Sudan University of Science and Technology

College of Graduate Studies

The Role of *Moringa Oleifera* as a Natural Antioxidants on Egg Laying Chicken Performance and Egg Quality

دور المورنيغا كمضاد طبيعي للأكسدة على أداء الدجاج البياض وجودة البيض

A Thesis Submitted for Partial Fulfillment for the Requirements of Master Degree in Animal Production

By

Samah Osman Omer Mohamed Sali

Supervisor

Prof. Mohammed Tigani Salih

January 2018

Dedication

This research is dedicated to my family; I suppose I might have completed the work because of having them around.

Also,

To my brothers, sisters and friends,

And Special dedicate to my supervisor Prof. Mohammed Tigani Salih.

With love and respect.

Samah

ACKNOWLEDGEMENTS

Firstly, thanks should be to Allah who supports me in my life.

I would like to thank my parents for their continued support, advice and love they have always been there for me through the good times and the bad they always instilled in me the importance of an education and hard work and without them I would never have been able to achieve my dreams.

I would like also thank my supervisor Prof. Mohamed Tigani Salih who has guided, support and challenged me to break in this field of research and deserves much of the credit for providing much needed insights of direction.

Profound thanks to Mr. Salah Abdoun for his support and provide the Moringa oleifera basis for research.

I wish to thank my friends Abdalaziz, Randa Abdelmoneim, Eglal Siddeg, Haythm Osman, Amal, Ahmad Belo, mohammed, faisal, Nazar, Sadam, Mirganey and Mohamed Sidahmed.

Thank are also extended to the people in the department of animal production.

Abstract

An experiment was carried out to investigate the effect of *Moringa oleifera* leaf powder (MOLP), as a natural antioxidant , added to feed egg production and egg quality parameters of Hy line layers .A total of 60 single Hy line , 17 weeks were used in the experiment . They were randomly allocated to four treatment group, according to the level of MOLP introduced in the diet . Group A served as the control (0% MOLP) group B (0% MOLP+OIL) group D (1% MOLP) and group C (1% MOLP+ OIL) respectively.

The hens were managed in an open-sided house in a battery cage system and were kept in cages of 50X40X45 cm. Each treatment had with 5 replicates, comprising three hens per experimental unit, in a complete randomized design. All birds were provided with laying diet containing 2,800 Kcal/Kg, 17% CP, 1.45% calcium and 0.47% phosphorus. Water was offered *ad labium* through nipple drinking. The birds were subjected to a 16 h light and 8 h dark per day for the entire period of the experiment. The results indicated that egg production was not significantly increased with increasing the level of MOLP in the experimental diet. While, eggs weight was significantly increased. However, there were a numerical rather than a significant improvement in egg weight throughout the duration of the experiment, where MOLP with Moringa oil show the high egg weight followed by the egg weight of MOLP treatment without Moringa oil added. The shell thickness (mm) of layers fed MOLP treatment with or without Moringa oil added were almost similar and significantly ($p<.01$) higher than thickness of eggs produced by birds fed the control diets with or without oil throughout the duration of the experiment. The yolk color of eggs of the experiment layers was measured weekly and the results show that addition of MOLP

resulted in a significant increase in egg yolk color from score one to the score four .

Haugh units were used as an indicator of egg freshness and it was found that the values of these units were significantly high for fresh eggs as well as stored eggs produced by layers fed MOLP with or without supplementation of moringa oil at different ages and store for 3, 7 and 14 days throughout the duration of the experiment.

المستخلص

اجريت هذه الدراسه لمعرفة تأثيرمسحوق اوراق المورينقا (*Moringa oleifera*) في عليقة الدجاج البياض علي انتاجية وجودة البيض. استخدمت في هذه التجربة 60 دجاجه بياضه , عمر 17 اسبوع وزعت عشوائيا الي اربعه مجموعة معاملات , بناءً علي مستوى المورينقا المضافه في العليقه . مجموعه (أ) غذيت بعليقه خاليه من المورينقا (الشاهد) بينما مجموعه (ب)عليقه مضاف اليها زيت المورينقا , وعليقه (ج) تحتوي علي عليقه مضاف اليها 1%مورينقا ,وعليقه (د) تحتوي علي عليقه مضاف اليها 1%مورينقا مع زيت المورينقا .ادير القطيع في نظام مفتوح ,وفي بطاريات ابعادها 50x40x45سم ,كل معامله بها 5 مكررات وكل مكرر به 3 دجاجات في تصميم كامل العشوائيه . كل الطيور تمت تغذيتها علي عليقه بياض تحتوي علي 2800 كيلو كالوري/كجم و17% بروتين خام و 1.45% كالسيوم و 0.47% كالسيوم , مع توفير الماء باستخدام نظام الحلمات, وتعريضها يوميا الي 16 ساعه ضوء و 8 ساعات ظلام اثناء فترة التجربه .أوضحت النتائج أن إنتاج البيض لم يزد معنويا مع إضافة أوراق المورينقا في النظام الغذائي ، في حين زاد وزن البيض وسمك القشره بشكل ملحوظ عند إضافة المورينقا طوال مدة التجربة. تم قياس لون صفار البيض أسبوعيا، وأظهرت النتائج أن إضافة أوراق المورينقا أدى إلى زيادة كبيرة في لون صفار البيض من الدرجة الأولى إلى الدرجة الرابعة.

تم استخدام وحدات (Haugh unit) كمؤشر لنضارة البيض ووجد أن إضافة أوراق المورينقا مع أو بدون زيت المورينغا ذاد من قيم هذه الوحدات بشكل ملحوظ للبيض الطازج والبيض المخزن لمدة 3 ، 7و 14 يوم في مختلف الأعمار طوال مدة التجربه .

List of contents

Subject	Page No
Dedication	I
ACKNOWLEDGEMENTS	II
Abstract	III
Abstract in Arabic	V
List of contents	VI
List of Tables	VIII
List of figures	X
List of Appendixes	XII
CHAPTER ONE	
INTRODUCTION	1
CHAPTER TWO	
LITERATURE REVIEW	3
2.1 Origin and distribution of Moringa:	3
2.2. Uses of *Moringa oleifera*:	3
2.3. Nutritional value of *Moringa oleifera* leaf:	5
2.4. Chemical analysis of *Moringa oleifera*	6
2.5. Mode of action of *Moringa oleifera*	7
2.6. Medicinal uses of *Moringa oleifera*	8
2.7. Effect of moringa leaf meal on the laying performance of hens	9
2.8. Selenium in plants and soils	10
2.9. Organic and Inorganic Selenium	10
2.10. Use of selenium for poultry	12
2.10.1. layers	12

2.10.2.Broilers	15
2.11. Practical approaches to improve selenium status of poultry selenium -enriched eggs	15
2.12. Effect of Organic selenium on egg weight and egg production	17
2.13.Organic selenium and egg quality	18
2.14.Selenium and egg freshness	18
2.15.The Haugh unit as a measure of the quality of an egg	19
2.16. Carotenoids	20
2.17. lutein	20
CHAPTER THREE	
MATERIAL AND METHODS	23
3.1 Experimental site (Study area) and period**:**	23
3.2 Preparation of *Moringa Oleifera* leaves powder:	23
3.3 Experimental stock	23
3.4 Housing and feeding of the experimental birds**:**	23
3.5 Experimental Measurements **:**	26
CHAPTER FOUR	
Results	27
CHAPTER FIVE	
Discussion	52
CHAPTER SIX	
CONCLUSION AND RECOMMENDATION	56
6.1.Conclusion	56
6.2. RECOMMENDATION	57
CHAPTER SEVEN	
References	58
Appendix	75

List of Tables

Table(1)	Composition of experimental diets used in the Experiment
Table (2)	Egg production(week 1 to week 4)
Table (3)	Egg production (week 5 to week 7)
Table (4)	Effect of moringa leaf with or without moringa oil on egg weight
Table (5)	Effect of moringa leaf with or without moringa oil on shell weight(gm)
Table (6)	Effect of moringa leaf with or without moringa oil on shell thickness (mm)
Table (7)	Effect of MOLP with or without Moringa oil on Haugh unit of fresh eggs
Table (8)	Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 3 days
Table (9)	Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 7 days
Table (10)	Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 14 days
Table (11)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 24th wk of age and stored for 3, 7, or 14 days
Table (12)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 25th wk of age and stored for 3, 7, or 14 days
Table (13)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 26th wk of age and stored for 3, 7, or 14 days
Table (14)	Effect of MOLP with or without moringa oil on HU of

	eggs obtained at 27th wk of age and stored for 3, 7, or 14 days
Table (15)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 28th wk of age and stored for 3, 7, or 14 days
Table (16)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 29th wk of age and stored for 3, 7, or 14 days
Table (17)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 30th wk of age and stored for 3, 7, or 14 days
Table (18)	Effect of moringa leaf with or without moringa oil on color (24 wk)
Table (19)	Effect of moringa leaf with or without moringa oil on color (25wk)
Table (20)	Effect of moringa leaf with or without moringa oil on color (26 wk)
Table (21)	Effect of moringa leaf with or without moringa oil on color (27 wk)
Table (22)	Effect of moringa leaf with or without moringa oil on color (28 wk)
Table (23)	Effect of moringa leaf with or without moringa oil on color (29 wk)
Table (24)	Effect of moringa leaf with or without moringa oil on color (30 wk)

List of Figures

Figure (1)	Egg production(week 1 to week 4)
Figure (2)	Egg production (week 5 to week 7)
Figure (3)	Effect of moringa leaf with or without moringa oil on egg weight
Figure (4)	Effect of moringa leaf with or without moringa oil on shell weight(gm)
Figure (5)	Effect of moringa leaf with or without moringa oil on shell thickness (mm)
Figure (6)	Effect of MOLP with or without Moringa oil on Haugh unit of fresh eggs
Figure (7)	Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 3 days
Figure (8)	Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 7 days
Figure (9)	Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 14 days
Figure (10)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 24th wk of age and stored for 3, 7, or 14 days
Figure (11)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 25th wk of age and stored for 3, 7, or 14 days
Figure (12)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 26th wk of age and stored for 3, 7, or 14 days
Figure (13)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 27th wk of age and stored for 3, 7, or 14

	days
Figure (14)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 28th wk of age and stored for 3, 7, or 14 days
Figure (15)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 29th wk of age and stored for 3, 7, or 14 days
Figure (16)	Effect of MOLP with or without moringa oil on HU of eggs obtained at 30th wk of age and stored for 3, 7, or 14 days
Figure (17)	Effect of moringa leaf with or without moringa oil on color (24 wk)
Figure (18)	Effect of moringa leaf with or without moringa oil on color (25wk)
Figure (19)	Effect of moringa leaf with or without moringa oil on color (26 wk)
Table (20)	Effect of moringa leaf with or without moringa oil on color (27 wk)
Figure (21)	Effect of moringa leaf with or without moringa oil on color (28 wk)
Figure (22)	Effect of moringa leaf with or without moringa oil on color (29 wk)
Figure (23)	Effect of moringa leaf with or without moringa oil on color (30 wk)

List of Appendixes

Figure (24)	Images of experimental layers
Figure (25)	picture on the left show yolk color van and on the right show digital
Figure (26)	The yolk color van
Figure (27)	showed the effect of MOLM on eggshell thickness of laying hens
Figure (28)	showed the effect of MOLM on egg yolk color of laying hens
Figure (29)	Moringa tree

CHAPTER ONE

INTRODUCTION

Nutrition is known to play a very important role in maintaining animal health, productive and reproductive performance of farm animals. Which certain extend could be achieved by the use of specific feed additives. A number of feed additives have been used worldwide in the poultry industry for so many years (Jang *et al*., 2007). The common feed additives used in poultry diets include antimicrobials, antioxidants, emulsifiers, binders, pH control agents and enzymes (Poultry Consultancy, 2012)**.** *Moringa oleifera* is one of such interesting plants which is reported to contain some significant levels of natural antioxidants, such as vitamin E, selenium and carotenoids (Khalafalla *et al*., 2010). *Moringa oleifera* leaves were known to be low in anti-nutritional factors and have been used in poultry feed with various performance results, depending on their nutritional value and inclusion level in the diet (Kaijage *et al*., 2003; Kakengi *et al*., 2007; Nuhu, 2010; and Olugbemi *et al*.,.2010).The presence of vitamin C, vitamin E, carotenoids, flavonoids and selenium makes *Moringa oleifera* a potential antioxidant (Moyo *et al*., 2012). *Moringa oleifera* leaf meal has high carotenoids content (Price., 2000). In addition, Moringa has been reported to possess quality sources of several nutrients including protein, calcium, Magnesium, Potassium, Iron, Vitamin A, and Vitamin C (Foidl *et al*., 2001; Marcu, 2005; Rweyemamu, 2006). Many researchers have noticed its nutritional and medicinal benefits (Dahot., 1988; Makkar and, Becker., 1997; Anwar & Bhanger., 2003; Anwar *et al*., 2007). Yameogo *et al*. (2011) reported that, on a dry matter basis, Moringa oleifera leaves contained 27.2% protein, 5.9% moisture, 17.1% fat, and 38.6% carbohydrates.

Adequate selenium (Se) supplementation is considered a crucial factor in maintaining the high productive and reproductive characteristics of

commercial poultry (Surai, 2002). Selenomethionine increases egg production, improves fertility, antioxidant status, accelerating growth after feather loss in young laying hens and has a positive influence on the storage ability of eggs (Edens, 2002). Moreover, organic sources of Se have been explored as an alternative to inorganic selenium (Payne, *et al*., 2005). The supplementation of hens' food with organic selenium not only improves their health and productivity, but can also be a natural way to produce functional food, respectively the production of eggs enriched with selenium (Yaroshenko *et al*., 2003 and Sara *et al*., 2008). In addition, Selenium supplementation increases the hatchability of fertile eggs and number of hatched chicks (Cantor and Scott, 1974; Davtyan *et al*., 2006 and Petrosyan *et al*., 2006). Carotenoids have been used for many years in poultry industry as a mean of pigmenting eggs and meat (Leeson and Summers, 1997). In addition, Incorporation of vitamin E into poultry diets has been shown to provide oxidative stability and increase the quality of their eggs and reduce the development of off flavors while increasing egg production (Ajuyah *et al*., 1993; Buckley *et al*., 1995; Cherian *et al*., 1996). Therefore, there is a dearth of information on the utilization of *Moringa Oliefera* and its effect on laying performance and egg quality of poultry.

1.1. Research Objectives:

The overall objective of this study is to determine the effect of *Moringa Oliefera* leaves meal on egg productivity,egg weight and quality of layers as measure by Haugh unit.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and distribution of Moringa:

Moringa (*Moringa Oleifera*) is native to the Indian subcontinent and has become naturalized in the tropical and subtropical areas around the world, The tree is known by such regional names as Benzolive, Drumstick tree, Horseradish tree, Kelor, Marango, Mlonge, Mulangay, Saijihan and Sajna (Fahey, 2005). The plant thrives best under the tropical insular climate, it can grow well in the humid tropics or hot dry lands and can survive in less fertile soils and it is also little affected by drought (Anwar *et al.,* 2007). Moringa (*Moringa oleifera Lam*) belongs to the moringaceae family, and is considered to have its origin in the north-west region of India, south of the Himalayan Mountains (Makkar and Becker, 1997). It is now widely cultivated and has become naturalized in many locations in the tropics (Fahey *et al.,* 2001). There are thirteen species of moringa trees in the family moringaceae and *Moringa oleifera* is the most widely cultivated species. It was further stated that they are native to India, the Red Sea area and/or parts of Africa including Madagascar. *Moringa oleifera* is indigenous to Northern India and Pakistan and was introduced throughout the tropics and sub-tropics becoming naturalized in many African countries (Kristin, 2000). This rapidly-growing tree also known as horseradish tree or drumstick tree was utilized by the ancient Romans, Greeks and Egyptians (Bosch, 2004).

2.2. Uses of *Moringa oleifera*:

Moringa oleifera is considered as one of the World's most useful trees, as almost every part of the Moringa tree can be used for food, medication and industrial purposes (Khalafalla *et al.,* 2010). People use its leaves, flowers and fresh pods as vegetables, while others use it as livestock feed (Anjorin *et*

al., 2010). Leaves could serve as a valuable source of nutrients for all age groups. For example, in Haiti and Senegal, health workers have been treating malnutrition in small children, pregnant and nursing women with Moringa leaf powder (Price, 1985). Anwar *et al.* (2007) reported that moringa leaf powder could be used as food or for medicinal and therapeutic purposes. It is used for improved wound healing, gastric ulcer, diarrhea, sore throat and cancer (Grever, 2001). In many countries, moringa leaves are used as traditional medicine for treating common ailments (Trees for Life, 2005). This tree has the potential to improve nutrition, boost food security and foster rural development (Hsu, 2006). Also , most people in South Africa, however, are not aware of the potential benefits of Moringa , but recently, a high degree of renewed interest was placed on the nutritional properties of Moringa in most countries where it was not native (Reyes *et al*., and 2006; Oduro *et al*., 2008). This could be due to the claims that it increases animal productivity as it has nutritional, therapeutic and prophylactic properties (Fahey, 2005).

In animals, nutrition plays a major role in animal's ability to overcome the detrimental effects of parasitism and diseases (Anwar *et al.,* 2007). A well-nourished animal resists diseases even when exposed to infection than the one, which is already weakened through malnutrition. To gain immunity, the animal needs energy, proteins for manufacture of antibodies and cells, minerals (zinc, copper and iron) and vitamins A and E in communicating messages in parts of the animal's body to fight infections (Conroy, 2005).

There are considerable variations among the nutritional values of Moringa, which depend on factors like genetic background, environment and cultivation methods (Brisibe *et al.,* 2009). The nutritional composition of Moringa of the South African ecotype has not previously been evaluated; the profile of chemical composition, fatty acids, amino acids and vitamins. Amino acids, fatty acids, minerals and vitamins are essential in animal feed.

These nutrients are used for osmotic adjustment; activate enzymes, hormones and other organic molecules that enhance growth, function and maintenance of life process (Anjorin *et al.,* 2010).

Nutritional composition of the plant plays a significant role in nutritional, medicinal and therapeutic values (Al- Kharusi *et al*., 2009). Scanty literature is available on the uses of *Moringa oleifera* plant parts as sanitizers or preservatives in foods. However, a very important step in the screening of a plant material for sanitizing/preservative activity is to evaluate its antimicrobial activity against food – borne microorganisms (Bukar *et al.,* 2010).

2.3. Nutritional value of *Moringa oleifera* leaf:

The leaves have immense nutritional value such as vitamins, minerals and amino acids (Anwar *et al*., 2007). *Moringa oleifera* leaves are known to be great source of vitamins and minerals being served raw, cooked or dried. Fugile (2005) reported that 8 g serving of dried leaf powder will satisfy a child within ages 1-3 years with 14% of the protein, 40% of the calcium, 23% of the iron, and nearly all vitamin A that the child needs in a day. One 100 g portion of leaves could provide a woman with over a third of her daily need of calcium and give her important quantities of iron, protein, copper, sulphur, and B-vitamins. It is estimated that only 20-40% of vitamin A content will be retained if leaves are dried under direct sunlight, but that 50-70% will be retained if leaves are dried in the shade (Subadra *et al.,* 1997).

