in the present study were affected positively by the dietary substitution of SBM by MOLM at different levels in dual purpose Koekeok hens. Weight of sampled eggs and albumen weight, egg length, Albumen height, yolk index, and yolk color, shell weight and yolk height, yolk weight were all improved especially at 5% MOLM inclusion. This was consistent with the finding of Etalem *et al.* (2014) in Cassava root chips and *M. oleifera* leaf meal as an alternative feed ingredient in the layer ration at 5% inclusion levels of MOLM in Dominant Cz layers. Improvement of albumen height in the present study was also agreed with the findings observed by Price (2000) and

Kaijage *et al*. (2004) but inconsistent for improvement of yolk index. The higher the yolk index and Haugh unit, the more desirable is the egg quality (Fayeye *et al.,* 2005).

The values obtained for yolk index ranged from 0.27-0.34 in which the lowest one was observed in control group and higher one was for birds fed on 5% MOLM. This was similar to the value reported by Odunsi *et al*. (2002), but lower than the range of 0.44-0.47 reported by Garba *et al*. (2010). They reported that the range value of yolk index in hens from 0.30-0.55 was an indication of the good internal quality of egg produced from leaf meal based diets, while Oluyemi and Robert (2000) reported that the yolk index which is a measure for determining the quality of an egg yolk should be between 0.30-0.50. The yolk index recorded in the present study was within the recommendation range (Mellau, 1999). Bhatnagar *et al.* (1996) reported that inclusion of *Moringa* at lower levels in Isa brown breed improved egg quality but higher levels of inclusion resulted in lower productivity and poorer egg quality indices. They conversely found non-significant effect on egg weights at 0%, 5% and 10% inclusion levels, but egg weight was lowest at 20% inclusion level.

Improvement of egg weight in the current study was similar with Kakengi *et al*. (2007) where substitution of sunflower with MOLM at 5 % levels in the diet showed a positive effect on egg weight. The decrease in weight at higher levels of MOLM inclusion in the present study and others were also not clear but probably was due lower energy and CP availability and also associated with lower digestibility of CF component reported in various other leaf meals (North, 1990).

Nobakht and Mehmannavaz (2010) showed that increasing yolk weight was the main reason for the increment in albumen weight and this might explain the increase in albumen weight in groups fed diets supplemented with MOLM. Nobakht and Moghaddam (2012) also noted a positive correlation between Haugh unit and quality of egg components (yolk and albumin). Egg albumen height and egg weight are indices for evaluation of Haugh unit. Indeed, increase in egg weight is related to increase in albumen weight and yolk weight. Increasing of egg weight due to increase in weight of albumen

and yolk especially for $T_2$ might be the main cause of improvement in Haugh unit in the present study.

The MOLM is a good pigmenting agent of poultry products due to its rich *xanthophylls* content (Etalem *et al*., 2013; Olugbemi *et al.,* 2010b). In the present study, the yolk color showed an increasing trend as the amount of MOLM increased in the ration. This was supported by the findings of different researchers with 5 and 10% inclusion of MOLM in the layer ration (Kakengi *et al.*, 2007; Olugbemi *et al.,* 2010b and Abou-Elezz *et al*., 2011). Egg yolk color is a very important factor in consumer satisfaction and influences human appetite (Amerine *et al.,* 1995), with a preference for golden yellow to orange yolk color (Hasin *et al*., 2006). Similarly, Jacob *et al.* (2000) noted that yolk color is a key factor in any consumer survey relating to egg quality.

The intense yellowish yolk color recorded in our study for eggs produced from birds on diets containing MOLM confirms its viability as a yolk-coloring agent, which can enhance the marketability of the eggs. The significant effect of substituting SBM by MOLM at different levels on egg width, egg shape index, shell thickness, Haugh unit, and yolk length in dual purpose Koekoek hens in the present study disagrees with Etalem *et al*. (2014). There is a direct relation between dietary protein supply and egg size (Niekerk, 2014). Lowering total protein, methionine or other essential amino acid supply can reduce egg weight. But the significant effect of MOLM on egg weight, yolk index Haugh unit and shell thickness in the current study was in line with Adeyemo *et al*., (2012) on effect of varied dietary crude protein levels with balanced amino acids on performance and egg quality characteristics of layers.

