MULBERRY (*Morus alba*) LEAF MEAL IN INDIGENOUS CHICKEN LAYER DIETS: EFFECT ON EGG PRODUCTION AND QUALITY

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirement for the Master of Science Degree in Animal Nutrition of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for an award of a degree in this or any other University.

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Recommendation

This thesis has been submitted to Graduate School with our approval as supervisors.

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DEDICATION

This work is dedicated to God almighty for giving me knowledge, understanding and a sound mind to tackle this project. To my supervisors Dr. King'ori and Dr. Ambula thank you for the great support and dedication throughout the project. To my husband for the encouragement and push to succeed and for all the sacrifices you have made for me. To my father, mother, niece and sister thank you for your prayers and encouraging words. To my domestic manager thank you for taking care of my little babies as I concentrated on my work. To my babies Talia and Jake, and all my friends without you none of my success would be possible.

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ABSTRACT

Soybean meal is the main protein ingredient in poultry feeds. It is expensive, inadequate in supply and does not have sufficient β-carotene which gives the yellow yolk colour preferred by most consumers. Mulberry leaf meal (MLM) has been identified as a locally available alternative protein source in poultry feeds and contains β-carotene that may improve egg production and yolk colour. However, the optimal level of inclusion in layer diets is unknown and there is need for it to be determined. This study therefore determined the effects of inclusion of MLM as a protein source in indigenous chicken (IC) diets on feed intake, egg production and quality. Sixty, 29-week-old IC were used in this study. The Experimental diets contained 16% CP, calculated metabolizable energy of 2800kcal ME/KG and SBM was substituted by MLM at the following levels: 0% (Diet 1), 5% (Diet 2), 10% (Diet 3) and 15% (Diet 4). The chickens were offered the experimental diets for 8 weeks. Objective one determined the effects of inclusion of mulberry leaf meal in the diets on feed intake, feed conversion ratio (FCR), and egg laying percentage. Data on feed intake and egg production and FCR were collected daily. Feed conversion ratio data was calculated by dividing the weight of feed consumed by the weight of eggs produced. In objective two, a sample of five eggs per treatment were used to assess the external qualities (shape index, egg shell thickness and egg shell ratio). Objective three determined the internal egg qualities (yolk colour, index and yolk albumin ratio) and cholesterol content. Data collected was analyzed using generalized linear model (SAS, 2009). Mean separation was done using least significance difference at 5%. The results from experiment one showed that inclusion of MLM at 10% increased egg production, feed intake and decreased FCR. In experiment two, inclusion of MLM in the diets had no effect on external qualities except shell thickness that decreased. For objective three, inclusion of MLM in diets had no effect on internal qualities and cholesterol content except yolk colour intensity that increased to deep yellow. In conclusion, MLM should be incorporated at 10% in layer chicken diets to improve egg production and quality.

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LIST OF ABBREVIATIONS AND ACRONYMS

AA	Amino acid
ADFI	Average daily feed intake
CA	Carbonic anhydrase
CF	Crude fibre
CP	Crude protein
CRD	Completely randomized design
DCP	Dicalcium phosphate
EAC	East African Community
FCR	Feed conversion ratio
FI	Feed intake
GLM	Generalized linear model
G	Grams
HDL	High density lipoprotein
HU	Haugh unit
IC	Indigenous chicken
KALRO	Kenya Agricultural and Livestock Research Organisation
KARI	Kenya Agricultural Research Institute
KCAL	Kilocalorie
KJ	Kilojoule
LDL	Low density lipoprotein
ME	Metabolizable energy
MG	Milligram
MLM	Mulberry leaf meal
MM	Millimetre
NSRC	National Sericulture Research Center
RYCF	Roche yolk colour fan
SADC	Southern Africa Development Community
SBM	Soybean meal
TAP	Tatton Agriculture Park

CHAPTER ONE

INTRODUCTION

1.1 Background information

Indigenous chicken are commonly reared in Kenya with an estimated population of 32 million birds, according to the 2019 Household and livestock census (KNBS, 2019). The low productivity of indigenous chicken with a mean annual egg production of 60 small eggs (Mengesha, 2012) for an average household of 5 people is primarily attributed to low genetic potential, poor nutrition, and in adequate disease control. Nutrition is of major influence and well managed hens can produce about 150 eggs per year (Okitoi *et al*., 2008). The main target product from indigenous chicken are eggs which are produced under various feeding regimes to meet the growing increase in demand for IC eggs (Omiti & Okuthe, 2009). Consumers have a preference for quality eggs available in the market (Nyachoti *et al*., 1997). Evaluation of egg quality is done through grading using interior and exterior quality. Exterior evaluation considers cleanliness, shape, texture, and soundness. Interior evaluation considers the yolk and the albumin quality. Grading is a form of quality control used to assess a variable commodity or product into a number of classes (Wu *et al*., 2014). Some defects affect the internal and external qualities of an egg therefore affecting the market preference (King'ori, 2012).