The leaves are rich in protein, carotene, iron and ascorbic acid and the pod is rich in the amino acid lysine (CSIR, 1962). Moringa is also rich in carotenoids, ascorbic acid, iron and in the two amino acids, methionine and cystine generally deficient in other feeds (Makkar and Becker, 1996). The extracted and unextracted moringa leaves gave crude protein values of 43.5 and 25.1% respectively, suggesting that both the extracted and unextracted

leaves are good sources of protein for livestock. As expected, the crude protein and fibre contents of the extracted leaves were higher than those of the unextracted leaves due to the loss of some cell solubles and lipids during the treatment with 80% ethanol, the crude protein, crude lipids and ash values of 26.4%, 6.5% and 12%, respectively were reported for the unextracted leaves by(Gupta *et al.,*1989) Also, higher levels of NDF (28.8%) and ADF (13.9%) were reported (Gupta *et al.,* 1989). The variations in the reported values may be due to differences in agro-climatic conditions or to different ages of trees, and possibly not due to different stages of maturity. The young leaves are used as fresh green vegetable and are commonly cooked and eaten like spinach as well as to make soup or salads in many parts of India. The leaves are an exceptionally good source of protein, provitamin A, vitamins B and C, minerals (particularly iron) and rich source of essential amino acids such as ethionine, cystine, tryptophan and lysine (Makkar and Becker, 1997;Sa´nchez-Machado *et al*., 2010).

2.4. Chemical analysis of *Moringa oleifera*:

Yameogo *et al*. (2011) reported that, on a dry matter basis, *Moringa oleifera* leaves contained 27.2% protein, 5.9% moisture, 17.1% fat, and 38.6% carbohydrates. Anwar and Rashid, (2007) noticed that on a dry matter basis, *Moringa oleifera* seeds contained 34.80% ether extract, 31.65% protein, 7.54% fiber, 8.90% moisture, and 6.53% ash contents.

Busani Moyo *et al*., (2011). Showed that, the dried leaves had the following mineral contents: calcium (3.65%), phosphorus (0.3%), magnesium (0.5%), potassium (1.5%), sodium (0.164%), sulphur (0.63%), zinc (13.03 mg/kg), copper (8.25%), manganese (86.8 mg/kg), iron (490 mg/kg) and selenium (363 mg/kg).

Sarwatt *et al*. (2004) reported that Moringa leaves are potentially inexpensive protein for livestock feeding containing 80% DM, 29.7% CP, 22.5% CF,

4.38% EE, 27.8% Ca and 0.26% phosphorus. Ayssiwede *et al*. (2011) revealed that the crude protein and fiber content of *Moringa oliefera* leaves were 28.5% and 11.7%, respectively.

The leaves of *Moringa oleifera* has high protein content which is between 20 – 33% on a dry weight basis, the protein is of high quality having significant qualities of all the essential amino acid as reported by (Foidl and Paull, 2008).

Makkar and Becker (1997) found that the essential amino acid contents of the leaves and sulfur containing amino acids of the kernel were higher than the amino acid pattern of the FAO reference protein, but other essential amino acids of the kernel were deficient. .

2.5. Mode of action of *Moringa oleifera*:

Antimicrobial and antioxidant effects of Moringa oleifera were examined by some researchers. Jabeen *et al*. (2008) mentioned that the antimicrobial properties of the Moringa oleifera seed extracts may be due to lipophilic compounds. These compounds may attach to the cytoplasmic membrane. The authors suggested that extracts of *Moringa oleifera* seeds may contain antibiotic metabolites, such as carboxylic acid, 2,4-diacetyl phloroglucinol, and cell wall-degrading enzymes and chitinases. The antioxidant effect of *Moringa oleifera* leaf extract and fruit was explained by Luqman *et al*. (2012), who noticed that it was due to the presence of polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates, which remove free radicals, activate antioxidant enzymes, and inhibit oxidases.

Moringa oleifera leaves have been reported to be a rich source of carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German,

2000; Siddhuraju and Becker, 2003). *Moringa oleifera* is one of such interesting plant which is reported to contain some significant levels of natural antioxidants, such as vitamin E, selenium (Khalafalla *et al.*, 2010) and tannins, which are reported to decrease the rate of lipid and pigment oxidation. Moringa is rich in carotenoid, ascorbic acid, iron and in the two amino acids generally deficient in other feeds i.e methionine and cystine (Makkar and Becker, 1996). *Moringa oleifera* leaf meal has high carotene content. Price (2000) found that it contains 16.3 mg carotenoids/100 grams. Carotenoids are used in physiological processes as antioxidants, but also have a protective and recycling role for other antioxidants like vitamins E and A (Surai and Speake, 1998).

2.6. Medicinal uses of *Moringa oleifera*

Apart from the medicinal uses, *Moringa oleifera* was reported to be a good source of vitamins and amino acids (Olugbemi *et al*; 2010) and was claimed to boost immune systems (Jayavardhanan *et al*., 1994;Fuglier, 1999; Olugbemi *et al*., 2010). The leaves and green fresh pods are used as vegetables by man and are rich in carotenoids and ascorbic acid (vitamin C) with a good profile of amino acids (Makkar & Becker, 1996). The Moringa seed oil is high in (80.4%) polyunsaturated fatty acid (Anwar and Rashid, 2007; Ogbunugafor *et al.,* 2011). Moringa oleifera extract was reported to have antibacterial properties and conclusion was made to investigate it as a phytotherapeutic agent to combat infectious agents (Patel, 2011). Most parts of the plant have been used in folk medicine in Africa and South Asia (Fahey, 2005).

The medicinal effects of the plant was ascribed to their possession of anti-oxidants, which are known to suppress formation of reactive oxygen species (ROS) and free radicals (Sofidiya *et al*., 2006; Ogbunugafor *et al*., 2011). Moringa oleifera leaves have reported that they were important feed

resources relatively rich in nutrients, energy and vitamins (Makkar and Becker, 1996 and 1997; Nuhu, 2010; Ayssiwede *et al*., 2011; Mutayoba *et al*., 2011). Also, they were known to be very poor in anti-nutritional factors and have been used both in ruminants (Foidl *et al*., 2001; Sarwatt *et al*., 2002) and in poultry feeding with various performance results depending on their nutritional value and inclusion level in the diet (Kaijage *et al*., 2003; Kakengi *et al*., 2007; Nuhu, 2010; Olugbemi *et al*.,.2010).

2.7. Effect of moringa leaf meal on the laying performance of hens:

Melesse *et al*. (2011) reported that use of Moringa stenopetala leaf meal in the diet of Rhode Island Red chicks produced significant ($P < 0.05$) increase in feed and crude protein intake, average weight gain feed efficiency ratios, and protein efficiency ratios when compared to a control diet. The authors related these findings to the presence of readily available protein in moringa leaf meal, which is convenient for monogastric animals, and also to the higher levels of methionine and other essential amino acids when compared to the soybean meal of a control diet. The authors concluded that inclusion of Moringa stenopetala leaf meal in amounts of up to 6% in the diet of growing chicks to replace expensive conventional protein sources has no negative effects on the chicks.

Olugbemi *et al*. (2010) noticed that supplementation 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, feed conversion ratio, and laying percentage. Egg weight significantly increased as a result of the supplementation of Moringa oleifera leaf meal with cassava chip when compared to a control diet (free of Moringa oleifera leaf meal and cassava chip). Abou-Elezz *et al*. (2011) mentioned that inclusion of different levels of Moringa oleifera leaf meal (0%, 5%, 10%, and 15%) in the laying hens'

diets linearly decreased egg-laying percentage and egg mass, while egg weight and feed intake showed a quadratic trend with the increased levels of Moringa oleifera leaf meal with the absence of a significant effect on feed conversion ratio. Generally, Kakengi *et al*. (2007), Olugbemi *et al*. (2010), and Abou-Elezz *et al*. (2011) agreed that produce adverse effects. In a study by Kakengi *et al*. (2007) it was revealed that Moringa could be used as a source of plant protein since it was highly accepted even at high inclusion levels in commercial layers diets.

2.8. Selenium in plants and soils:

Plant foods are the major dietary sources of selenium in most countries. In Finland, Sweden and New Zealand, the prevalence of selenium deficient soils make it necessary for selenium-enriched fertilizers to be used in agriculture (Rayman 2000). Animals that eat grains or plants that were grown in selenium-rich soil have higher levels of selenium in their muscle. The decline in blood selenium concentration in many countries has potential public health implications, particularly in relation to chronic diseases prevalent to the Western world such as cancer and cardiovascular disease (Rayman 2000; Brown and Arthur 2001). The underlying rationale is that Selenium-biofortified wheat is a potential strategy for increasing the dietary intakes of selenium, thus improving human health for most of the population.

The bioavailability of organic selenium compounds in foods is generally high (apparent absorption of 70–95%), particularly from plant foods, where most selenium is in the selenomethionine form while selenite, on the other hand, is more likely to be 60–70% absorbable (Lyons *et al*. 2003).

2.9. Organic and Inorganic Selenium:

There are principal differences in assimilation and metabolism of organic and inorganic selenium forms. For example, selenite is passively absorbed in

the intestine as a mineral, used for immediate synthesis of some selenoproteins and the rest is released from the body with faeces and urine. In contrast, organic selenium is absorbed as an amino acid, similar to methionine. A portion is used for immediate synthesis of selenoproteins, similarly to selenite, but another portion of the organic selenium is incorporated non-specifically in newly synthesised proteins in place of methionine.

It is deposited, therefore, in muscle or eggs, and as a result Se reserves in the body are established. Therefore the main advantage of organic selenium is improved retention in the tissues which provides Se reserves for the body. These reserves are especially important in stress conditions when the Se requirement increases while its dietary supply usually decreases with lower feed consumption. This is not the case when inorganic selenium is added to the diet since animals are not able to synthesise selenomethionine (Schrauser, 2000).

In stress conditions muscle protein catabolism by proteasomes can release SeMet which can then serve as a source of Se for newly synthesied selenoproteins such as GSH-Px, thioredoxin reductase and methionine sulphoxide reductase. Those enzymes can deal with overproduction of free radicals and prevent the decrease in productive and reproductive performance of farm animals. It has been proven that selenium from both selenite and SeMet is readily available for synthesis of the selenoenzyme GSH-Px in rat tissues. There are also several lines of evidence confirming the idea that selenium accumulated in tissues in the form of SeMet can be available for selenoprotein synthesis.

(Surai, 2000) indicated that chicks hatched from eggs enriched with selenium from Sel-Plex® had higher liver GSH-Px activity not only at hatching, but more importantly, even at 5 days post hatch. This could be explained by usage of SeMet accumulated in tissues as a result of Se transfer

from the egg during embryogenesis. Secondly, the bioavailability of the Se pool in maintaining liver GSH-Px activity during a period of Se deprivartion, following excess selenite or SeMet loading was assessed in rats (Ip and Hayes, 1989). We must remember that selenomethionine is a storage form of selenium in the body. During protein catabolism in proteasomes selenomethionine can be released and used for additional selenoprotein synthesis and this could result in maintenance of high productive and reproductive performances of farm animals or good health of companion animals in stress conditions.

2.10. Use of selenium for poultry:

Selenium is a dietary essential nutrient for poultry. The Se requirement for the laying hen ranges from 0.05 to 0.08 ppm depending on daily feed intake while the broiler's requirement is 0.15 ppm (NRC, 1994). Natural feedstuffs often will meet these requirements, but as previously mentioned, there is considerable variation in Se content of natural feedstuffs. Therefore, it is common practice in the poultry industry in the U.S. to supplement the diet with some form of Se.

The maximum level of Se supplementation allowed in poultry diets is 0.30 ppm (NRC, 1994; AAFCO, 2003). This supplementation has historically come from inorganic sources of Se, primarily Sodium Selinate (SS), but in 2000, the Food and Drug Administration (FDA) approved the use of Se-enriched yeast (SY). There have been several reports comparing the use of organic Se with inorganic Se in broilers and laying hens, which will be discussed below.

2.10.1. layers:

Selenium (Se) is an essential dietary nutrient for laying hens. Rutz , 2003 pointed out that supplementation of Se to the layers' diet increased Se concentration in shell leading to improved shell quality. While Increased Se concentration in egg yolk and egg white leading to improved internal egg

quality during storage, as well as decreased lipid peroxidation in egg yolk during storage increased egg yolk, albumin and shell weight.

The Se requirement for laying hens ranges from 0.05 to 0.08 ppm depending on daily feed intake (NRC, 1994). However, Se content of feed grains widely varies from region to region (NRC, 1994), and thus it is common practice in the poultry industry to supplement laying hen diets. The maximum allowed Se inclusion level in the United States is 0.30 ppm. Selenium is required for maintenance of health, growth, and physiological functions. Traditionally, Se has been added to poultry diets via inorganic sources, such as sodium selenite. Research has shown that organic Se such as selenocysteine, selenomethionine (SM), or Se-enriched yeast (SY), as supplemental sources of Se is more bioavailable than Se in sodium selenite (Cantor *et al.*, 1982). Moreover, organic sources of Se have been explored as an alternative to inorganic supplementation (Payne, *et al.*, 2005). The use of organic Se results in less Se being transferred to the environment through feces, and more Se is deposited into body tissues and eggs, therefore use of Se yeast in laying hen diets is very effective for increasing the Se content of eggs (Cantor and Scott, 1974; Ort and Latshaw, 1978; Swanson, 1987; Davis *et al.*, 1996; Cantor *et al.,* 2000 Patton, 2000, Payne *et al*., 2005 and Utterback, *et al*., 2005). Low Se content of human diets has been correlated with higher incidences of cancer and other diseases (Allan, *et al.,* 1999); therefore, Se yeast fed to laying hens may add value to market eggs (Utterback, *et al*., 2005). The enrichment of eggs with organic selenium represents a commercially valuable use for the future. The supplementation of hens' food with organic selenium not only improves their health and productivity, but can also be a natural way to produce functional food, respectively the production of eggs enriched with selenium (Yaroshenko *et al*., 2003 and Sara *et al.,* 2008). In addition, Selenium supplementation increases the hatchability of fertile eggs and number of hatched chicks

(Cantor and Scott, 1974; Davtyan *et al*., 2006 and Petrosyan *et al*., 2006). As a result, Se has an important role in poultry fertility and embryonic development.

Several researchers have indicated no difference in daily egg production due to Se supplementation or source (Cantor and Scott, 1974, Latshaw and Osman, 1975, Ort and Latshaw, 1978, Cantor *et al*., 2000; Patton, 2000). The increase in whole egg Se when diets are supplemented with Se has been reported by several authors and is very consistent (Cantor and Scott, 1974; Latshaw and Osman, 1975; Ort and Latshaw, 1978; Latshaw and Biggert, 1981; Martello and Latshaw, 1982; Swanson, 1987; Davis *et al*., 1996; and Cantor *et al*., 2000). Several reports also have indicated that yolk or white Se concentrations are increased depending on Se source, but these reports are slightly variable. Latshaw and Osman (1975), Martello and Latshaw (1982), Swanson (1987), and Davis *et al*. (1996) reported that eggs from hens fed diets supplemented with selenomethionine (SM) had higher Se in the white than those fed SS or selenocysteine. Ort and Latshaw (1978) indicated that the Se level of yolks was greater when hens were fed SS, but Swanson (1987) and Davis *et al*. (1996) indicated that SM increased yolk Se more than SS. Latshaw and Biggert (1981) and Cantor *et al*. (2000) reported that whole egg, egg white, and egg yolk Se levels were greater in hens fed SM compared with those fed SS. Dietary selenium intake affects the selenium content of eggs. An increase in the selenium content of layer diets from 0.3 ppm to 0.5 ppm has been found to increase selenium concentrations per gram of egg weight from 340 ng to 565 ng (Boruta *et al*. 2007). The selenium content of eggs is also determined by the dietary selenium source (Kenyon& Spring 2003). In layers fed selenium-enriched yeast, the selenium content of eggs increased from 0.3 ppm to 2.7 ppm. A lower increase in the selenium content of eggs, from 0.3 ppm to 0.7 ppm, was noted in laying hens fed inorganic selenium (Payne *et al*. 2005). In many bird species, selenium is

accumulated in the egg white (0.785-18.98 Mg/egg). In quail and chicken eggs, the highest level of selenium ions are found in the yolk (1.35-4.80 Mg), while in ostriches in the eggshell (390.2Mg) (Golubkina & Papazyan 2006).

2.10.2.Broilers:

studies have shown that supplementation with organic selenium decreased drip loss, improved FCR ,better feathering, prevention of peroxide accumulation during meat storage as well as decreased mortality due to ascities and improved chicken growth in stress conditions (Edens, 1997; 2002). In several broiler performance trials, organic selenium supplementation showed a positive effect on weight gain and FCR compared to controls (Spring 2008). The improved membrane integrity from organic selenium supplementation is linked to better osmotic regulation and control, reducing the amount of water lost from the tissue as either oedema in living animals or drip loss in meat. Water holding capacity is also an important characteristic of meat, as it determines the level of exudative loss in packaging and during cooking, and the juiciness of meat. The researchers have suggested that the prooxidant effect of inorganic selenium is detrimental to cell membranes causing higher levels of drip loss. Organic selenium exerts an antioxidant effect on the birds' cellular membranes and tissue structures resulting in less exudative losses from meat.

2.11. Practical approaches to improve selenium status of poultry selenium -enriched eggs:

Poultry diets are supplemented with Se in order to provide a margin of safety against deficiency and to maintain high productive and reproductive performance. In this respect the sources/forms of dietary Se are of great importance. During evolution, all animals, including birds, have adapted to metabolise the organic form of Se (Surai and Dvorska, 2001a). Indeed, feed

ingredients only contain Se in organic forms, mainly as SeMet. This means that for a bird or animal adapted to this form of Se, the digestive system is ill prepared for inorganic Se (selenite or selenate). This fact is underscored by the fundamental differences in absorption and metabolism of inorganic and organic Se sources. SeMet is actively absorbed in the intestine as an amino acid employing similar processes as methionine. In contrast, inorganic Se is passively absorbed. The chemical similarity between SeMet and methionine allows the body to use them interchangeably in protein synthesis. This makes it possible to build Se reserves in the body (mainly in muscle). Since SeMet is not synthesized by animals but only by plants (Schrauzer, 2000), there is no Se reserve in the body when inorganic Se is used. This principal difference can explain why organic Se is more effective than inorganic sources, especially during stress conditions.

Consider two different scenarios for the Se supplementation of poultry. The first and most common scenario is the addition of inorganic Se to diets containing low levels of naturally occurring organic Se. When overproduction of free radicals occurs as a result of stress conditions, the body responds to by attempting to mobilise antioxidant reserves and more importantly by synthesising additional selenoproteins. In this scenario the main limitation is the absence of Se reserves and a restricted ability to synthesise additional selenoproteins. Therefore in this scenario we would expect a decrease in productive and reproductive performance of poultry as well as compromised immunity defence.

Increased Se concentration in the egg as a result of dietary organic Se was associated with better antioxidant protection of chicks during embryonic development and during the first week post-hatch (Surai, 2000a). Commercial results from UK breeder farms confirmed the practical nature of enhancing Se status in this manner. An improvement in hatchability (0.5-

2.0%) was noted and associated with a substantial decrease in mortality for the first 10 days post-hatch (Surai, 2000).

2.12. Effect of Organic selenium on egg weight and egg production

Maysa *et al*., (2009) showed that , egg production percentage, egg weight , egg quality (haugh units) egg yolk index and egg thickness were significantly increased with for hens fed Sel-Plex ^{TM} Supplementation compared with those in control group.

Natasha *et al*., (2012) reported that, in three experimental groups(molted laying hens 80 weeks of age) , group1 received 0.30 mg Se kg $^{-1}$ from sodium selenate and groups 2 and 3received 0.38 mg Se kg $^{-1}$ and 0.46 mg Se kg $^{-1}$ from sodium selenate and selenium yeast. The egg production were significantly higher in the supplemental groups supplemented with selenium yeast in feed. While egg weight (g) was significantly higher in the experimental groups 2 and 3. i.e egg weight (g) in control group was 68 g less than what obtained in groups 2 (72g) and group 3 (72g) that received selenium yeast in feed. Egg production intensity was increased in groups 2 and 3 (91.95% and 91.98%) of Lying hens fed with feed supplementation with organic selenium in relation with the group 1 of laying hens fed with feed supplementing with mineral selenium (82%)**.** Sara *et al* ., (2008). Indicated that addition of organic selenium (Sel- Plex ^{TM})in laying hens diets within the second stage (aged between 48-62 weeks) improved egg production, and egg weight.