The range of Haugh unit observed in this study was 75-87 in which lowest was from the control group and highest one was from 5% MOLM. The higher the value of the Haugh unit, the better the quality of eggs, which are classified according to the United States Department of Agriculture (USDA) as AA (100 to 72), A (71 to 60), B (59 to 30) and C (below 29) (USDA, 2000), in which the refrigerated eggs are classified as AA. Oluyemi and Robert (2000) also reported that unit score of 72 and above has been graded as the

best quality. This showed that, the quality of the egg regarding the Haugh unit in the current study was under AA grade in diets which contain 5%, 10% and 15% MOLM.

Good shell thickness is an important bioeconomic trait in commercial egg production as it may help to reduce the percentage of cracked eggs and decrease the rate of loose for producers. ISA (2009) reported that the minimum shell thickness of 0.35 and shape index of 74 is indicators of quality egg. In the present study, shell thickness was in the range of 0.29-0.38 while shape index was 75-80. The lowest shell thickness was from the control group while the highest one was from birds fed 5% MOLM. In addition, the lowest shape index was observed on $T_2$ while the highest shape index was from $T_1$ (control group). This implies that, substitution of SBM by MOLM at 5% inclusion level results in better egg quality in terms of shell thickness and shape index.

Shell quality cannot be maintained for long without adequate levels of calcium, phosphorus and Vitamin D in the layer diet. Other micronutrients including Magnesium, Iron, Copper, Manganese, Zinc, Vitamin K and certain amino acids function in calcium transport and bone matrix turnover. Even some B vitamins (Folic acid, Niacin, B12) have been associated positive effects on shell quality. This supports the current study due to the presence of these nutrients in MOLM contributes to the quality of egg shell (Hy-line, 2013). Scott and Silversides (2000) also reported the structural quality of the shell egg is important to the processor because eggs that are structurally sound will arrive to the consumer in the best condition. Furthermore, high interior quality is of importance to egg products manufacturers because it allows for better separation of components without crossover contamination.

There are reports of albumen quality increasing with increasing dietary protein and amino acid content (Hammershoj and Kjaer, 1999), increasing with increased dietary lysine concentration (Balnave *et al*., 2000) increasing with ascorbic acid supplementation (Franchini *et al.,* 2002) and increasing with vitamin E supplementation (Kirunda *et al.,* 2001; Puthpongsiriporn *et al*., (2001). Nys (2004) and Seuss-Baum (2007) reported that both internal and external egg quality is affected by nutrition or feed which is

supplemented to hens. For example, content in total proteins, essential amino acids, total lipids, phospholipids, phosphorus, iron, vitamin C, E, minerals affect positively. These findings were support the present study because *M. oleifera* contains those amino acids and vitamins and improve both internal and external egg quality.

### 5.5. Egg Shelf Life

The significant effect in shelf life of eggs in terms of pH of yolk and albumen at different storage time was in line with the findings of (Scott and Silversides, 2000; Silversides and Scott, 2001; Monia *et al.,* 2003; Silversides and Budgell, 2004, and Mahmoud *et al.,* 2012). Pappas *et al*., (2005) characterize the decline in albumen and yolk pH deterioration rate as a function of the antioxidant status of egg contents. They proposed that organic selenium enhances the egg's antioxidant status by upgrading the glutathione peroxidase activity in yolk and albumen. This in turns slows the process of lipid and protein oxidation during storage period; hence more valuable egg quality by extended storage time which is consistent with the antioxidant properties of *M. oleifera* (Thomson and Ali, 2003 and Mirunalini *et al.,* 2004).

*Moringa oleifera* is among the most promising species based on their high antioxidant activity, high contents of micro-nutrients and phytochemicals that could help in stability and shelf life of poultry product (Yang *et al.,* 2006; Jung *et al*., 2010). The flavonoids such as quercetin and kaempferol were identified as the most potent antioxidants in *Moringa* leaves (Foidl *et al*., 2001). Similarly, Siddhuraju and Becker (2003) noted their antioxidant activity was higher than the conventional antioxidants such as ascorbic acid, which is also present in large amounts in *Moringa* leaves and used to prolong shelf life of poultry products.

The total antioxidant capacity of fruit and vegetable extracts reflects concentrations of ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), beta-carotene (vitamin A precursor), various flavonoids, and other phenolic compounds (Pennington and Fisher, 2009). Some authors have demonstrated the high antioxidant activity of α-tocopherol,

ascorbic acid, and their high radical scavenging abilities (Kulisic *et al.,* 2004). Furthermore, Yeum *et al*. (2009) reported some synergistic effects among ascorbic acid, α-tocopherol, and ß-carotene have been against oxidation.