An advantage of grading is that it allows consumers to exercise a preference in relation to quality which may stimulate increased prices and sales. It also sets and maintains reliable standards giving confidence in the product and a favourable reputation is established safeguarding the market. Grading also facilitates the mechanical process of packaging and distribution and often enhances the appearance of the product. The price differentials that grading develops provide an incentive to the producer to adjust production methods.

Some of the qualities preferred in the market include a strong shell, consistent egg yolk, deep yellow colour of the egg yolk, physical appearance of the egg and long shelf life (Panigrahi, 1989). The quality of an egg will not only depend on the genetic make-up of the layer but also on the type of feed consumed. Forage based diets are meant to improve the quality of the eggs without imparting any negative effects on the egg. Feeds that improve on the above qualities are highly recommended depending on the nutritional content of the selected forage (Tufarelli *et al*., 2018).

Mulberry is a multi-functional plant, easy to grow and an excellent source of nutrients and phytochemicals. The leaves have long been known to have medicinal properties against heart diseases, diabetes and high blood pressure (Lim *et al*., 2013. Mulberry trees are commonly found in most rural compounds in Kenya. This is due to the many benefits associated with it which include; feeding silkworms (Tuigong *et al*., 2015) and livestock especially ruminant animals with leaves, making jam from the berries and consumption by people. The trees are also used for wood, fuel and timber. Mulberry leaf meal (MLM) is easily available; hence it is a means of providing a cheap source of protein for animals.

Mulberry has the potential to increase productivity level of small-scale farmers through feeding of poultry due to its availability and ease of establishment. Mulberry leaves contain β-carotene, which can be converted with varying efficiency by poultry to vitamin A and the xanthophylls, which can be a good source of the pigmentation in egg yolk (Srivastava *et al*., 2006).

Mulberry leaves reduce egg yolk cholesterol hence increasing the egg quality and reduce negative health effects for the consumers. Cholesterol increases the risk of having a heart attack or stroke to the consumers, the oxidized LDL-cholesterol causes inflammation of the surrounding tissues that leads to disease and organ damage in the body (Berger *et al*., 2015). Poor feeding regime and poor handling methods lead to production of low-quality eggs. These eggs have low off take rates and low shelf life that leads to great losses to farmers, retailers and wholesalers.

This study therefore aimed at improving the performance of indigenous chicken and quality of the eggs released into the market through inclusion of MLM in the diets.

1.2 Statement of the problem

Soybean meal is the main protein ingredient in poultry feeds. It is expensive, inadequate in supply and of variable quality. It also does not have sufficient β-carotene which gives the egg yolk the deep yellow colour. Eggs with deep yellow colour are most preferred by most consumers. Low -quality eggs (external and internal quality) have low demand and fetch poor prices which lead to low income and poverty among farmers. Manipulation of egg yolk colour in eggs from hens fed soybean-based diets to deep yellow is often done using synthetic β-carotene which is an added cost. This results in high cost of production as well as production of low-quality eggs (external and internal quality) hence leading to low income and poverty among farmers.

1.3 Objectives

1.3.1 Broad objective

To contribute to sustainable egg production and quality through improved nutrition by use of mulberry leaf meal in indigenous chicken layer diets.

1.3.2 Specific objectives

i. To determine the effect of inclusion of mulberry leaf meal (MLM) in indigenous chicken (IC) layer diets on performance.
ii. To determine the effect of inclusion of MLM in IC layer diets on external egg quality.
iii. To determine the effect of inclusion of MLM in IC layer diets on internal egg quality.

1.4 Hypotheses

i. Inclusion of MLM in IC layer diets has no significant effect on feed intake and egg production.
ii. Inclusion of MLM in IC layer diets has no significant effect on external egg quality.
iii. Inclusion of MLM in IC layer diets has no significant effect on internal egg quality.

1.5 Justification of the study

Over the recent years there has been increasing competition for protein foodstuffs between human beings and non-ruminant livestock. These feedstuffs e.g., soybean and fishmeal, are scarce and their incorporation in feeds makes them expensive while the low-quality ones are mainly used in feed compounding. This results in high cost of production as well as production of low-quality eggs (external and internal quality) hence leading to low income and poverty among farmers. Consumers mostly consider price and size of the eggs when purchasing eggs. The physical characteristics of the egg that most consumers prefer are deep yellow yolk color and jumbo eggs. These factors have led to the need for identification and evaluation of locally available alternative protein feed resources for poultry in order to improve productivity, egg quality and hence reduce the cost of production. However, some plants are good sources of protein and natural β-carotene.