Stanley *et al*., (2004) found that hen-day egg production and egg weight from the Sel-Plex™ fed hens were significantly higher ($P \leq 0.05$) than the control group.

Ebenebe *et al*., (2013) indicated that inclusion of Moringa at lower levels improved egg production and egg quality but higher levels of inclusion

resulted in lower productivity and poorer egg quality indices.

2.13.Organic selenium and egg quality:

Maysa *et al*., (2009) Egg shell thickness (mm) of laying hens at week 50of eg, as affected with organic selenium (Sel- Plex ^{TM}) 0.1 mg/kg In diet was improved 39 mm more than control group 35nmm (Klecker *et al*., (2001) and Sara *et al*., (2008) indicated that the administration of organic selenium in laying hen diets increased egg shell thickness and reduced the number of eggs with shell abnormalities, consequently improved eggshell quality.

The results of shell thickness was reported by Renema (2006) who indicated that shell thickness was higher in the Sel-Plex™ group than in the control group after 9 weeks of treatment, while shell thickness in the group given inorganic selenium was intermediate. Spring (2006) showed that organic selenium supplementation in broiler breeders and layers improve egg quality and anti-oxidative properties.

2.14.Selenium and egg freshness:

Egg freshness is one of the most important parameters of consumer perception and demand. Egg quality decreases during storage. This process is associated with biochemical changes in composition and in the structure of the egg. In experiments conducted in Japan (Wakebe, 1998) inclusion of selenomethionine (Se-Met) in the layer diet, supplying 0.3 ppm Se/kg feed increased GSH-Px activity in the egg yolk and white.

Haugh units were used as an indicator of egg freshness. The value was high on day one in both treatment and control groups with difference due to Se supplementation. These data indicate the possibility of improving maintenance of egg quality during storage, with a commercial advantage during egg transportation and storage. There are implications for incubated hatching eggs as well, since prolonged egg storage is associated with decreased hatchability. Therefore, the inclusion of organic Se in the diet of

laying hens could be used to maintain egg quality during storage.

2.15. The Haugh unit as a measure of the egg quality:

The Haugh unit is a measure of the internal quality of an egg. The test was introduced by Raymond Haugh in 1937, and is considered the most significant measure of egg quality.

An egg is weighed, then broken onto a flat surface (breakout method), and a micrometer used to determine the height of the thick albumen (egg white) that immediately surrounds the yolk. The height, correlated with the weight, determines the Haugh unit, or HU, rating. The higher the number, the better the quality of the egg (fresher, higher quality eggs have thicker whites). The formula is as below:

HU = 100 log10 (h - 1.7w0.37 + 7.6)

Where:

HU = Haugh unit

h = observed height of the albumen in millimeters

w = weight of egg in grams

The Haugh unit value ranges from 0 - 130 and it can be ranked as below:

AA : 72 or more

A : 71 - 60

B : 59 - 31

C : 30 or less

Improvement in egg antioxidant status with selenium supplementation has been reported by (Wakebe, 1998) who observed higher selenium, vitamin E and higher Haugh unit readings in these eggs. Gajčević *et al*. (2009) reported that blood glutathione peroxidase (GSHPx) activity was higher and egg Haugh unit readings and TBARS (thiobarbituric acid reactive substances) values were better in the organic selenium supplemented eggs compared to controls.

The colour intensity of the egg yolk supplemented with the natural carotenoid, capsanthin and capsorubin was consistently higher in selenium eggs (Wong 2005) compared to the control as determined by the Roche Yolk Color Fan test and this was due to the protective antioxidant effect of selenium.

2.16. Carotenoids:

Carotenoids are class of natural fat soluble pigments found mainly in plants, algae, and various microorganisms. More than 750 carotenoids have been identified in nature (Britton *et al*., 2004), and thus plays important role in the coloration of many plants, invertebrates, fishes, amphibians, reptiles, and birds (Goodwin, 1984).

Carotenoids have been used for many years in poultry industry as a mean of pigmenting eggs and meat (Leeson and Summers, 1997). Their spectral qualities result in change in color of fat depots; depending on the actual xanthophyll pigment and the concentration in the birds' diet, they impart colors ranging from yellow to intense orange. Inclusion of a carotenoid mixture in the laying hen diets was associated with increased lutein, citranaxanthin, canthaxanthin and carotenoic acid accumulation in the egg yolk (Surai and Speake, 1998) and hen tissues (Surai *et al*., 1999b). Eggs of hens have high level of carotenoids are characterized by higher hatchability in comparison to eggs with low carotenoid levels (Inborr, 1996; Kemp *et al*., 2001).

2.17. lutein:

Lutein is a group of natural pigments known as xanthophylls and it is mainly found in fruits, vegetables and eggs (Johnson, 2004). Its colour is yellow-orange and can be used in poultry diets to pigment egg yolks. Its supplementation in human diet can reduce risk of several diseases like age related macular degeneration and cataracts (Ribaya-Mercado and Blumberg,

2004). Lutein is highly bio-available from fats, fat-soluble compounds; in eggs it is mainly present in egg yolk (Chung *et al*., 2004). Several authors reported that lutein supplementation in layer diet cause an increased lutein concentration in Plasma (Wu *et al*., 2009) and contents of eggs (Leeson and Caston, 2004). Lutein is mostly found in marigold (Tagets erecta) and its extraction by solvent extraction method has been used to produce lutein additives (Breithaupt, 2002). Some authors (Li *et al*., 2007) reported a better stability of esterified lutein than that of free lutein, under the same challenge of light and heat. Both free and esterified lutein can be added in the diets of layer to increase the lutein contents of eggs (Wu *et al*., 2009).

Over the last 10 years there has been increased awareness of the role of xanthophylls in human health, and in particular the roles of lutein and zeaxanthin in prevention of certain eye disorders.

Macular degeneration is the leading cause of blindness in developed countries, resulting in progressive and irreversible loss of central region vision. The most effective prevention to date is increasing our intake of lutein, which accumulates in the macular region of the eye and seems to aid in prevention of such blindness. Lutein and zeaxanthin are able to absorb blue light striking the retina, which is thought to initiate degeneration of the delicate surface membrane (Landrum and Bone, 2001). Lutein may also play a role as an antioxidant in macular surface membranes (Rapp *et al.*, 2000).

Moeller *et al.* (2000) suggested that xanthophyll intake might also influence development of cataracts. Eggs, although not normally the richest source of pigments, contain highly available and stable pigments that could be of importance in preventing cataract and macular degeneration. Landrum and Bone (2001) suggested the intake of lutein and zeaxanthin in North Americans is less than 1 mg/d, which is much less than the preventive levels being tested for these nutrients (Grando *et al*., 2003).

Eggs normally contain 0.3 to 0.5 mg of total xanthophylls, with just over half present as lutein (Steinberg *et al*., 2000). Lutein is a xanthophyll carotenoid found naturally in marigold flowers, corn, and dark-green leafy vegetables. Recent evidence suggests that lutein may be beneficial in the protection against numerous human diseases, such as macular degeneration, heart disease, and cancer (Ribaya-Mercado and Blumberg, 2004). Due to its suggested role in disease prevention, research has been conducted on the development of lutein-enriched eggs, through supplementation of the layers diet (Leeson and Caston, 2004). Since lutein is a fat-soluble compound, the lutein composition of eggs from layers can be manipulated by adjusting the level of lutein in the layers diet (Leeson and Caston, 2004). Moringa is rich in carotene ,ascorbic acid, iron and in the two amino acids generally deficient in other feeds i.e methionine and cystine (Makkar and Becker, 1996).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Experimental site (Study area) and period:

This experiment was conducted at the Poultry Unit of the Sudan University of science & technology(SUST), college of agriculture studies, Department of animal production, Shambat, Sudan.

3.2 Preparation of *Moringa Oleifera* leaves powder:

Moringa olifera leaves were obtained from Salah Abdoun farms, Khartoum Bahri, El Samrab farm. The moringa leaves were harvested, and then dried under shade to maintain the nutritious properties and greenish color. The dried leaves were then milled using a hammer mill to produce *Moringa Oleifera* leaves powder (MOLP) suitable for incorporation into layers diets.

3.3 Experimental stock :

60 Hy line chicken (17 weeks old) were used in the experiment. The chicken were purchased from Elkhadarah Company, Then the flock was transferred to Poultry Unit in SUST, college of agriculture studies, department of animal production, Shambat.

3.4 Housing and feeding of the experimental birds:

The experimental birds were managed in an open-sided house in a battery cage system; each three birds were kept in an individual cage (50 x 40 x 45 cm). The cage pens were galvanized metal wire in double-decker rows providing 390 cm^2/hen. For this experiment, one upper deck of cages and one lower deck of cages were used. Each pen had a nipple waterer. A continuous, galvanized metal feed trough was divided by replicate to insure that the hens were not able to consume feed assigned to the adjoining replicate. A wire divider was inserted in the egg collection area to prevent eggs from separate replicates being mixed. Feed (in mash form) and water

were provided *ad libitum* throughout the experiment. The lighting schedules were similar to guidelines set in the Hy-Line W-36 Commercial Management Guide (Hy-Line International, 2003) and maintained on a 16 h L: 8 h D lighting program for the entire period of the study. Each treatment was replicated five times (15 birds per treatment) in a complete randomized design (CRD).

The flock were allowed 2 weeks of adaptation priod. All birds were provided with layer diet containing 2,800 kcal/kg diet, 17.00% CP, 1.45% calcium and 0.47% phosphorous.The feeding program consisted of layer basal diets (Table 1) that was formulated to meet the bird's dietary nutrient requirements (NRC, 1994). Four dietary treatment groups were produced from the basal feed as follows:

A= control without moringa oil (negative control)

B= control with Moringa oil (positive control)

C= diets contained 1% MOLP with no moringa oil

D= diets contained 1% MOLP with moringa oil

Table.1.Composition of experimental diets used in the Experiment

	control+ no Oil	control+ Mor oil	Moringa no Oil	Moringa +oil
Dura	67.60	65.45	**66.94**	64.60
G.N Meal	11.10	11.10	**10.8**	11.00
Hatch	0.00	0.00	**1%**	**1%**
lime Stone	0.00	0.00	**8**	8.00
Molasses	1.00	0.50	**1**	0.50
Wheat Bran	4.35	6.42	**4.36**	6.48
lime	9.02	9.10	**1.14**	1.15
L. Conc. Koudijs	5.00	5.00	**5**	5.00
Dcal	0.68	0.68	**0.53**	0.55
Veg. Oil	0.10	0.10	**0.1**	0.10
Salt	0.20	0.20	**0.2**	0.20
CHOLINE	0.05	0.05	**0.05**	0.05
ANTI TOXINS	0.20	0.20	**0.2**	0.20
ORGANIC ACID	0.20	0.20	**0.2**	0.20
Lysine1	0.30	0.30	**0.3**	0.30
Meth1	0.20	0.20	**0.18**	0.17
Total	**100.00**	**100.00**	**100.000**	**100.00**

Calculated analysis

	ME	CP	Lysine	Meth	ca	Av.P
1% Moringa+ no mor oil	2,808	17	0.88	0.45	3.82	0.42
control+ Moringa oil	2,808	17	0.88	0.48	3.83	0.44
control without mor oil	2,808	17	0.87	0.47	3.80	0.44
1% Moringa + mor oil	2,808	17	0.89	0.45	3.83	0.42

3.5 Experimental Measurements :

Egg production was recorded daily with weekly measurement of feed intake, egg weight. 3eggs per cage (replicate) per week were randomly taken from each treatment group for egg quality measurements. Eggshell thickness and yolk color and Haugh Unit. The average eggshell thickness was determined by measuring the thickness at the large end, small end and in the middle of the egg, using Electronic Digital Vernier (figure 1) and the mean of the three measurements were taken as the average shell thickness. The weekly collected eggs were used for determination of albumen quality of fresh eggs, by measuring the Haugh units of each egg using the equation bellow:

Haugh Unit (Hu) = $100 \text{ Log } (H + 7.5 - 1.7W^{0.37})$

Where H is observed height of albumen in mm and W is the weight of the egg.

To examine the effects of storage duration on albumen quality and yolk color, the rest of collected eggs was stored at room temperature for 3, 7 and 14 days before Haugh unit and yolk color measurements.

CHAPTER FOUR

Results

This experiment compared the effects of feeding layers MOLP with or without supplementation of moringa oil. The effects MOLP on the percentage of hen day egg production (Tables 2, 3 and Fig 1, 2) show no significant difference between different treatments. Egg weight results (Table 4 and Fig 3) followed a similar trend to percentage of hen day egg production, where no significant difference between different treatments was detected. However, there were a numerical improvement in egg weight throughout the duration of the experiment, with MOLP with moringa oil show the high egg weight followed by the egg weight of MOLP treatment without moringa oil added.

The shell thickness (mm) and shell weight of layers fed MOLP treatment with or without moringa oil added were almost similar and significantly ($P<.01$) higher than thickness of eggs produced by birds fed the control diets with or without oil, throughout most of the seven weeks duration of the experiment (Table 5,6 and Fig 4, 5).

Haugh units were used as an indicator of egg freshness and it was found that the values of these units were significantly high for fresh eggs as well as stored eggs produced by layers fed MOLP with or without supplementation of moringa oil at different ages and store for 3, 7 and 14 days throughout the duration of the experiment (Table 6, 7,8,9,10 and Fig 6,7,8,9). However, as time progressed during storage time, Haugh units in the two control groups declined sharply whilst, the decrease was more moderate in the MOLP with or without supplementation of moringa oil groups. The same finding were observed for eggs obtain from hens at the same age (at 24^{th}, 25^{th}, 26^{th}, 27^{th}, 28^{th}, 29^{th} and 30^{th} of age) and stored for the same above duration (Tables 11,12,13,14,15,16,17 and Figs 10,11,12, 13,14,15,16).

The yolk color of eggs of the experiment layers was measured weekly for five consecutive weeks (Tables18,19,20,21,22,23,24) and Fig (17,18,19,20,21,22.23) and the results show that addition of MOLP resulted in a significant increase in in egg yolk color from the first degree according to Roche yolk color fan for both of the control group, to 4 degree for the egg yolk of birds fed MOLP. The addition of oil show no further increase in the color.

Effect of moringa leaf with or without moringa oil on egg production

Table (2): Egg production (week 1 to week 4)

Treatment	Egg production
1% Moringa without oil	78±2.29[a]
Control + Moringa oil	72±2.19[a]
Control without oil	79±2.18[a]
1% Moringa with oil	72±2.19[a]
P-value	0.4952[NS]
$Lsd_{0.05}$	0.1765
SE±	0.06353

Values are mean±SD.

Means having different superscripts are significantly different (P≤0.05).

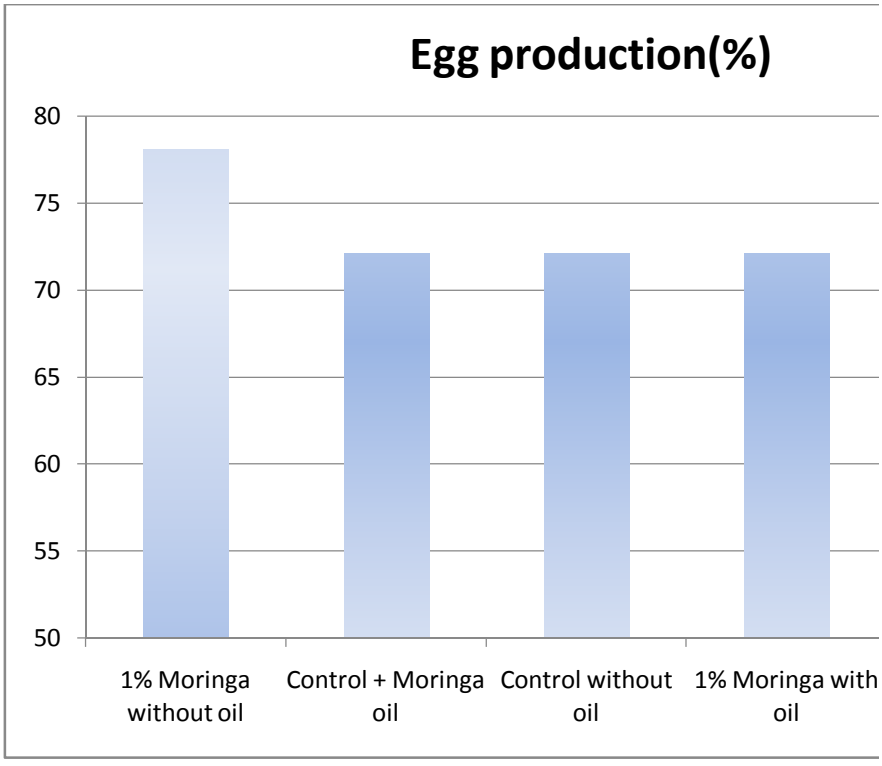

Figure (1) Egg production (week 1 to week 4)

Table (3): Egg production (week 5 to week 7)

Treatment	Egg production
1% Moringa without oil	57±2.45[a]
Control + Moringa oil	59±2.31[a]
Control without oil	62±2.35[a]
1% Moringa with oil	64±2.38[a]
P-value	0.3841[NS]
$Lsd_{0.05}$	0.1646
SE±	0.0592

Values are mean±SD.

Means having different superscripts are significantly different (P≤0.05).