In addition, Zheng *et al.* (2009), Andjelkovic *et al*. (2006) and Mukai *et al. (*2005) noted that phenolic compounds have a high antioxidant activity through three mechanisms: free-radical scavenging activity, transition-metal-chelating activity.), and/or singlet-oxygen quenching capacity. The improvement in shelf life of eggs in the present study as a result of MOLM inclusion could be related to the presence of the above mentioned antioxidants in MOLM. Feeding of MOLM for dual purpose Koekoek hens in the present study improves the shelf life of an egg by minimizing the rapid increasing of the yolk pH. During storage of eggs, the pH of the albumen increases and this is thought to be related to the deterioration of albumen quality. Similar with the present study, Benton and Brake (2000) noted that there is a significant effect of storage time on pH and albumen height.

Heath (1977) reported that in a newly laid egg the albumen pH lies between 7.6 and 8.5. Li-Chan *et al.* (1995) also noted during storage, the albumen pH increases at a temperature dependent rate to a maximum value of about 9.7. After 1month of storage, an albumen pH of 9.18. After 21 days of storage, the albumen had a pH close to 9.4, regardless of storage temperature between 3 and 35°C. In addition (Scott and Silversides (2000); Silversides and Scott (2001); Monia *et al*. (2003); and Silversides and Budgell (2004) also noted a shift in albumen pH from (8.35) at day 7 to (9.08) and (9.29) after 14 and 21days of storage, respectively.

This indicated that the result found in the present study was valuable regarding with improving shelf life of an egg in terms of pH of albumen. Yolk pH in the current study also lower than the value obtained by Li-Chan *et al.* (1995) reported in newly laid eggs, the yolk pH is in general close to 6.0; however, during storage it gradually increases to reach 6.4 to 6.9. This implies that, feeding of MOLM for dual purpose Koekoek hens in the present study improves the shelf life of an egg in terms of minimizing the rapid increasing of the yolk pH.

The decrease in albumen height, albumen weight and Haugh unit with storage time was consistent with the reports of (Keener *et al*., 2000); Silversides and Budgell, 2004). Storage time has inverse relationship with height of albumen. During egg storage, the quality of the vitelline membrane declines, making the yolk more susceptible to breaking and decreases its weight (Kirunda and McKee, 2000).

In the current study, there was a decrease in yolk weight but there was no breakage except eggs from the control group. This implies that *Moringa* has a potential of extending shelf life of poultry products. Niekerk (2014) reported that storage for one week at 25°C will reduce the Haugh Unit (HU) up to the limit of acceptable freshness (70 HU), whereas storage for one week at 8°C will result in eggs that are still very fresh (85 to 90 HU). This indicated that eggs from hens fed MOLM in the present study were still under the grade of AA according to USDA (2000) up to four week storage at room temperature.

The significant decrease of yolk and albumen measurements relating with storage time was in line with the finding of Jin *et al.* (2011); Gavril and Usturoi (2012) and Tebesi *et al. (*2012). The range of values observed in the current study differed from values reported by Tayeb (2012) on effects of storage length on egg quality parameters of laying hens. Wojdylo *et al*. (2007) noted that many herbs, spices, and their extracts have high antioxidant capacity, such as, Moringa (*M. oleifera*), Oregano (*O. vulgare*), Rosemary (*R. officinalis*), and Sage (*S. officinalis*) to improve quality of poultry products in terms of prolonging their shelf life and this was in line with the current study.

### 5.6. Fertility, Hatchability and Embryonic Mortality

The result obtained in the current study was inconsistent with the finding of Etalem *et al.* (2014) using *M. oleifera* leaf meal as an alternative feed ingredient in the layer ration which showed non-significant effect of MOLM on fertility, hatchability and embryonic mortality. Park *et al*. (2004); Mahmood and Al-Daraji, (2011) and Moyo *et al.* (2011) reported that *M. oleifera* leaf contains higher levels of zinc and vitamin E, which can play

a beneficial role in hatchability of eggs. Similarly, Durmus *et al.* (2004) noted increased hatchability with increasing zinc concentration in the diets of Brown parent stock layers.