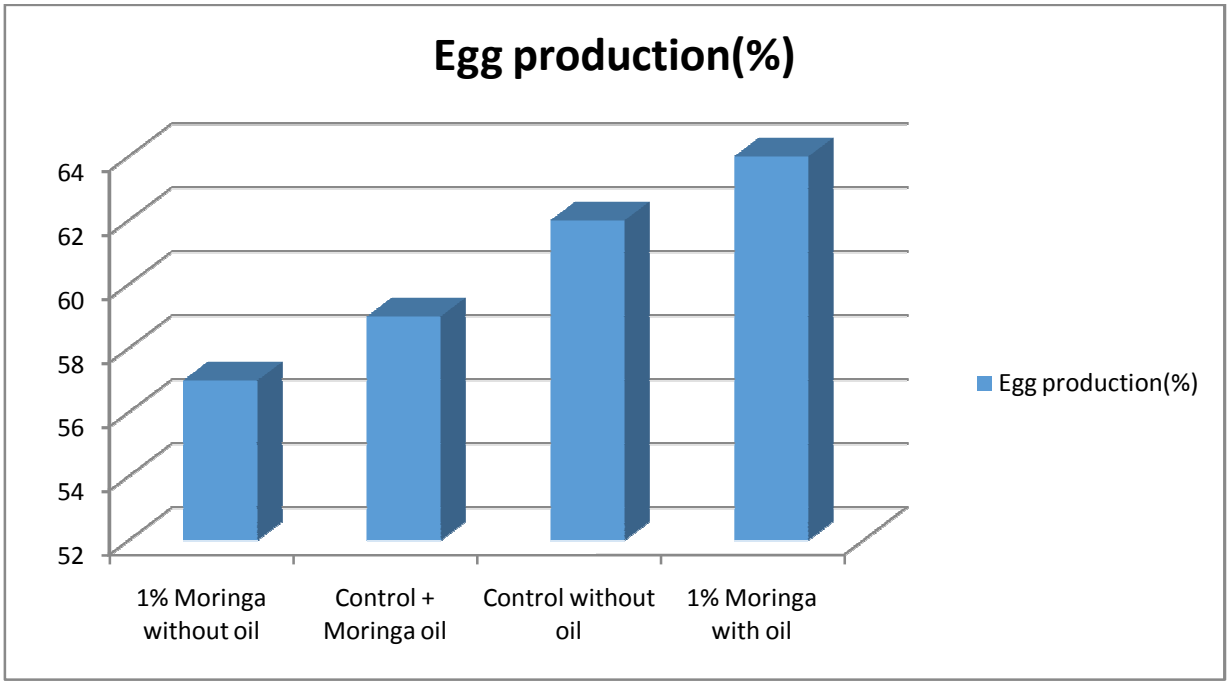

Figure (2) Egg production (week 5 to week 7)

Table (4) Effect of moringa leaf with or without moringa oil on egg weight (gm)

Treatments	Weeks						
	1	2	3	4	5	6	7
control without oil	46.00^{a} ±2.00	45.00^{b} ±1.00	47.33^{a} ±1.15	47.67^{a} ±4.16	46.67^{a} ±0.58	47.33^{a} ±2.08	46.67^{a} ±1.53
control+ Moringa oil	45.67^{a} ±0.58	46.00^{ab} ±1.00	48.00^{a} ±1.00	48.33^{a} ±1.53	48.00^{a} ±1.00	48.00^{a} ±2.00	48.00^{a} ±2.65
1% Moringa no oil	46.67^{a} ±0.58	47.00^{ab} ±1.00	48.67^{a} ±0.58	48.67^{a} ±0.58	49.00^{a} ±1.00	48.67^{a} ±0.58	49.00^{a} ±4.58
1% Moringa + oil	50.67^{a} ±4.51	50.33^{a} ±4.51	49.67^{a} ±2.08	48.67^{a} ±4.62	49.00^{a} ±3.00	48.67^{a} ±0.58	48.67^{a} ±0.58
P-value	0.1268^{NS}	0.0418*	0.244^{NS}	0.065^{NS}	0.3412^{NS}	0.543^{NS}	0.417^{NS}
$Lsd_{0.05}$	4.707	4.547	2.491	6.052	3.169	2.824	5.213
SE±	1.443	1.394	0.7638	1.856	0.9718	0.866	1.599

Mean(s) having different superscript(s) in a column are significantly different (P≤0.05) according to DMRT.

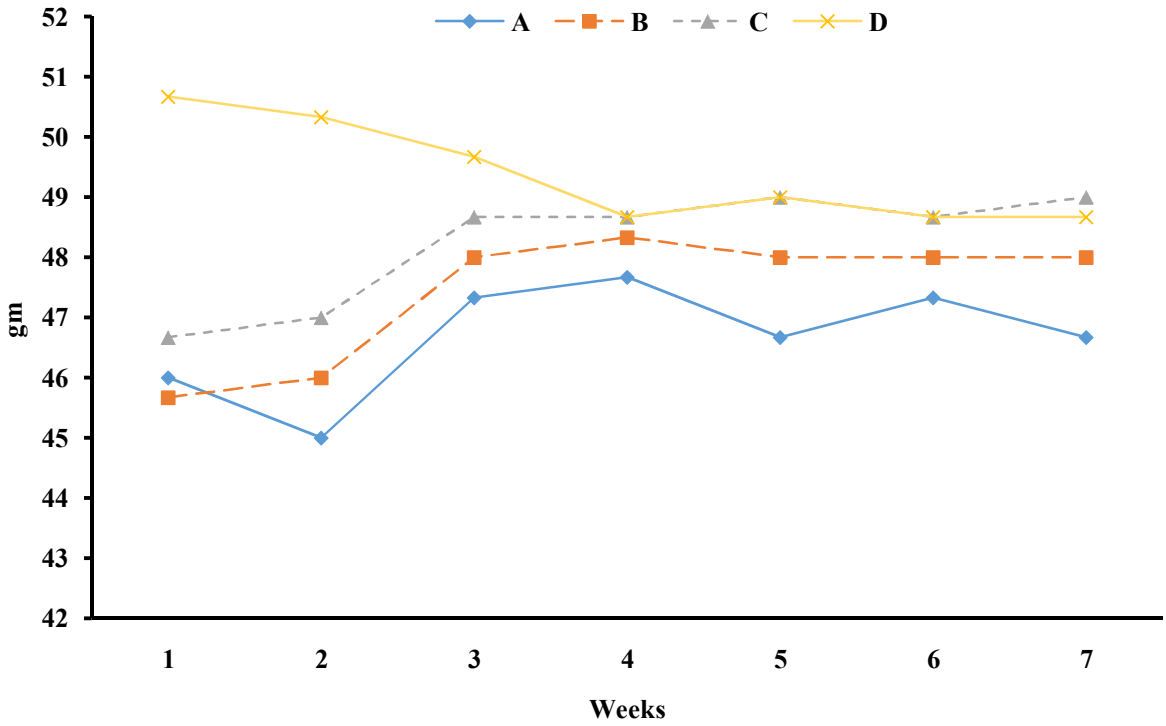

Figure (3) Effect of moringa leaf with or without moringa oil on egg weight(gm)

Table (5) Effect of moringa leaf with or without moringa oil on shell weight (gm)

Treatments	Weeks						
	1	2	3	4	5	6	7
comtrol without oil	6.00^{a} ±1.73	6.67^{a} ±1.15	5.33^{a} ±0.58	5.67^{a} ±1.15	6.00^{a} ±0.00	6.00^{a} ±0.00	5.67^{a} ±0.58
control+ Moringa oil	6.67^{a} ±0.58	5.67^{a} ±1.15	5.67^{a} ±0.58	6.33^{a} ±0.58	6.00^{a} ±0.00	6.33^{a} ±0.58	5.67^{a} ±0.58
1% Moringa no oil	7.00^{a} ±1.00	7.33^{a} ±0.58	6.00^{a} ±0.00	6.33^{a} ±0.58	6.67^{a} ±0.58	6.67^{a} ±1.15	6.00^{a} ±0.00
1% Moringa + oil	7.67^{a} ±0.58	7.33^{a} ±0.58	6.33^{a} ±0.58	6.67^{a} ±0.58	6.67^{a} ±0.58	7.00^{a} ±0.00	6.33^{a} ±0.58
P-value	0.1699^{NS}	0.533^{NS}	0.1631^{NS}	0.905^{NS}	0.1189^{NS}	0.33^{NS}	0.363^{NS}
$Lsd_{0.05}$	2.034	1.718	0.9414	1.438	0.7694	1.216	0.9414
SE±	0.6237	0.5269	0.2887	0.4408	0.2359	0.3728	0.2887

Mean(s) having different superscript(s) in a column are significantly different (P≤0.05) according to DMR

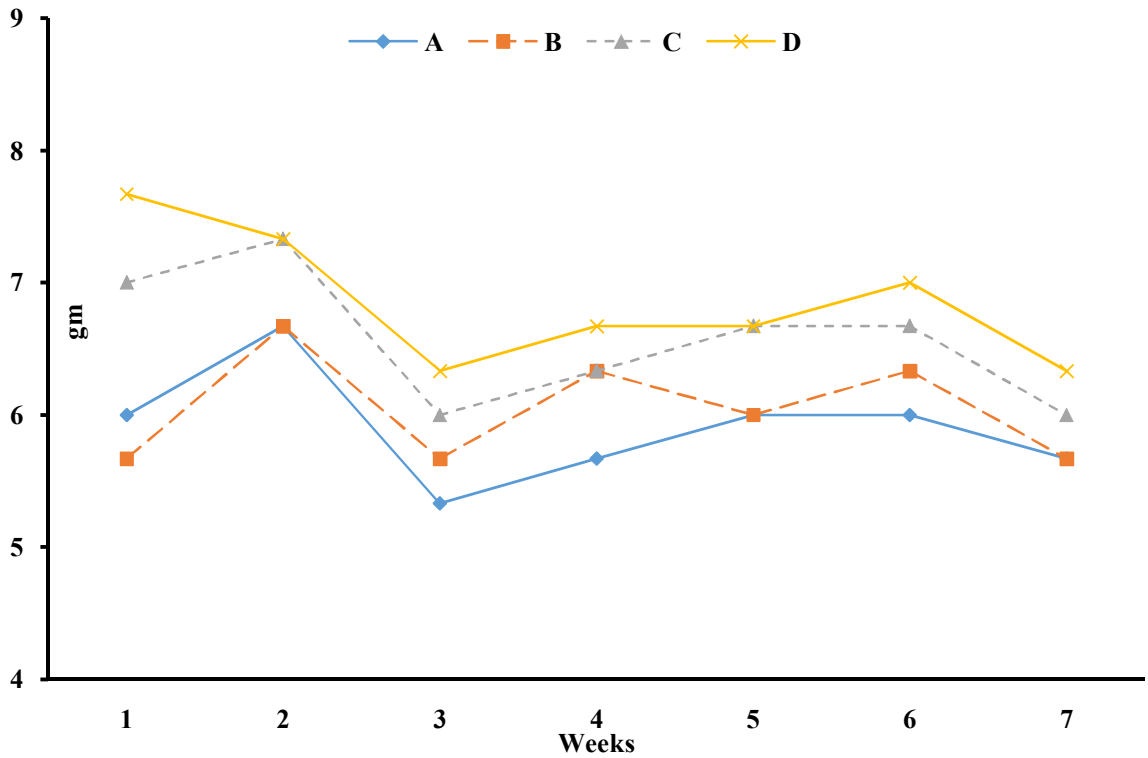

Figure (4) Effect of moringa leaf with or without moringa oil on shell weight(gm

Table (6): Effect of moringa leaf with or without moringa oil on shell thickness (mm)

Treatments	Weeks						
	1	2	3	4	5	6	7
comtrol without oil	0.33^{d} ±0.01	0.34^{c} ±0.01	0.35^{a} ±0.01	0.35^{c} ±0.02	0.35^{c} ±0.01	0.35^{d} ±0.02	0.32^{a} ±0.03
control+ Moringa oil	0.35^{c} ±0.00	0.34^{c} ±0.01	0.35^{a} ±0.01	0.35^{c} ±0.01	0.35^{b} ±0.01	0.36^{c} ±0.03	0.33^{a} ±0.03
1% Moringa no oil	0.36^{b} ±0.01	0.35^{b} ±0.02	0.41^{a} ±0.08	0.36^{b} ±0.01	0.36^{a} ±0.01	0.370^{b} ±0.01	0.35^{a} ±0.01
1% Moringa + oil	0.36^{a} ±0.01	0.36^{a} ±0.01	0.36^{a} ±0.02	0.36^{a} ±0.02	0.36^{a} ±0.02	0.39^{a} ±0.02	0.37^{a} ±0.03
P-value	0.0042^{**}	0.0495^{*}	0.2869^{NS}	0.0436^{*}	0.0361^{*}	0.0451^{*}	0.2488^{NS}
$Lsd_{0.05}$	0.0005954	0.0005954	0.0005954	0.0005954	0.0005954	0.0005954	0.0005954
SE±	0.0001826	0.0001826	0.0001826	0.0001826	0.0001826	0.0001826	0.0001826

Mean(s) having different superscript(s) in a column are significantly different (P≤0.05) according to DMRT

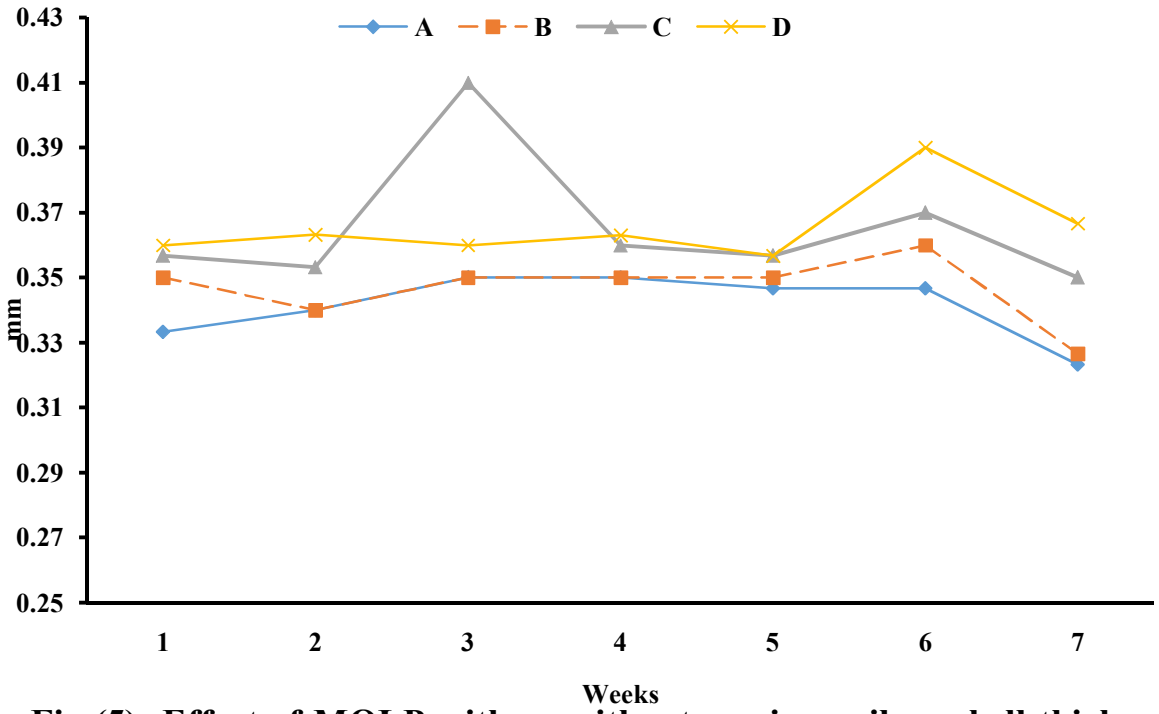

Fig (5): Effect of MOLP with or without moringa oil on shell thickness of eggs laid by hens at different ages (mm)

Table (7): Effect of MOLP with or without Moringa oil on Haugh unit of fresh eggs

Treatment	HU
Control without oil	89.86±2.79[b]
Control + Moringa oil	91.43±2.15[b]
1% MOLP + Moringa oil	95.14±1.77[a]
1% MOLP without Moringa oil	96.14±2.61[a]
P-value	0.0001[**]
$Lsd_{0.05}$	2.609
SE±	0.894

Values are mean±SD.

Means having different superscripts are significantly different (P≤0.05).

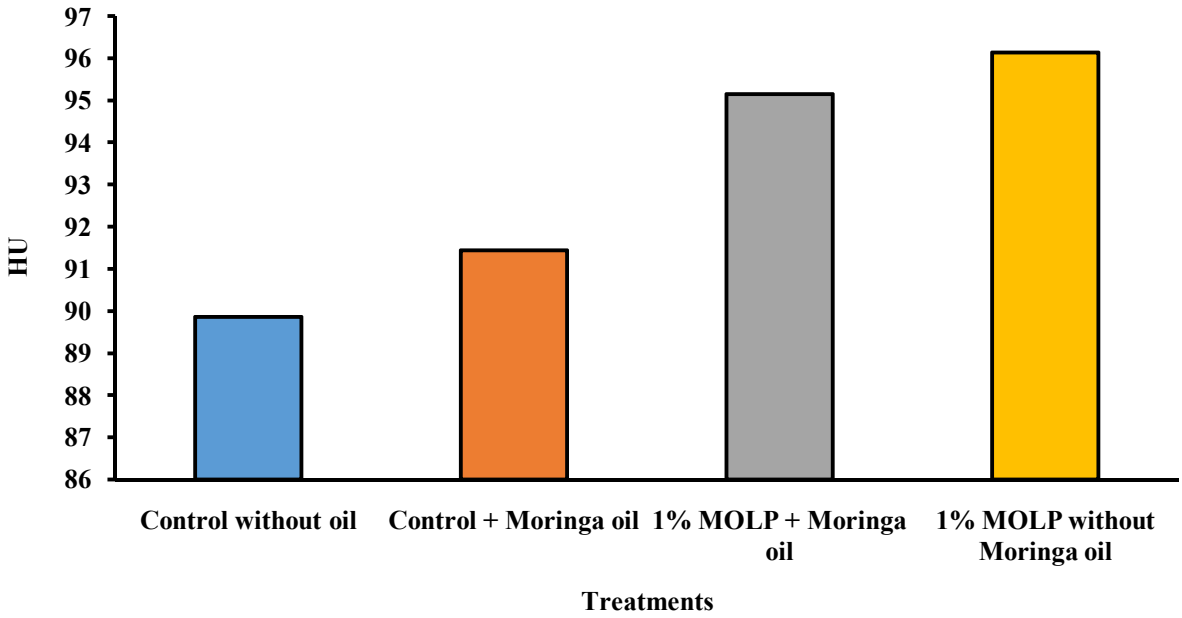

Fig (6): Effect of MOLP with or without Moringa oil on Haugh unit of fresh eggs

Table (8): Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 3 days

Treatment	HU
Control without oil	85.29 ± 2.43^{c}
Control + Moringa oil	87.57 ± 2.64^{bc}
1% MOLP + Moringa oil	90.43 ± 3.60^{ab}
1% MOLP without Moringa oil	93.14 ± 4.10^{b}
P-value	0.0009^{**}
$Lsd_{0.05}$	3.601
SE±	1.234

Values are mean±SD.

Means having different superscripts are significantly different (P≤0.05).

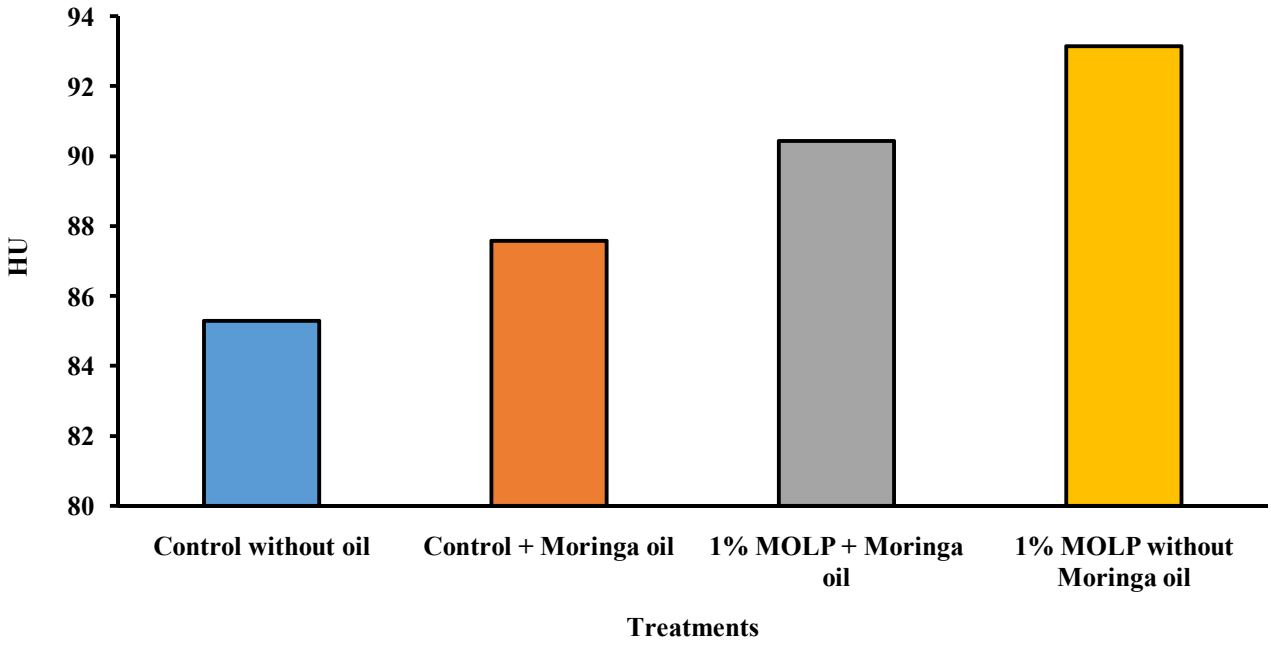

Fig (7): Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 3 days

Table (9): Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 7 days

Treatment	HU
Control without oil	82.71 ± 1.98^{c}
Control + Moringa oil	84.71 ± 2.43^{bc}
1% MOLP + Moringa oil	86.86 ± 3.13^{ab}
1% MOLP without Moringa oil	88.57 ± 3.10^{a}
P-value	0.0028^{**}
$Lsd_{0.05}$	2.983
SE±	1.002

Values are mean±SD.

Means having different superscripts are significantly different (P≤0.05).

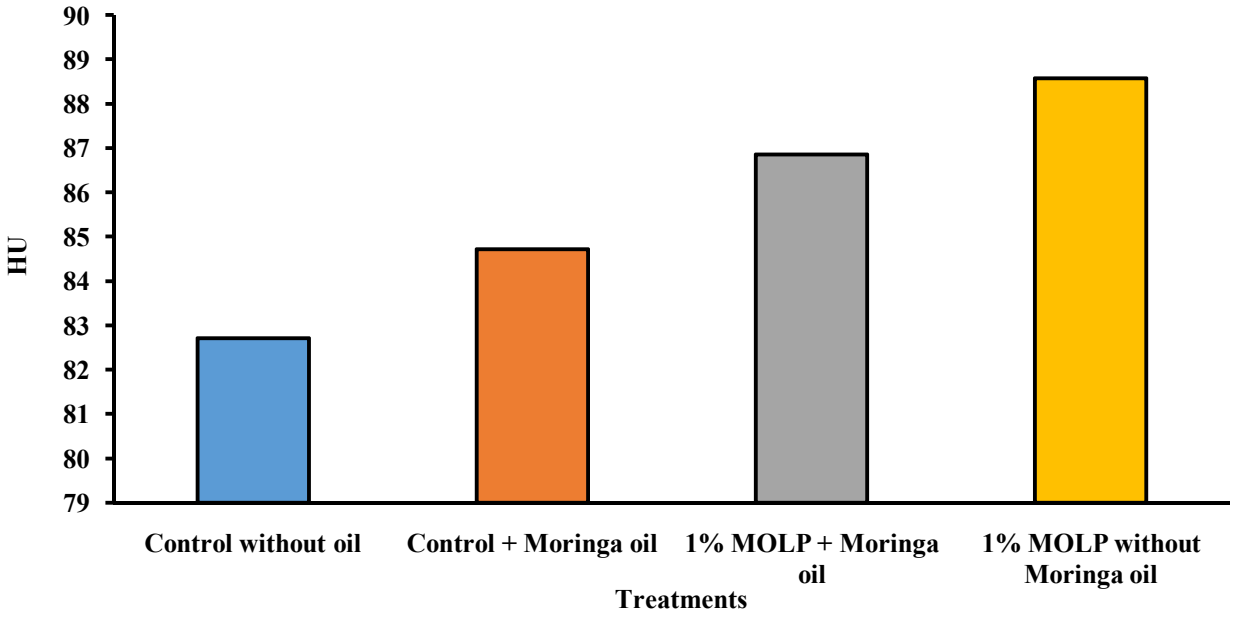

Fig (8): Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 7 days

Table (10): Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 14 days

Treatment	HU
Control without oil	78.50 ± 2.23^{c}
Control + Moringa oil	80.71 ± 3.35^{bc}
1% MOLP + Moringa oil	82.57 ± 3.31^{ab}
1% MOLP without Moringa oil	85.14 ± 3.13^{a}
P-value	0.0036^{**}
$Lsd_{0.05}$	3.353
SE±	1.149

Values are mean±SD.

Means having different superscripts are significantly different (P≤0.05).

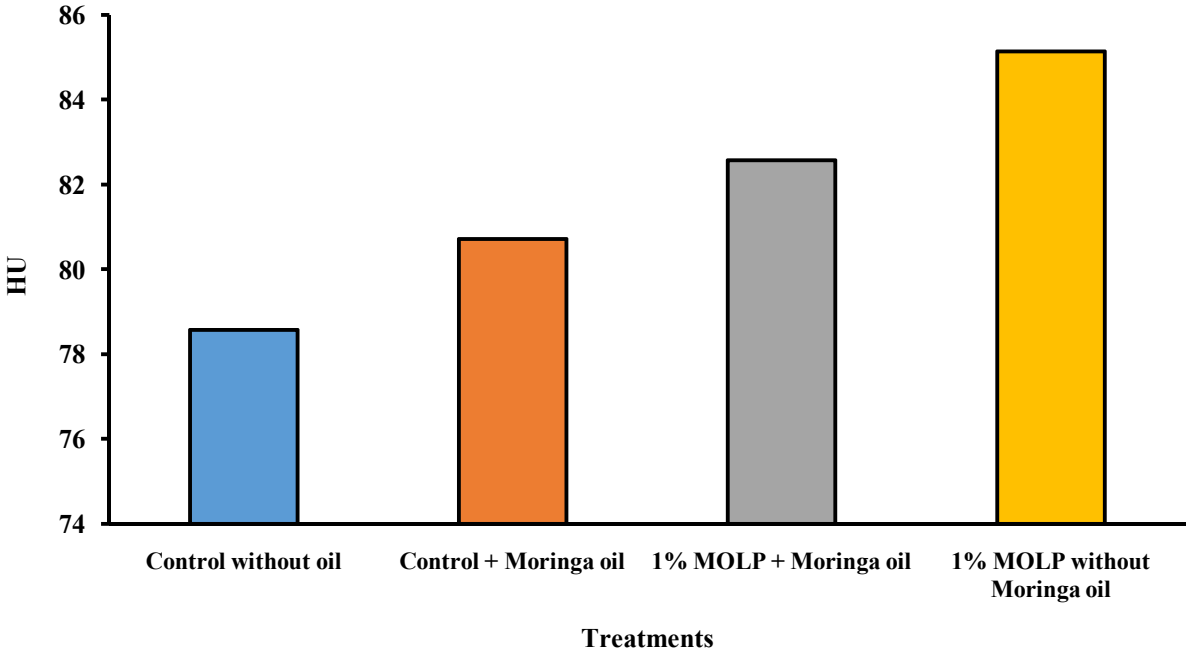

Fig (9): Effect of MOLP with or without Moringa oil on Haugh unit of eggs stored for 14 days

Table (11): Effect of MOLP with or without moringa oil on HU of eggs obtained at 24th wk of age and stored for 3, 7, or 14 days

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	89^{defgh} ±5.03	87^{gh} ±0.58	84^{hi} ±1.53	81^{i} ±6.00
control+ Moringa oil	91^{defg} ±1.53	92^{cde} ±1.53	88^{efgh} ±1.00	86^{h} ±0.00
1% Moringa no oil	98^{ab} ±1.53	97^{abc} ±0.00	92^{cdef} ±1.00	87^{fgh} ±5.00
1% Moring + oil	101^{a} ±1.00	101^{a} ±0.58	94^{bcd} ±4.51	89^{defgh} ±2.00
P-value	0.04396*			
$Lsd_{0.05}$	4.624			
SE±	1.605			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

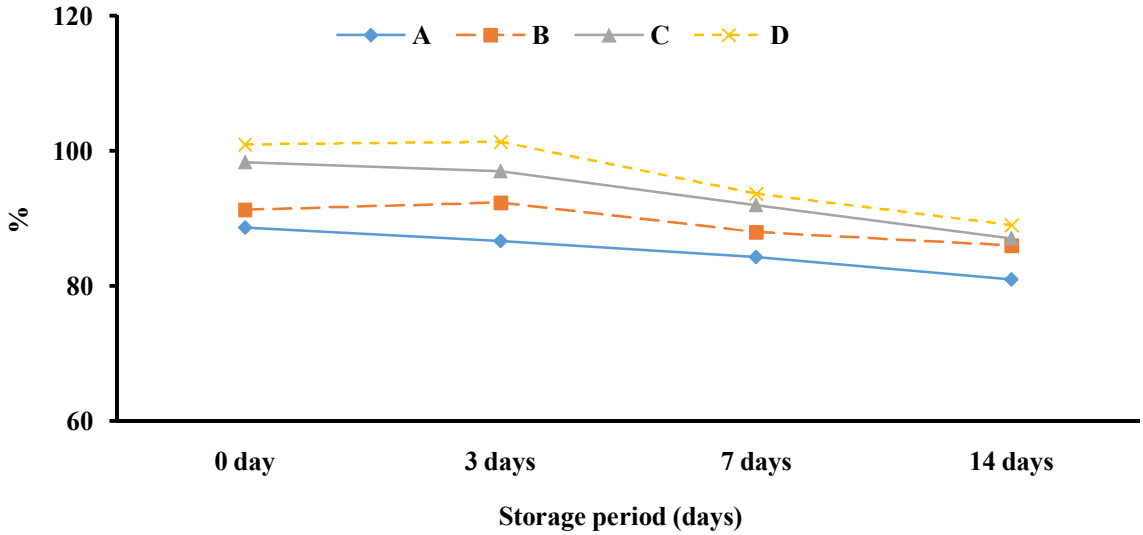

Figure (10): Effect of MOLP with or without moringa oil on HU of eggs obtained at 24th wk of age and stored for 3, 7, or 14 days

Table (12): Effect of MOLP with or without moringa oil on HU of eggs obtained at 25th wk of age and stored for 3, 7, or 14 days

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	84^{efg} ±0.58	83^{fg} ±0.00	81^{g} ±0.00	80^{g} ±0.58
control+ Moringa oil	87^{cde} ±2.89	87^{def} ±0.58	86^{def} ±1.00	83^{fg} ±1.00
1% Moringa no oil	95^{ab} ±7.09	93^{ab} ±1.53	89^{cd} ±2.00	86^{def} ±2.52
1% Moring + oil	95^{a} ±2.31	95^{a} ±0.58	91^{bc} ±1.00	88^{cde} ±0.00
P-value	0.03526*			
$Lsd_{0.05}$	3.742			
SE±	1.299			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

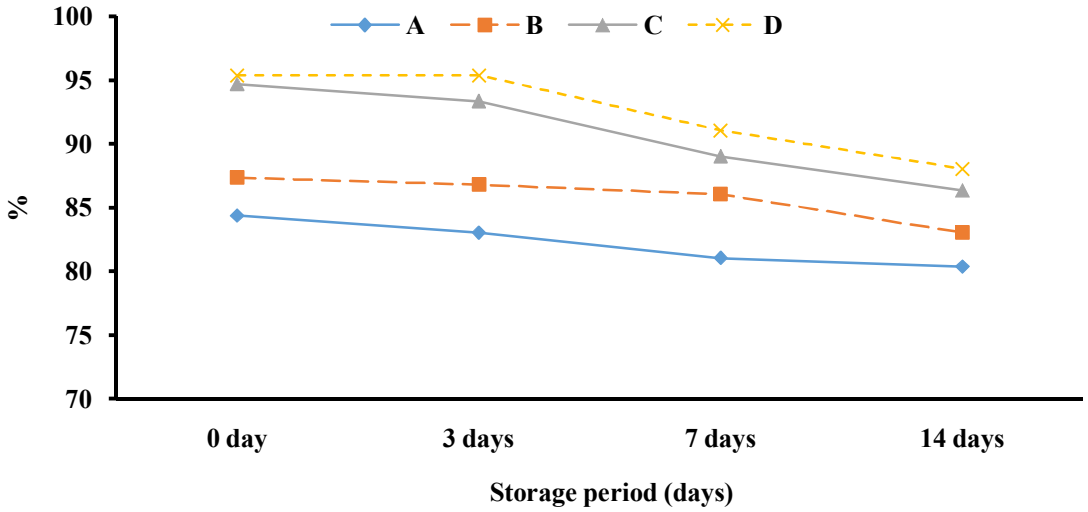

Figure (11): Effect of MOLP with or without moringa oil on HU of eggs obtained at 25th wk of age and stored for 3, 7, or 14 days

Table (13): Effect of MOLP with or without moringa oil on HU of eggs obtained at 26th wk of age and stored for 3, 7, or 14 days

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	90^{abcd} ±1.00	83^{fgh} ±1.00	82^{gh} ±5.13	81^{h} ±2.00
control+ Moringa oil	91^{abc} ±2.08	85^{efgh} ±1.15	83^{fgh} ±2.65	82^{gh} ±0.00
1% Moringa no oil	93^{a} ±3.21	86^{defg} ±2.00	85^{efgh} ±4.16	84^{efgh} ±2.52
1% Moringa + oil	93^{ab} ±3.00	89^{bcde} ±2.08	87^{cdef} ±0.00	86^{defg} ±1.73
P-value	0.02548*			
$Lsd_{0.05}$	4.144			
SE±	1.439			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

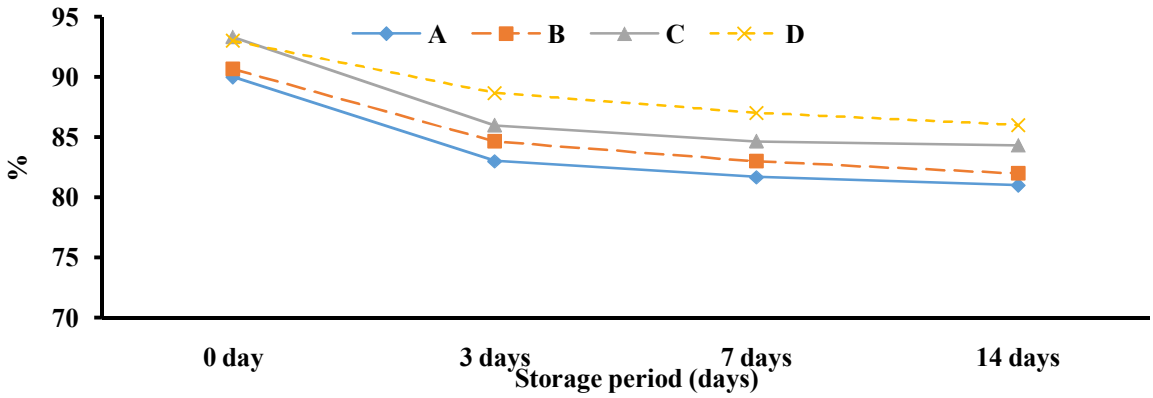

Figure (12): Effect of MOLP with or without moringa oil on HU of eggs obtained at 26th wk of age and stored for 3, 7, or 14 days

Table (14): Effect of MOLP with or without moringa oil on HU of eggs obtained at 27th wk of age and stored for 3, 7, or 14 days

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	92^{abcd} ±1.15	89^{cdef} ±1.15	86^{fgh} ±1.73	78^{i} ±1.53
control+ Moringa oil	93^{abc} ±4.00	89^{cdef} ±3.21	87^{defg} ±3.61	81^{hi} ±1.53
1% Moringa no oil	95^{ab} ±3.61	90^{bcdef} ±5.00	88^{cdefg} ±1.15	83^{ghi} ±4.36
1% Moringa + oil	96^{a} ±2.65	92^{abcde} ±0.58	89^{cdef} ±0.00	87^{efgh} ±3.51
P-value	0.01914*			
$Lsd_{0.05}$	4.698			
SE±	1.631			

Mean(s) having different superscript(s) in a column are significantly different (P≤0.05) according to DMRT.

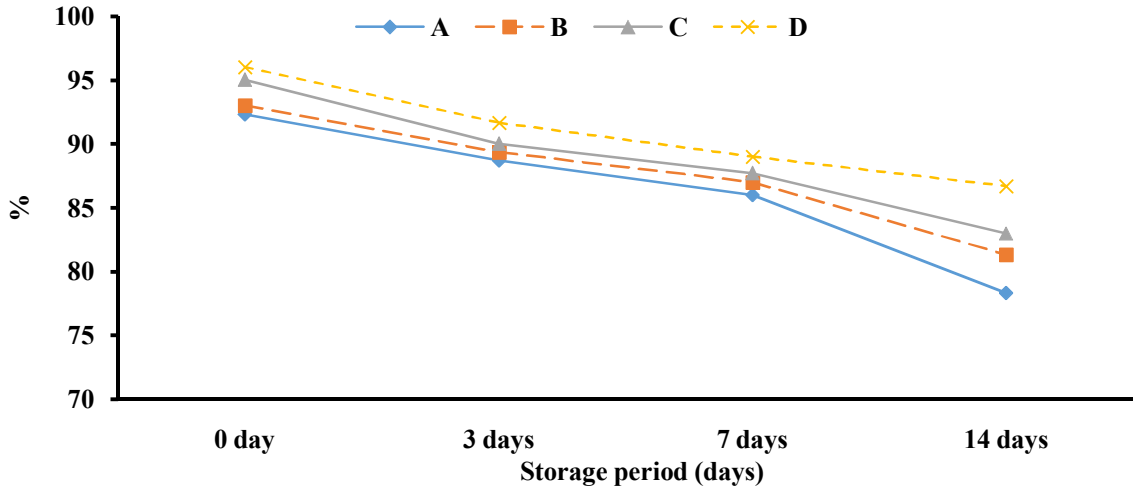

Figure (13): Effect of MOLP with or without moringa oil on HU of eggs obtained at 27th wk of age and stored for 3, 7, or 14 days

Table (15): Effect of MOLP with or without moringa oil on HU of eggs obtained at 28th wk of age and stored for 3, 7, or 14 days

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	91^{b} ±1.53	85^{cde} ±1.53	84^{cdef} ±2.00	78^{g} ±1.00
control+ Moringa oil	93^{ab} ±1.53	88^{bc} ±0.58	85^{cde} ±0.00	79^{fg} ±2.65
1% Moringa no oil	79^{fg} ±2.65	89^{bc} ±4.00	87^{bcd} ±4.16	80^{efg} ±1.15
1% Moringa + oil	98^{a} ±1.53	93^{ab} ±7.51	88^{bc} ±5.13	81^{defg} ±4.00
P-value	0.0005^{**}			
$Lsd_{0.05}$	5.292			
SE±	1.837			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

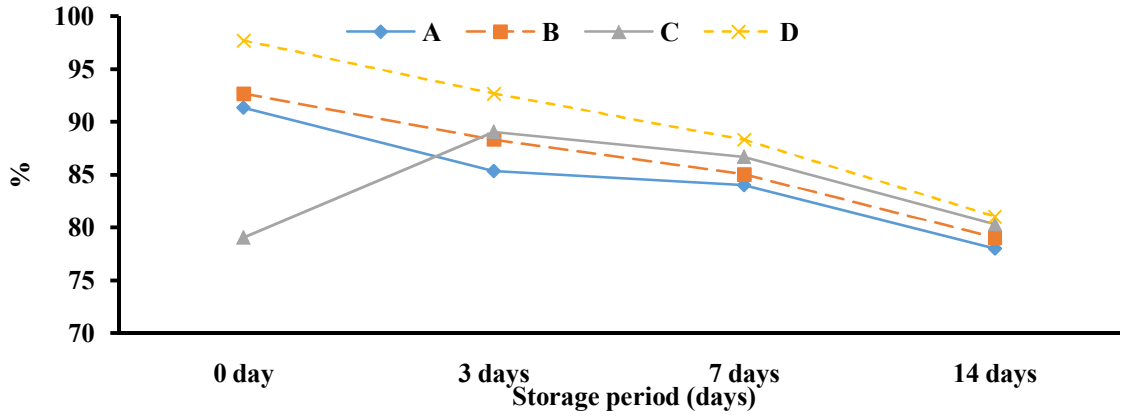

Figure (14): Effect of MOLP with or without moringa oil on HU of eggs obtained at 28th wk of age and stored for 3, 7, or 14 days