Brown and Pentland (2007) showed that zinc helps in protecting the structure of the genetic material or the DNA chromatin in the sperm nucleus, a structure important for successful fertilization. *Moringa* contains significant amount of iron, phosphorus, calcium, and is relatively rich in vitamin C (Agbaje *et al*., 2007). The current study is also in line with Adesola *et al.* (2012) who reported improved hatchability as a result of ascorbic acid supplementation to diets of indigenous Venda hens. However, the relatively poor hatchability and higher embryonic mortality observed in the control group of the present study might be due to a deficiency in critical nutrients, such as zinc, vitamin E, and so on, which are important for better hatchability as reported by (Park *et al.,* 2004; Mahmood and Al-Daraji, 2011).

Similarly, Davtyan *et al*. (2006), Petrosyan *et al*. (2006), Hanafy *et al*. (2009) and Agate *et al.* (2000) reported that organic selenium supplementation of laying hens diets improved the environment of the sperm storage tubules in the hen's oviduct, allowing the sperms to live longer, increasing the length of time the sperms can be stored and increasing the number of sperm holes in the yolk layer. Supplementation of plant leaves containing selenium increased fertility and hatchability % (Osman *et al*. (2010). Liao *et al*. (2013) also concluded that eggshell thickness affected hatchability. The physical characteristics of the egg like weight, shell thickness, length and width and shape index play an important role in the embryo development and successful hatching (Narushin and Romanov, 2002). These might explain the improvement fertility and hatchability in groups fed diets supplemented with MOLM in the present study, was due to these reasons.

In the present study, supplementation of layers with MOLM improved early and mid-embryonic mortalities but, late embryonic mortality was not affected by the dietary treatment. This was similar with the finding of Etalem *et al.* (2014) who reported on Cassava root chips and *M. oleifera* leaf meal as alternative feed ingredients in the layer

ration. ISA (2009), Deeming (2002) and Tona *et al*. (2005) reported that nutrition of the parent stock, care of the hatching egg before setting fumigation during the first days of incubation, shocking and trembling and insufficient turning are causes of early embryonic mortality. On the other hand, the turning and the care of the hatching eggs play a great part for mid and late embryonic mortality. But there are also factors which affect different stages of embryonic mortalities like, temperature, humidity and ventilation (ISA, 2009).

### 5.7. Chick Quality Parameters

In the current study chick quality parameters appeared to be not negatively affected by the dietary inclusion of MOLM at different levels as replacement of SBM. Chick weight, chick length and yield percentage were improved in the diets containing MOLM than the control group. Improvement of chick weight in the present study was concurred with the finding of Etalem *et al.,* (2014) who observed higher chick weight in hens fed 5% MOLM. Similarly, Coon *et al* (2006) noted that, addition of protein in chickens' diet improves chick weight at hatching. The present study regarding egg size and chick weight at hatching was in agreement with the findings of Abiola *et al.* (2008) and Malago and Baitilwake (2009), who noted a positive correlation between egg size and chick weight at hatching.

Hatchability and chick quality at hatching is directly related with quality parameters of eggs the better egg size, the better yolk, the better albumen and better shell thickness resulting in best hatchability with best chick quality (Kingori, 2011). Bray and Iton (1999), Wilson (2000), Silversides and Scott (2001), and Tona *et al.* (2002) have shown that egg weight is a dominant factor affecting chick weight at hatch. In the present study, inclusion of MOLM was improving chick quality by improving both internal and external egg quality parameters. Kenny and Kemp (2003) noted that, the developing embryo and the hatched chick are completely dependent for their growth and development on nutrients deposited in the egg.

### 5.8. Partial Budget Analysis

In the current study, feed cost increases as increases as the level of MOLM in the diet containing 5%, 10% and 15% MOLM. This was in line with the findings of Onibi *et al*. (2008) or Tendonkeng *et al*. (2011) in which the feed costs/kg live body weight of broiler finishers were increased with *Leuceana* or *Moringa* leaves meal inclusion in the diets. The net return in the present study was decreasing as increasing of MOLM. This is due to the decreasing of egg production in relating to increasing MOLM level beyond 5%. This was comparable to the finding of Zanu *et al.* (2012) who noticed that partial replacement of fish meal with *M. oleifera* leaf meal decreased the net revenue for broilers, according to their reduction in weight gain.

Controversially, Ayssiwede *et al.* (2010) noticed that the lowest feed cost/kg carcass was achieved when 8% and 16% of MOLM was introduced into the diets of the birds. Adeniji and Lawal (2012) reported as *Moringa* is profitable for Senegal chicken and feeding for rabbit up to 100% MOLM replacing groundnut.