Table (16): Effect of MOLP with or without moringa oil on HU of eggs obtained at 29th wk of age and stored for 3, 7, or 14 days

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	91^{cd} ±1.15	87^{ef} ±2.52	81^{h} ±0.58	75^{j} ±0.58
control+ Moringa oil	92^{bcd} ±1.73	88^{e} ±1.53	82^{h} ±0.58	76^{j} ±0.00
1% Moringa no oil	94^{ab} ±1.00	90^{d} ±0.58	83^{gh} ±1.15	79^{i} ±1.00
1% Moringa + oil	95^{a} ±2.08	93^{abc} ±0.58	85^{fg} ±1.53	83^{gh} ±1.53
P-value	0.0483*			
$Lsd_{0.05}$	2.161			
SE±	0.7501			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

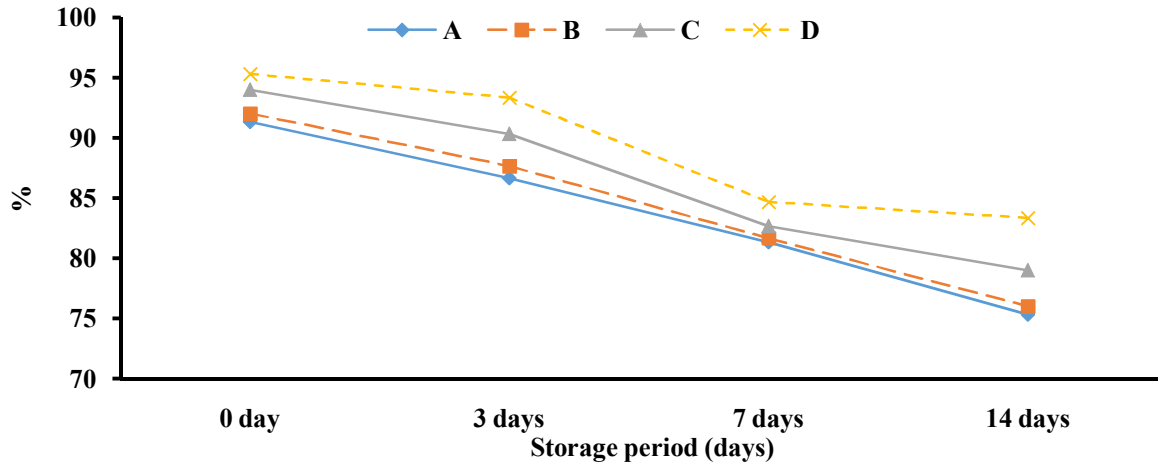

Figure (15): Effect of MOLP with or without moringa oil on HU of eggs obtained at 29th wk of age and stored for 3, 7, or 14 days

Table (17): Effect of MOLP with or without moringa oil on HU of eggs obtained at 30th wk of age and stored for 3, 7, or 14 days

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	92^{ab} ±2.08	83^{ef} ±0.58	81^{fg} ±0.58	77^{h} ±2.00
control+ Moringa oil	93^{a} ±2.52	84^{ef} ±1.00	82^{fg} ±0.00	78^{h} ±2.00
1% Moringa no oil	94^{a} ±2.08	88^{cd} ±0.58	84^{ef} ±1.00	79^{gh} ±1.53
1% Moringa + oil	95^{a} ±1.15	89^{bc} ±1.53	86^{de} ±0.00	82^{fg} ±5.00
P-value	0.00^{**}			
$Lsd_{0.05}$	3.139			
SE±	1.09			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

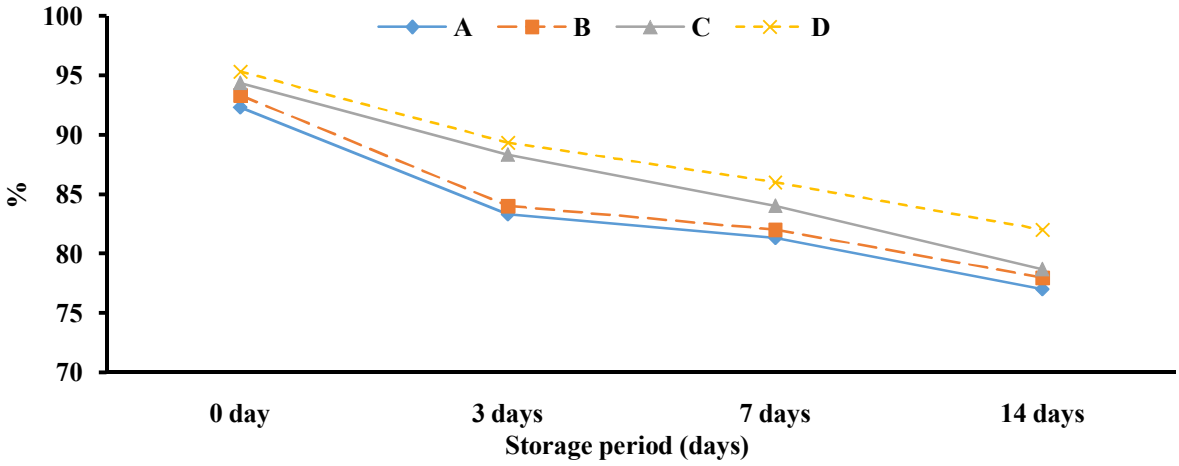

Figure (16): Effect of MOLP with or without moringa oil on HU of eggs obtained at 30th wk of age and stored for 3, 7, or 14 days

Table (18): Effect of moringa leaf with or without moringa oil on color (24 wk)

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
control+ Moringa oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
1% Moringa no oil	3.00^{b} ±0.00	3.67^{a} ±0.00	3.67^{a} ±0.00	3.67^{a} ±0.58
1% Moring + oil	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00
P-value	0.0292^{*}			
$Lsd_{0.05}$	0.4174			
SE±	0.1449			

Mean(s) having different superscript(s) in a column are significantly different (P≤0.05) according to DMRT.

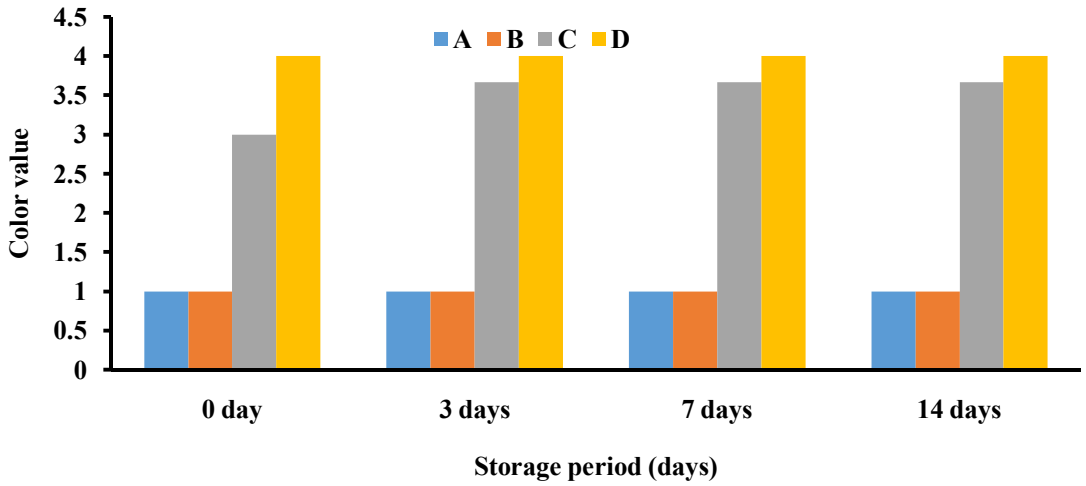

Figure (17): Effect of moringa leaf with or without moringa oil on color (24 wk)

Table (19): Effect of moringa leaf with or without moringa oil on color (25 wk)

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00
control+ Moringa oil	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00
1% Moringa no oil	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00
1% Moring + oil	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00
P-value	0.0481^{*}			
$Lsd_{0.05}$	0.0005259			
SE±	0.0001826			

Mean(s) having different superscript(s) in a column are significantly different (P≤0.05) according to DMRT.

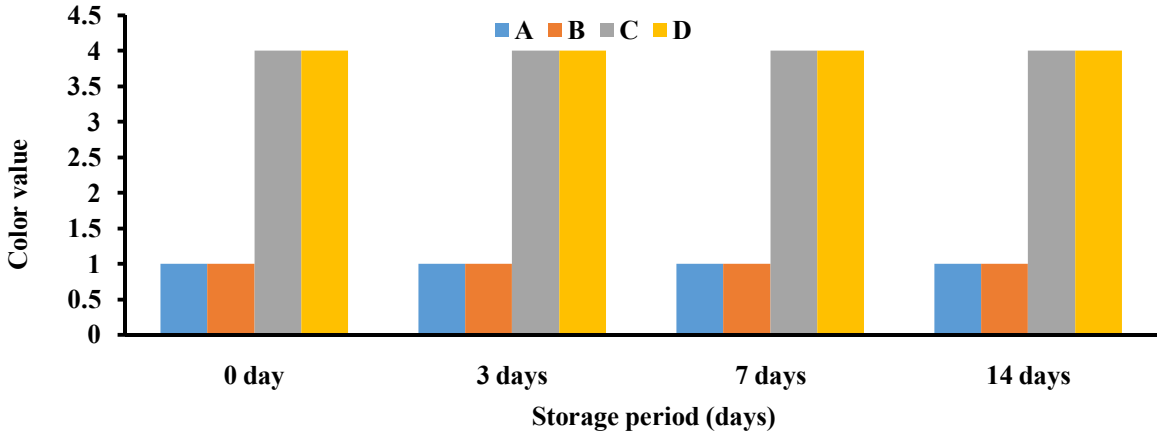

Figure (18): Effect of moringa leaf with or without moringa oil on color (25wk)

Table (20): Effect of moringa leaf with or without moringa oil on color (26 wk)

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00
control+ Moringa oil	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00	1.00^{b} ±0.00
1% Moringa no oil	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00
1% Moring + oil	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00	4.00^{a} ±0.00
P-value	0.0435^{*}			
$Lsd_{0.05}$	0.0005259			
SE±	0.001826			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

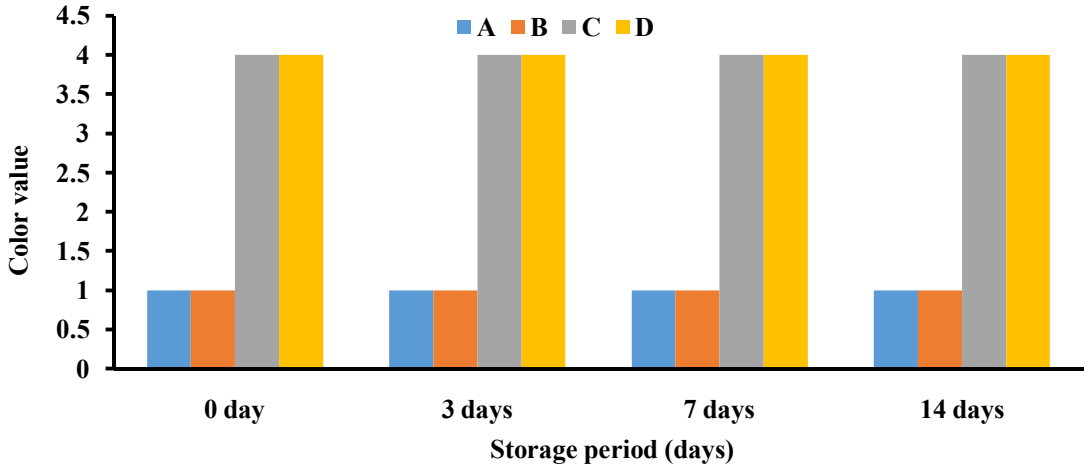

Figure (19): Effect of moringa leaf with or without moringa oil on color (26 wk)

Table (21): Effect of moringa leaf with or without moringa oil on color (27 wk)

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	1.00^{d} ±0.00	1.00^{d} ±0.00	1.00^{d} ±0.00	1.00^{d} ±0.00
control+ Moringa oil	1.00^{d} ±0.00	1.00^{d} ±0.00	1.00^{d} ±0.00	1.00^{d} ±0.00
1% Moringa no oil	3.33^{c} ±0.58	3.67^{bc} ±0.58	3.67^{bc} ±0.58	4.00^{ab} ±0.58
1% Moring + oil	4.33^{a} ±0.58	4.00^{ab} ±0.00	4.00^{ab} ±0.00	4.33^{a} ±0.58
P-value	0.0385^{*}			
$Lsd_{0.05}$	0.5363			
SE±	0.1862			

Mean(s) having different superscript(s) in a column are significantly different (P≤0.05) according to DMRT.

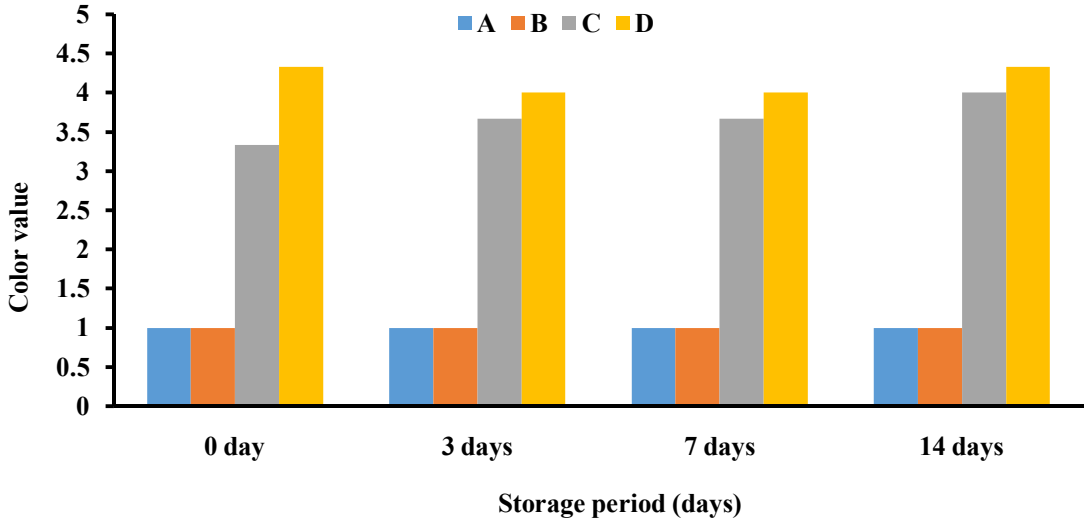

Figure (20): Effect of moringa leaf with or without moringa oil on color (27 wk)

Table (22): Effect of moringa leaf with or without moringa oil on color (28 wk)

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
control+ Moringa oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
1% Moringa no oil	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00
1% Moring + oil	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00	5.00^{a} ±1.00
P-value	0.0104^{*}			
$Lsd_{0.05}$	0.241			
SE±	0.08367			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

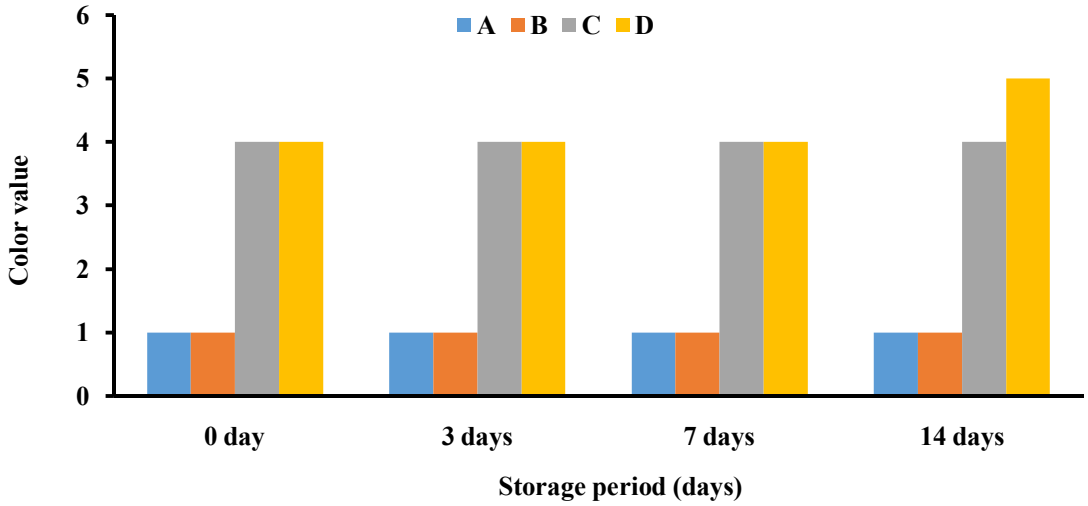

Figure (21): Effect of moringa leaf with or without moringa oil on color (28 wk)

Table (23): Effect of moringa leaf with or without moringa oil on color (29 wk)

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
control+ Moringa oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
1% Moringa no oil	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00
1% Moring + oil	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00	4.33^{a} ±0.58
P-value	0.0257^{*}			
$Lsd_{0.05}$	0.241			
SE±	0.08367			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

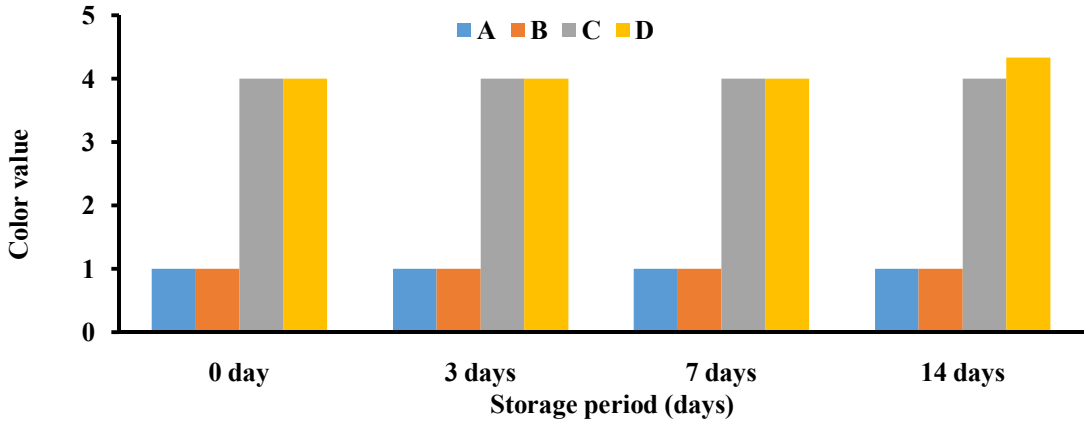

Figure (22): Effect of moringa leaf with or without moringa oil on color (29 wk)

Table (24): Effect of moringa leaf with or without moringa oil on color (30 wk)

Treatments	Storage period (days)			
	0	3	7	14
comtrol without oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
control+ Moringa oil	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00	1.00^{c} ±0.00
1% Moringa no oil	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00	4.00^{b} ±0.00
1% Moring + oil	4.00^{b} ±0.00	4.00^{b} ±0.00	4.33^{ab} ±0.58	4.67^{a} ±0.58
P-value	0.0103*			
$Lsd_{0.05}$	0.3408			
SE±	0.1183			

Mean(s) having different superscript(s) in columns and rows are significantly different (P≤0.05) according to DMRT.