# 6. CONCLUSION AND RECOMMENDATIONS

Generally, replacing of SBM by MOLM at 5% ($T_2$) dietary level improved feed conversion ratio, egg production parameters (hen day egg production, hen housed egg production total and average egg weight), egg quality parameters (egg length, egg width, egg shape index, albumen weight and height, yolk weight and height, Haugh unit, yolk color, yolk length and yolk index) egg shelf life, fertility and hatchability, embryonic mortality and chick quality. On the other hand, higher feed intake 10% MOLM ($T_3$) and T1 (0% MOLM) inclusion level and higher body weight change was obtained at T2 (5% MOLM). Higher yolk color was observed for $T_3$ (10% MOLM) and $T_4$ (15% MOLM). Even if MOLM had a potential of improving these parameters mentioned above, it was not profitable due to its high price than SBM.

Based on the above conclusion, the following recommendations are forwarded:

- From economic benefit point of view, it is not advised to use MOLM at the levels studied in poultry feeding. A further possibility of using MOLM is by increasing *Moringa oleifera* production in order to exploit its benefit in poultry feeding as a result its price will be decreasing.
- In our country, there are no such findings in the area of feeding of *Moringa oleifera* leaf meal for poultry to improve their productive and reproductive performance. So researchers should do further findings and give evidence about the nutritional value of MOLM in poultry for producers.

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## 8. APPENDICES

**Appendix table 1:** analysis of variance of total feed intake

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 0.62 | 0.21 | 21 | <.0001 |
| Error | 8 | 0.05 | 0.01 | | |
| Corrected total | 11 | 0.67 | | | |

**CV**=0.74

**Appendix table 2:** analysis of variance of body weight change

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 0.042 | 0.014 | 35 | <.0001 |
| Error | 8 | 0.003 | 0.0004 | | |
| Corrected total | 11 | 0.045 | | | |

**CV**=6.14

**Appendix table 3:** analysis of variance of Feed conversion ratio

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 0.614 | 0.204 | 20.4 | 0.0005 |
| Error | 8 | 0.083 | 0.010 | | |
| Corrected total | 11 | 0.697 | | | |

**CV**=5.88

**Appendix table 4:** analysis of variance of hen day egg production

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 675.89 | 225.29 | 26.76 | 0.0002 |
| Error | 8 | 67.42 | 8.42 | | |

| | | |
|---|---|---|
| Corrected total | 11 | 743.31 |

**CV**=5.57

**Appendix table 5:** analysis of variance of hen housed egg production

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 569.14 | 189.71 | 5.01 | 0.0304 |
| Error | 8 | 302.91 | 37.86 | | |
| Corrected total | 11 | 872.05 | | | |

**CV**=12.28

**Appendix table 6:** analysis of variance of average egg weight

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 58.91 | 19.63 | 20.03 | 0.0005 |
| Error | 8 | 7.87 | 0.98 | | |
| Corrected total | 11 | 66.78 | | | |

**CV**=1.95

**Appendix table 7:** analysis of variance of Haugh unit

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 245.67 | 81.89 | 40.94 | <.0001 |
| Error | 8 | 16.00 | 2.00 | | |
| Corrected total | 11 | 261.67 | | | |

**CV**=1.77

**Appendix table 8:** analysis of variance of yolk color

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 214.92 | 71.64 | 286.56 | <.0001 |

| | | | |
|---|---|---|---|
| Error | 8 | 2.00 | 0.25 |
| Corrected total | 11 | 216.92 | |

**CV**=6.18

**Appendix table 9:** analysis of variance of fertility

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 337.09 | 112.36 | 112.36 | <.0001 |
| Error | 8 | 8.00 | 1.00 | | |
| Corrected Total | 11 | 345.09 | | | |

**CV**=1.14

**Appendix table 10:** analysis of variance of hatchability

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 263.12 | 87.70 | 74.96 | <.0001 |
| Error | 8 | 9.33 | 1.17 | | |
| Corrected Total | 11 | 272.45 | | | |

**CV**=1.52

**Appendix table 11:** analysis of variance of average chick weight

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 13.68 | 4.56 | 14.71 | 0.0013 |
| Error | 8 | 2.47 | 0.31 | | |
| Corrected Total | 11 | 16.15 | | | |

**CV**=1.67

**Appendix table 12:** analysis of variance of average chick length

| Source | DF | Sum of squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 3 | 2.62 | 0.87 | 14.5 | 0.0013 |
| Error | 8 | 0.48 | 0.06 | | |
| Corrected Total | 11 | 3.1 | | | |

CV=**1.47**