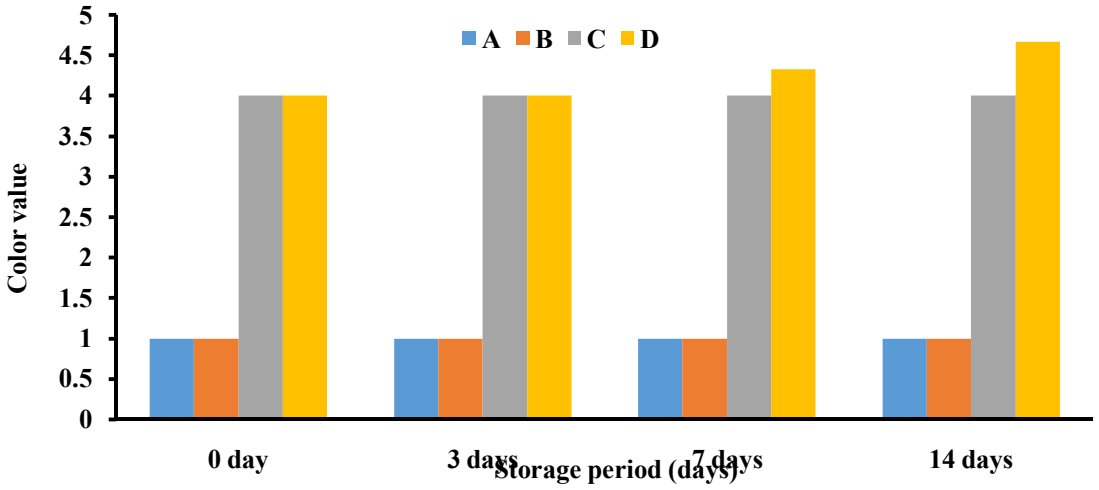

Figure (23): Effect of moringa leaf with or without moringa oil on color (30 wk)

CHAPTER FIVE

Discussion

Discussion

The improved performance observed when poultry layers are fed MOLP has been attributed to organic selenium and lutein and their capacity to improve the egg quality as measured by shell thickness, Haugh Unit and yolk color (Wakebe,1999, Pappas *et al* 2005, Surai *et al*., 2006, Cantor and Scott 1974). The present study confirms these positive effects of MOLP feeding on egg quality and further demonstrates that this positive effect does not include egg production and egg size. Results for percentage hen-day production agree with those of Cantor *et al*. (2000) and Patton (2000) who reported no difference in percentage hen-day production when hens were fed a basal diet supplemented with selenium_selenite_or selenium enriched yeast. However, our results disagree with those of Cantor and Scott (1974) who reported an increase in percentage hen-day production for hens fed 0.10 ppm of selenomethionine relative to no supplementation, and recent finding at our laboratory by (Abdel Aziz, 2016) who reported an increase in percentage hen-day production for hens fed low level of MOLP relative to no supplementation.

Storage of eggs is common practice in commercial poultry production, both in the case of hatching eggs and table eggs. Egg storage has several benefits, such as reducing the number of individual incubations and providing flexibility to meet market fluctuation demands. Therefore, eggs are usually kept in a storage room for several days. The goal of storage is to arrest embryonic development until the eggs are loaded into the incubator. However, storage can alter some characteristics of the egg including loss of water, carbon dioxide, and a subsequent increase in the pH of the albumen (Decuypere *et al*., 2001). Furthermore, the age, ambient temperature and

production stage of a hen affect shell structure and consequently, the rate of diffusion through the pores of the eggshell. Such changes may effects the shelf life of table eggs and contribute to the reduction in hatchability (Etches, 1996).

At oviposition, the egg contains a high concentration of carbon dioxide, which stars to escape after laying and during storage, leading to 7% rise in the pH of the albumen during the first 3 days of storage (around a pH of 8.95 for fresh eggs to 9.15 after 3 d of storage). This rise in pH is important for pH-dependent enzymes, which control early embryogenesis activity, and as consequence resulted in 97.14% hatchability. Further storage of hatching egg (up to 7d) lead to excess carbon dioxide loss and as consequence, 3% more rise in albumen pH (9.41). At this high pH the initiation of embryo development negatively affected and hatchability % drop from 97.17% to 91.42% (Decuypere *et al*., 2007). Furthermore, increase in pH as result of storage of hatching eggs for more than 3 days results in dissociation of two of the albumen's proteins in particular (lysozyme and ovomucin), which in turn reduces viscosity of the albumen and associated decrease in HU (Scott and Silversides, 2000; Niemiec *et al*., 2001; Silversides and Scott, 2001).

The high HU noted throughout the duration of our Present study, as result of feeding MOLP, was significantly greater at oviposition (even before storage of egg) and the consequence of that on carbon dioxide loss and rise in pH. This positive preservation of egg freshness may be due to the Se-dependent antioxidant enzyme glutathione peroxidase present in the yolk and albumen (Gaal *et al*., 1995), since increased in this selenium dependent enzyme activity known to slow down the rate of lipid and protein oxidation during egg storage and thus leading to better egg quality before and during storage.

The improvement in eggshell quality noted in the present study was similar to that observed previously by Siske *et al*. (2000) who reported increased eggshell strength when organic forms of Mn, Se, and Zn were substituted

into the diet for one-half of the inorganic forms of these minerals, Organic Se is also reported to improve the integrity of vitelline membrane surrounding the egg yolk (Scheideler *et al*., 2010; Kirunda *et al*. 2001). Therefore, Increase in eggshell well reduce co_2 and water loss from the albumen to the outside environment, while improvement of the vitelline membrane strength prevent water penetration from the albumin with around 88% moisture content into the yolk where the moisture content is 49 %. This improvement in vitelline membrane strength could also help reduce losses in a breaker plant separating yolk from albumen and could improve the shelf life of carton eggs such that the whole egg yolk would not break upon cracking and preparing the egg for consumption.

Improvement in eggshell thickness using MOLP will have a very important impact on table egg industry in the tropic countries, since high ambient temperature in this region increase eggshell breakage, which accounts for a loss of 7 to 8% for the eggs industry, of the total eggs laid (Hamilton, 1978).

Egg yolk pigmentation, is considered one of the most important factor in the evaluation of egg quality (Hèrnandez *et al*., 2001): the food industry requires highly pigmented yolks for the production of egg based products more appealing for consumers. Poultry cannot synthesize carotenoids and they must absorb them from the diet (Schiedt, 1998; Blanch, 1999).

The high lutein content of MOLP in the treatment groups of our present study, significantly increased the color on Roche yolk fan, from score one to the score four. Color is one of the most important factors that affect consumer choices, bright yolk color is considered as an indicator of freshness, good health and performance of the flock. Therefore, this bright yolk color is a value added when adding MOLP to the layer feed, especially in countries like in Sudan, where poultry feed depend on sorghum as the main source of energy, since it lack this type of pigmentation. Recent evidence suggests the role of lutein in human prevention of macular

degeneration, cataract, cancer & cardiovascular disease as well improving the Immune response.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1.Conclusion

Dietary MA as a source of DHA is useful for enhancing the n-3 FA content, and thus the nutritional quality of shell eggs, without significantly impacting consumer acceptance. Dietary MA may also be useful in certain geographical regions for enhancing yolk color. Additional health benefits to the consumer from yolk carotenoid consumption remain to be investigated.

Storage of eggs is common practice in commercial poultry production, both in the case of hatching eggs and table eggs. Egg storage has several benefits, such as reducing the number of individual incubations and providing flexibility to meet market demands; however, storage can alter some characteristics of the egg including loss of water, carbon dioxide, and a subsequent increase in the pH of the albumen (Decuypere *et al*., 2001). Furthermore, the age and production stage of a hen affect shell structure and, consequently, the rate of diffusion through the pores of the eggshell. Such changes may also contribute to the reduction in hatchability associated with egg storage (Etches, 1996).

At oviposition, the egg contains a high concentration of carbon dioxide which stars to escape after laying and during storage, leading to a rise in the pH of the albumen. This is important because early developmental activity is controlled by pH-dependent enzymes. Excess carbon dioxide loss causes the albumen to have an excessively high pH and this is negatively affects the initiation of embryo development. If the loss of carbon dioxide is too low, the pH of the albumen will also be too low resulting in eggs which are too fresh" and not hatch as well as those stored

”for 3-4 days (Table 1). This process of carbon dioxide loss is also temperature-dependent and may be stimulated by cooling after oviposition (Lapao *et al*., 1999; Tazawa and Whittow, 2000).

6.2. Recommendation

- From the experimental results and general observation it can be recommended that MOLM can be used as feed additives in diets.
- The use of broiler broth improves the shell and thus reduces the broken eggs on the farms and increases the number of eggs total.
- Increase the shell quality of albumin which helps in storage for the longest possible period and increase the hatching rate.
- Improved shell yolk used in dried egg industry.
- The further researches needed to be done on the other egg quality attributes as well as fertility and hatchability that were not measured in this study.

Chapter seven

References

AAFCO (2003). Association of American Feed Control Officials Incorporated, Olympia, WA.

Abou-Elezz FMK, Sarmiento-Franco L, Santos-Ricalde R, Solorio-Sanchez F (2011). Nutritional effects of dietary inclusion of Leucaena leucocephala and *Moringa oleifera* leaf meal on Rhode Island Red hens' performance. Cub J Agri Sci 2011; 45: 163–169.

Ajuyah, A. O., R. T. Hardın and J. S. Sım (2001). Effect of dietary full-fat flax seed with and without antioxidant on the fatty acid composition of major lipid classes of chicken meats. Poult. Anim. Physiol. A. Anim. Nutr. 85:335-341.

Al-Kharusi LM, Elmardi MO, Ali A, Al-Said FAJ, Abdelbasit KM, AlRawahi S (2009). Effect of mineral and organic fertilizers on the chemical characteristics and quality of date fruits. Int. J. Agric Biol., 11: 290-296

Allan,C.B., Lacourciere, G. M. and Stadtman, T. C. (1999). *Responsiveness of selenoproteins to dietary selenium. Annu. Rev. Nutr. 19:1–16.*

Anjorin TS, Ikokoh P, Okolo S (2010). Mineral composition of Moringa oleifera leaves, pods and seeds from two regions in Abuja, Nigeria. Int. J. Agric Biol., 12: 431-434.

Anwar F, Bhanger MI (2003). Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. J Agri Food Chem 51: 6558–6563.

Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera* (2007). a food plant with multiple bio-chemical and medicinal uses. Phytother Res; 21:

17–25.

Anwar F, Rashid U (2007). Physico-chemical characteristics of Moringa oleifera seeds and seed oil from a wild provenance of Pakistan. Pak J Bot 2007; 39: 1443–1453.

Ayssiwede, S.B.,J.C. Zanmenou, Y . Issa, M.B. Hane. A. Dieng, C.A.A.M. Chrysostome, M.R. Houinato, J.L. Hornik and A. Missohou (2011). Nutrient composition of some unconventional and local feed resources available in Senegal and recoverable in indigenous chickens or animal feeding. Pak. J. Nutr., 10:707-717.

BLANCH, A. (1999). Getting the color of yolk and skin right. World's Poult. Sci. J. 15: 32–33.

BORUTA A., SWIERCZEWSKA E., ROSZKOWSKI T (2007). Effect of organic and inorganic forms of selenium on morphological composition of eggs and selenium content in egg mass. Medycyna Wet. 63: 238-241. (in Polish).

Bosh, C.H. (2004). USDA National Nutrient Database for standard reference. In: Grubben, G.J.H. and Denton, O.A. (eds). PROTOA Foundation, Wageningen Netherlands/CTA, Wageningen, Netherlands,pp 392- 393.

Breithaupt DE, Wirt U, Bamedi A (2002). Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (Tagetes erecta L.) and several fruits by liquid chromatography-mass spectrometry. Journal Agriculture and Food Chemistry 2002; 50:66-70.

Brisibe EA, Umoren UE, Brisibe F, Magalhaes PM, Ferreira JFS, Luthria D, Wu X, Prior RL (2009). Nutritional characterization and antioxidant capacity of different tissues of Artemisia annua L. Food Chem. 115: 1240-1246.

Britton G, Liaaen-Jensen S, Pfander H, eds, (2004) Carotenoids Handbook. Birkhäuser Verlag, Basel.

Brown, K.M. and Arthur, J.R. (2001). Selenium, selenoproteins and human health: a review. Public Health Nutr. 4(2B): 593–599

Buckley, D. J., P. A. Morrıssey and J. I. Gray (1995). Infuence of dietary vitamin E on the oxidative stability and quality of pig meat. J. Anim. Sci. 73:3122- 3130.

Bukar, A., Uba, A. and Oyeyi, T.I. (2010): Phytochemical analysis and antimicrobial activity of parkia biglobosa (Jacq.) Benth. extracts against some food – borne microorganisms. Advances in Experimental Biology (In press)

Busani Moyo, Patrick J. Masika, Arnold Hugo and Voster Muchenje (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African Journal of Biotechnology Vol. 10(60), pp. 12925-12933.

C.S.I.R. (1962). The wealth of India. A dictionary of Indian raw materials and industrial products. Raw materials, volume 6: L-M, New Delhi, CSIR, India.

Cantor, A. H. and Scott, M. L (1974). The effect of selenium in the hen's diet on egg production, hatchability, performance of progeny and selenium concentration in eggs. Poult. Sci. 53:1870–1880.

Cantor, A. H., P. D. Moorehead, and M. A. Musser (1982). Comparative effects of sodium selenite and selenomethionine upon nutritional muscular dystrophy, selenium-dependentglutathione peroxidase, and tissue selenium concentrations of turkey poults. Poult. Sci. 61:478-484.

Cantor, A. H.; Straw, M. L.; Ford, M. J.; Pescatore, A. J. and Dunlap, M. K. (2000). Effect of feeding organic selenium in diets of laying hens on egg selenium content. Page 473 in Egg Nutrition and Biotechnology.

J. S. Sim, S. Nakai, and W. Guenter, ed. CABI Publishing, New York, NY.

Cherıan, G., F. H. Wolfe and J. S. Sım (1996). Feeding dietary oils with tocopherols. Effects on internal qualities of eggs during storage. J. Food Sci. 61:15-18.

Chung HY, Helen MR, Elizabeth JJ (2004). Lutein bioavailability is higher fromlutein-enriched eggs than from supplements and spinach in men. The Journal of Nutrition 2004; 134:1887-93.

Conroy C (2005). Participatory livestock Research- A guide, Intermediate Technology Development Group, London.

Dahot MU (1988). Vitamin contents of flowers and seeds of *Moringa oleifera*. Pak J Biochem 1988; 21: 21–24.

Davis, R. H.; Fear, J. and Winton, A. C. (1996). Interactions between dietary selenium, copper, and sodium nitroprusside, a source of cyanide in growing chicks and laying hens. Br. Poult. Sci. 37:87–94.

Davtyan, D.; Papazyan, T. and Nollet, L. (2006). Dose response of Se added as sodiumselenite or Sel-Plex on male sperm quality and breeder productivity. XII European Poultry Conference, Verona, Italy, 10-14 September.

Decuypere E, Bruggeman V. (2007). The endocrine interface of environmental and egg factors affecting chick quality. Poultry Sci. 86:1037–1042

Decuypere al.,(2001).E. Decuipere, K. Tona, V. Bruggeman, F. Bamelis The day-old developing chick. Brit. Poult. Sci. 41:235.

Dillard CJ, German JB. (2000). Phytochemicals: nutraceuticals and human health: A review. J Sci Food Agric **80**: 1744– 1756.

Ebenebe CJ, Anigbogu CC, Anizoba MA, Ufele AN (2013). Effect of various levels of Moringa leaf meal on the egg quality of Isa Brown

breed of layers. Advances in Live Science and Technology 14: 45-49.

Edens, F.W. (2002). Practical applications for selenomethionine: broiler breeder reproduction. In: Nutritional Biochemistry in the Feed and Food Industries, Proceedings of Alltech's 18th Annual Symposium (K.A. Jacques and T.P. Lyons, eds). Nottingham University Press, UK, pp. 29-42.

Etches, R.J., (1996). Monitoring Reproductive Success. Vol. 11, CAB International, Oxon, UK., pp: 298-309.

Fahey JW, AT Zalcmann, and P Talalay (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56(1): 5-51. [corrigendum: *Phytochemistry* 59: 237].

FAHEY, J.W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. In Trees for Life Journal, vol. 1, pp. 5.

Foidl, N. and Paull, R. (2008). "*Moringa oleifera*". In: The Encyclopedia of Fruit and Nuti (ABI, Oxfordshire.uk Pp 509 – 512.

Foidl, N., Makkar, H.P.S and Becker, K., (2001). Potential of *Moringa oleifera* for agricultural and industrial uses. In: The Miracle Tree. The Multiple Attributes of Moringa. Fugile, L.G. (ed). 31:45-76.

FUGLIER, L.J. (1999). The Miracle Tree: *Moringa oleifera*, Natural Nutrition for the Tropics. Church World Service, Dakkar, Senegal, pp. 68.

Gaal, M. Mezes, R.C. Noble, J. Dixon, B.K. (1995). Speake Development of antioxidant capacity in tissues of the chick embryoComp Biochem Physiol B, 112 (1995), pp. 711-716.

Gajčević Z., Kralik G., Has-Schön Elizabeta, Pavić V. (2009): Effects of organic selenium supplemented to layer diet on table egg freshness and selenium content. Italian Journal Animal Science 8, 189–199.

GOLUBKINA N.A., PAPAZYAN T.T.(2006). Selenium distribution in eggs of avian species. Comp. Biochem.Physiol., Part B 145: 384-388.

Goodwin, Charles (1984). Notes on Story Structure and the Organization of Participation. In Structures of Social Action. M. Atkinson and J. Heritage, eds. Pp. 225-246. Cambridge: Cambridge University Press.

GRANDO, F., B. OLMEDILLA, I. BLANCO, (2003). Nutritional and clinical relevance of lutein in human health. Br. J. Nutr. 90, 487-502.

Grever, T., (2001). Utilization of selected forest products and medicinal plants in four villages in the buffer zone of Bach Ma National Park, Vietnam (in Germany). BSc-dissertation, University of Greifswald, Germany.

Gupta, K., Barat, G.K., Wagle, D.S. and Chawla, H.K.L. (1989). Nutrient contents and anti-nutritional factors in conventional and non-conventional leafy vegetables. Food chemistry, 31: 105-116.

HAMILTON, R. M. c. (1978). The effects of dietary protein level on productive performance and egg quality of four strains of White Leghorn hens. Poult. Sci. 57: 1355-1364

Haugh, R. R. (1937). The Haugh unit for measuring egg quality. US Egg Poult. Mag. 43:522-555, 572-573.

Hayes, Bruce. (1989). The prosodic hierarchy in meter. In Paul Kiparsky and Gilbert Youmans, eds., Rhythm and Meter. Orlando: Academic Press, 201-260.

Hernández JA, Talavera JM, Martínez-Gómez P, Dicenta F, Sevilla F. (2001) Response of antioxidant enzymes to plum pox virus in two apricot cultivars. Physiol Plant. 2001;111:313–321.

Hsu R (2006). Moringa oleifera medicinal and Economic uses. International course on Economic botany, National Herbarium, Leiden, The

Netherlands.

Inborr, J., (1996). Astaxantin-a carotenoid with great potential Poult. Int., 35: 54-60.

Jabeen R, Shahid M, Jamil A, Ashraf M (2008). Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pak J Bot 2008; 40: 1349–1358.

Jang, I.S., Ko, Y.H., Kang, C.Y., Lee, C.Y., (2007). Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed Sci. and Tech. 134:304-315.

Kaijage J. T. (2003). Effect of substituting sunflower seed meal with *Moringa oleifera* leaf meal on the performance of commercial egg strain chickens and egg quality characteristics. Dissertation of Award of Msc Digree in Tropical Animal Production at Sokoine University, Morogoro, Tanzania: pp 142.

Kakengi A M V, Kaijage J T, Sarwatt S V, Mutayoba S K, Shem M N and Fujihara T (2007). Effect of *Moringa oleifera l*eaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. Livestock Research for Rural Development. Volume 19, Article 120.

Kemp, C., L. Wylie and C. Fisher (2001). Broiler breeder nutrition, nutrient transfer and broiler performance. Proceedings of the 13th European Symposium on Poultry Nutrition, October 1-4, 2001, Blankenberge, Belgium, pp: 61-67.

Kenyon, S.; Spring, P. and Tucker, L. (2003). Egg selenium Concentrations in breeder hens fed diets with selenium derived from different sources. pp 1060-1063 in Proc. 16th Eur. Symp. on the Quality of Egg Poultry Meat and 10th Eur. Symp. on the Quality of Egg and Egg Products, Saint Brieuc, France

Khalafalla, M.M., Abdellatef, E., Dafalla, H.M., Nassrallah, A.A., Aboul-Enein, K.M., Lightfoot, D.A., EI-Deeb, F.E. & EI-Shemy, H.A. (2010). Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma. Afr. J. Biotechnol. 49, 8467-8471.

Kidd, M. T. (2003). A treatise on chicken dam nutrition that impacts on progeny. World's Poult. Sci. J. 59:475–494.

Kirunda DFK, Scheideler SE, McKee SR (2001) The Efficacy of Vitamin E (DL-alpha-tocopheryl acetate) Supplementation in Hen Diets to Alleviate Egg Quality Deterioration Associated with High Temperature Exposure. Poult Sci 80, 1378-1383

Klecker D.; Zantlokaul, M. and Zeaman, L. (2001). Effect of organic selenium, zink and manganese on reproductive traits of laying hens and cockerels on the quality parameters of eggs. Proceedings of the 13th European Symposiumon Poultry Nutrition, Blankenberge, Belgium, October 2001.

Kristin, D. (2000). The moringa tree. Echo technical note. Florida, U.S.A.

Landrum, J. T., and R. A. Bone. (2001). Lutein, zeaxanthin, and the macular pigment. Arch. Biochem. Biophys. 385:28–40.

LAPÃO, C., L.T. GAMA and M.C. SOARES (1999). Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. Poult. Sci. **78**, 640-645.

Latshaw JD and Biggert MD (1981). Incorporation of Selenium into Egg Proteins After Feeding Selenomethionine Or Sodium Selenite.Poultry Science 60, 1309-1313.

Latshaw, J. D., and M. Osman (1975). Distribution of selenium in egg white and yolk after feeding natural and synthetic selenium compounds.

Poult. Sci. 54:1244-1252.

Latshaw, J. D., J. F. Ort, and C. D. Diesem (1977). The selenium requirements of the hen and effects of a deficiency. Poult. Sci. 56:1876-1881.

Leeson S, Namkung H, Caston L, Durosoy S and Schlegel P (2008). Comparison of selenium levels and sources and dietary fat quality in diets for broiler breeders and layer hens. Poultry Science 87, 2605-2612.

Leeson, S., and J. D. Summers. (1997). Commercial Poultry Nutrition. Publ. Univ. Books, Guelph, ON.

Leeson, S., and L. Caston. (2004). Enrichment of eggs with lutein. Poult. Sci. 83:1709-1712.

Li Y, *et al*. (2007). An enzyme-coupled assay for amidotransferase activity of glucosamine-6-phosphate synthase. *Anal Biochem* 370(2):142-6

Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D (2012). Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant and scavenging potential using in vitro and in vivo assays. Evi Bas Compl Alt Med 2012: 519084.

Lyons, G., Stangoulis, J. and Graham, R. (2003). High-selenium wheat: biofortification for better.

Makkar HPS, Becker K (1997). Nutrients and antiquality factors in different morphological parts of *Moringa oleifera* tree. J Agri Sci 1997; 128: 311–322.

MAKKAR, H.P.S. and BECKER, K. (1996). Nutritional value and an nutritional components of whole and extracted *Moringa oleifera* leaves. In Animal Feed Science and Technology, vol. 63, p. 211-228.

Marcu, M.G. and Pharm, D. (2005). Miracle Tree. KOS Health Publications, 466 Foothill Blvd number 251, La Canada, CA. 91011.

Martello, M. A., and J. D. Latshaw (1982). Utilization of dietary selenium as indicated by prevention of selenium deficiency and by retention in eggs. Nutr. Rep. Int. 26:43-50.

Maysa, M.Hanafy, Elsheikh and Abdalla., (2009).The organic selenium suuplementation on productive and physiological performance in a local strain of chicken. eygpt. poul. sci. vol(29)(iv):(1061-1084).

Melesse A, Tiruneh W, Negesse T (2011). Effects of feeding *Moringa stenopetala* leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate. Trop Subtrop Agroeco 2011; 14: 485–492

Moeller, S. M., P. F. Jacques, and J. B. Blumberg. (2000). The potential role of dietary xanthophylls in cataract and agerelated macular degeneration. J. Am. Coll. Nutr. 19:522S–527S.

Moyo, B., Oyedemi, S., Masika, P.J., and Muchenje, V., (2012). Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf meal extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera*/Sunflower cake. Meat Sci.02:29.

Mutayoba SK, Dierenfeld E, Mercedes VA, Frances Y, Knight CD (2011). Determination of chemical composition and anti-nutritive components for Tanzanian locally available poultry feed ingredients. Int J Poult Sci. 2011;10:350–357.

Natasha Gjorgovska1, Filev Kiril2, Levkov Vesna1, Kostadinov Tosho1. (2012). The effect of different levels of seleniumin feed on egg production, egg quality and selenium content in yolk. lucrări ştiinţifice - seria zootehnie, vol. 57.

Niemiec, J., M. Pinska, E. Swierczewska, J. **Riedel, and A. Boruta. (2001).** The effect of storage on egg quality and fatty acid content in PUFA-enriched eggs. J. Anim. Feed Sci. 10:267–272.

NRC (1994). Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washinton, DC.

Nuhu, F. (2010). Effect of Moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. Msc. Thesis, Animal Science Department of the Faculty of Agriculture and Natural Resources/Kwame Nkrumah University of Science and Technology: Kumasi, pp: 107.

Oduro I, Ellis WO, Owusu D (2008). Nutritional potential of two leafy vegetables: Moringa oleifera and Ipomoea batatas leaves. Sci. Res. Essays. 3(2): 057-060.

Ogbe AO, Affiku JP (2012). Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition Micro Biotech Food Sci 2011/2012; 1: 296–308.

Olugbemi TS, Mutayoba SK, Lekule FP (2010). Evaluation of *Moringa oleifera* leaf meal inclusion in cassava chip based diets fed to laying birds. Livest Res Rur Dev 2010;; 22: article 118.

OLUGBEMI, T.S, - MUTAYOBA, S.K. and LEKULE, F.P. (2010). Effect of Moringa (*Moringa oleifera*) Inclusion in Cassava based diets to broiler chickens. In International Journal of Poultry Science, vol. 9, no. 4, p. 363-367.

Ort, J. F., and Latshaw, J. D. (1978). The toxic level of sodium selenite in the diet of laying chickens. J. Nutr. 108:1114–1120.

PATEL, J.P. (2011). Antibacterial activity of methanolic and acetone extract of some medicinal plants used in India folklore. In International Journal of Phytomedicine, vol. 3, p. 261-269.

Patton, N. D (2000). Organic selenium in the nutrition of laying hens: Effects on egg selenium content, egg quality and transfer to developing

chick embryos. Ph.D. Diss., Univ. Kentucky, Lexington.

Payne, R. L.; Lavergne, T. K. and Southern, L. L. (2005). Effect of inorganic versus organic selenium on hen production and selenium concentration. Poult. Sci. 84:232–237.

Petrosyan, A.; Papazyan, T. and Nollet, L. (2006). Administration of Se as Sel-Plex on top of Sodiumselenite still improves fertility of hatchability of a broiler breeder flock. XII European Poultry Conference, Verona, Italy, 10-14 September.Poult. Sci. J. 15: 32–33.

Price, L.M. (2000). The moringa tree. Available: <http://www.echonet.org> [Consulted: April 18, 2011.progeny. British Poultry Science (In press) Public Health Nutr. 4(2B): 593–599.

Rapp, L. M., S. S. Maple, and J. H. Choi. (2000). Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina.

Rayman, M.P. (2000). The importance of selenium to human health. *Lancet* 356: 233–241

Renema, R. A. (2006). Creating the ideal hatching egg: quality, efficiency and fertility. Nutritional biotechnology in the feed and food industries: Proceedings of Alltech's 22nd Annual Symposium, Lexington, Kentucky, USA.

Reyes Sanchez N, Sporndly E, Ledin I (2006). Effects of feeding different levels of foliage from *Moringa Oleifera* to creole dairy cows on intake, digestibility, milk production and composition. Livest. Sci ., 101(1-3): 24-31.

Ribaya-Mercado JD, Blumberg JB (2004). Lutein and zeanthin and their potential roles in disease prevention. Journal of the American College Nutrition 2004; 23(6):567-87S.

RUTZ, C. (2003). Assessing the breeding season diet of goshawks Accipiter gentilis: biases of plucking analysis quantified by means of continuous radio-monitoring. J. Zool. 259: 209-217.

Rweyemamu, L.M.P. (2006). Challenges in the development of micronutrient-rich food ingredients from Soya beans and *Moringa oleifera* leaves: Moringa and other highly nutritious plant resources: Strategies, standards and markets for a better impact on nutrition in Africa, Accra, Ghana. 16-18.

Sa´ nchez-Machado, D.I., Nu´ nez-Gaste´ lum, J.A., Reyes-Moreno, C., Ramı´rez-Wong, B. & Lo´ pez-Cervantes, J. (2010). Nutritional quality of edible parts of Moringa oleifera. Food Analytical Methods, 3, 175–180.

Sara, A.; Bennea, M.; Odagiu, A. and Pantă, L. (2008). Effects of the organic selenium (Sel-Plex) administered in laying hens' feed in second laying phase on production performances and the eggs quality. Bulletin UASVM Animal Science and Biotechnologies, 65:1-2

SARWATT, S.V, - KAPANGE, S.S. and KAKENGI, A.M.V. (2002). The effects on intake, digestibility and growth of goats when sunflower seed seed cake is replaced with *Moringa oleifera* leaves in supplements fed with Chloris gayana hay. In Agroforestry systems, vol. 56, p. 241-247.

Sarwatt, S.V., Milang'ha, M.S., Lekule, F.P. and Madalla, N. (2004). *Moringa oleifera* and cotton seed cake as supplements for smallholder dairy cows fed napier grass. Livestock Research for Rural Development. 16:38.www.google.com, Accessed (28 November 2012).

Scheideler SE, Weber P, Monsalve D. (2010). Supplemental vitamin E and selenium effects on egg production, egg quality, and egg deposition of α-tocopherol and selenium. J Appl Poult Res 19, 354-360

SCHIEDT, K. (1998). Absorption and metabolism of carotenoids in birds, fish and crustaceans. in: Biosynthesis and Metabolism. Carotenoids. Birkhauser Verlag, Basel, Switzerland. Vol. 3. pp 285–358.

Schrauzer G. N. (2000). Selenomethionine: a review of its nutricional significance, metabolism and toxicity. J. Nutr. 130:1653–1656.

Scott, M. L. and J. N. Thompson (1971). Selenium content of feedstuffs and effects of dietary selenium levels upon tissue selenium in chicks and poults. Poult. Sci. 50:1742-1748.

Scott, T. A. and F. G. Silversides (2000). The effect of storage and strain of hen on egg quality. Poult. Sci. 79:1725-1729.

Siddhuraju P, Becker K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (Moringa oleifera Lam.). J Agric Food Chem **15**: 2144–2155.

Silversides, F. G. and T. A. Scott. (2001). Effect of storage and layer age on quality of eggs from two lines of hens. Poult. Sci. 80:1240-1245.

Siske V, Zeman L, Klecker D (2000). The egg shell: A case study in improving quality by altering mineral metabolism - naturally.In: Lyions TP, Jacques KA, editor. Biotechnology in the feed industry. Proceedings of Alltech's 16th Annual Symposium. T. P. Nottingham, UK: Nottingham University Press. p.327

Skřivan M., Ševčíková S., Dlouhá G., Tůmová E., Ledvinka Z. (2006): Enhancement of vitamin E and A in animal products. Patent Application. Office of Industrial Proprietorship, Czech Republic. Skřivan M., Šimáně J., Dlouhá G., Doucha J. (2006b): Effect of dietary sodium selenite, Se-enriched yeast and Se-enriched alga Chlorella on

egg Se concentration, physical parameters of eggs and laying hens production. Czech J. Anim. Sci., 51, 163–167

SOFIDIYA, M.O, - ODUKOYA, O.A, - FAMILONI, O.B. and INYA-AGHA, S.I. (2006). Free-radical scavenging activity of some Nigerian medicinal plant extracts. In Pakistan Journal of Biological Science, vol. 9, p. 1438-1441.

Spring, P .(2006). Poultry health through nutrition. World Poultry 22:26-29.

Spring, P. (2008). Maximising meat quality with selenium. *Int. Pol. Prod.* 15(5): 7–11

Stanley, V. G.; Krueger, W. F. and Sefton, A. E. (2004). Single and combined effects of yeast cell wall residue and Sel-Plex® on production and egg quality of laying hens. Poult. Sci. 83:200.

STEINBERG, W., M.A. GRASHORN, A.M. KLUENTER, J. SCHIERLE (2000): Comparative pigmentation efficiency of two products containing either apo-ester or tagetes extracts on egg yolks and liquid eggs. Arch. Geflugelk. 64, 180-187.

Subadra, S. and Monica, J. (1997). Retention and storage stability of beta carotene in dehydrated drumstick leaves (Moringa oleifera). International Journal of food science and nutrition, 48(6): 373-379.

Sura P.F. (2000). Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. British Poultry Science 41:235-243.

Surai P.F. (2002) Natural Antioxidanyts in Avian Nutrition and Reproduction.Nottingham University Press, Nottingham.

Surai P.F. (2002). Selenium in poultry nutrition 2. Reproduction, egg and meat quality and practical applications. World's Poultry Science Journal 58:431-450.

Surai, P.F. (2006). Selenium in Nutrition and Health.

Surai, P.F. and Sparks, N.H.C. (2001). Comparative evaluation of the effect of two maternal diets on fatty acid, vitamin E and carotenoid in the chick embryo. Brit. Poult. Sci. 42:252-259

Surai, P.F. (2000). Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. B. Poult. Sci., 41: 235-243.

Surai, P.F., I.A. Ionov, E.F. Kuchmistova, R.C. Noble and B.K. Speake, (1998). The relationship between the levels of alpha-tocopherol and carotenoids in the maternal feed, yolk and neonatal tissues: Comparison between the chicken, turkey, duck and goose. J. Sci. Food Agric., 76: 593-598.

SURAI, P.F. (1999). Tissue-specific changes in the activities of antioxidant enzymes during the development of the chicken embryo. British Poultry Science, 40: 397–405.

Surai,P., Lonov,I., Kuchmistova, E., Noble, R and. Speake,B.(1998). The relationship between the level of α-tocopherol and carotenoids in the maternal feed, yolk and neonatal tissues: comparison between chicken, turkey, duck and goose. J. Sci. Food Agric.76:593-598.

Swanson, C. A. (1987). Comparative utilization of selenite, selenomethionine, and selenized yeast by the laying hen. Nutr. Res. 7:529–537.

The Poultry Consultancy. (2009). Poultry feed formulation: Defining a feed additive. www.thepoultryconsultancy.com.

Trees for life, (2005). Moringa book. Available at http://www.treesforlife.org.

Utterback, P. L.; Parsons, C. M.; Yoon, I. and Butler, J. (2005). *Effect of* supplementing selenium yeast in diets of laying hens on egg selenium

content, Poult. Sci., 84: 1900-1901.

Wakebe, M. (1998). Feed for chicken and for hen. Japanese Patent Office, Patent number JP10023864A2. Jan 27, 1998.

Wong, H.K. (2005). Vitamin E and selenium enriched eggs for health. Paper presented at ScienceWorld's Poult Sci J, 57 , pp. 127-138

Wu, J., A.D. Del Genio, M.-S. Yao, and A.B. Wolf (2009). WRF and GISS SCM simulations of convective updraft properties during TWP-ICE. *J.* Geophys. *Res.*, 114, D04206, doi:10.1029/2008JD010851.

Yameogo CW, Bengaly MD, Savadogo A, Nikiema PA, Traore SA (2011). Determination of chemical composition and nutritional values Moringa oleifera leaves. Pak J Nutr 2011; 10: 264–268.

Yaroshenko F.A.; Dvorska, J. E.; Surai, P. F. and Sparks, N.H.C. (2003). Selenium/ vitamin E enriched eggs: nutritional quality and stability during storage. Poster presented at Alltech's 19th Annual Symposium on Nutritional.

Appendixes

Figure.24 Images of experimental layers

Figure 25. The picture on the left show yolk color van and on the right show digital

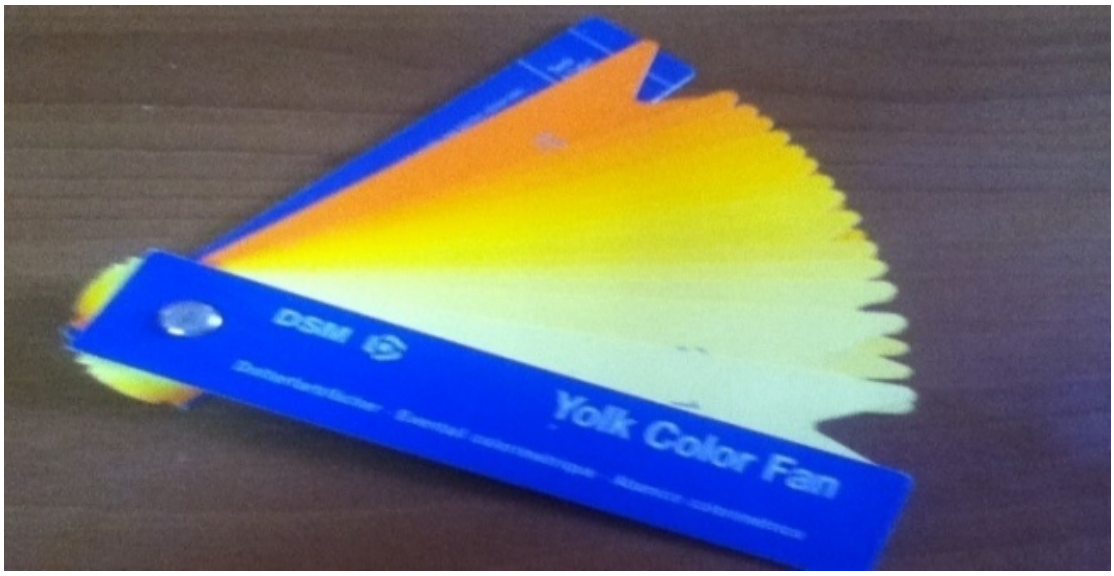

Figure 26. The yolk color van

Figure(27): showed the effect of MOLM on eggshell thickness of laying hens

Figure (28). showed the effect of MOLM on egg yolk color of laying hens

Figure(29): Moringa tree