Mulberry leaf meal has been identified as locally available alternative protein source for poultry feeds that may improve egg productivity and egg quality. Mulberry tree is hardy, deep rooted and does well in almost all types of soils and it is locally available in Kenya because of favorable climatic conditions. It is drought tolerant and can thrive in arid and semi-arid lands (ASALs), which make up 88% of the Kenya land mass. Mulberry leaves are rich in protein (15-35%), minerals [2.42-4.71% calcium (Ca); 0.23-0.97% phosphorus (P)], metabolizable energy (4728-9372 KJ/kg) and β-carotene (3.91-14.79mg/100g). Therefore, this study evaluated the effect of incorporation of MLM as a protein and β-carotene source in indigenous chicken layer diets on feed intake, egg production, yolk colour, egg weight, egg shell thickness and cholesterol content.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of poultry industry in Kenya

Poultry keeping is one of the most popular livestock enterprises in Kenya due to its low capital space requirements. Most rural families in Kenya keep poultry. According to the 2019 Household and livestock census, the Kenyan poultry population was estimated to be over 38 million birds with 78% being free-ranging indigenous chicken, 21% commercial layers and broilers, while the rest being other poultry species (ducks, turkeys, pigeons, ostriches, guinea fowls and quails) (KNBS, 2019). Turkeys are reared commercially in small numbers compared to chicken as well as ducks and quails (Upton, 2000). The main genotypes of commercial layers are Isa Brown and Ross, while commercial broiler genotypes include Arbor Acres, Hybro, Cobb (United Kingdom) and Hypeco (Holland). There are two genotypes of turkeys – local small bronze and buff type and the commercial large white and buff types. Ducks are of the Muscovy type while guinea fowls are the helmeted type (Omiti & Okuthe, 2009).

Kenya has a well-developed commercial poultry industry in Africa providing a major source of animal proteins in many diets (Nyaga, 2007). Poultry meat and eggs contribute to a well-balanced diet, as there are few cultural or religious taboos that hinder the consumption of these products. Poultry also plays important socio-cultural roles in the Kenyan society. For example, poultry are slaughtered during religious festivals such as Easter and Christmas for Christians and Ramadhan for Muslims. The sector is also linked with sports and culture; cockerel fighting is a big attraction in some communities in Kenya especially Luhya community. Part of the income derived from poultry farming is appropriated as government revenue, representing 30% of the agricultural contribution (25%) to GDP. The rest forms an important pathway out of poverty, especially among the rural population (Omiti & Okuthe, 2009).

2.2 Overview of indigenous chicken (IC) production in Kenya

Indigenous chicken farming in Kenya is a widespread type of poultry farming that is mainly found in the rural and peri-urban areas. Indigenous chicken are kept by 90% of rural households while broilers and layers are mainly reared by urban and peri-urban farmers who target the ready market (KNBS, 2019). Local chicken breeds are the most abundant livestock species in Kenya with a population of 30 million birds. They are evenly distributed across the country with an exception of the urban and arid areas as presented in table 2.1.

Table 2.1 *Population and distribution of indigenous chicken in Kenya*

County	Population	County	Population
Mombasa	230,630	Samburu	189,650
Kwale	856,756	Trans-Nzoia	1,378,444
Kilifi	923,152	Uasin-Gishu	1,359,750
Tana-River	182,688	Elgeyo Marakwet	535,348
Lamu	257,198	Nandi	1,208,268
Taita-Taveta	665,686	Baringo	1,073,898
Garissa	176,720	Laikipia	1,013,616
Wajir	144,142	Nakuru	1,059,023
Mandera	302,788	Narok	956,040
Marsabit	42,478	Kajiado	180,062
Isiolo	104,384	Kericho	175,062
Meru	1,254,189	Bomet	174,564
Tharaka-Nithi	836,386	Kakamega	986,946
Embu	1,112,404	Vihiga	54,568
Kitui	956,412	Bungoma	452,475
Machakos	1,289,614	Busia	62,064
Makueni	1,062,413	Siaya	84,320
Nyandarua	1,476,374	Kisumu	143,398
Nyeri	1,469,778	Homa Bay	88,972
Kirinyaga	1,351,312	Migori	82,358
Murang'a	1,244,108	Kisii	245,242
Kiambu	1,323,954	Nyamira	128,858
Turkana	86,316	Nairobi	489,466
West-Pokot	848,358		
TOTAL	**30,320,632**		

Source: KNBS (2019)

The distribution is mostly influenced by availability of feed resources, human population and environmental conditions. Indigenous chicken are mostly kept under a free-range system in small flocks of less than 30 birds. They are more robust and adapted to local conditions than the hybrids but have a lower productivity rate (Nyaga, 2007). On average, each household in Kenya keeps about 12 chickens, mainly for domestic consumption. They play a vital gender role for women, widows and orphaned children in terms of cash incomes and savings, food security, nutrition and socio-cultural activities (Omiti & Okuthe, 2009). Indigenous chicken are highly adapted to the harsh scavenging conditions, poor nutrition and disease and parasite challenges. The reactions are closely linked with anatomy and physiological features, which have developed as a result of natural selection (Mengesha, 2012).There is a relatively high mortality rate because of lack of drinking water, disease control, and predators. On average, farmers lose up to 40 percent of their stock annually due to these controllable challenges (King'ori *et al.*, 2010).

Indigenous chicken are characterized by low production performance and late maturity. The mean annual egg production is estimated to be 60 small eggs (Mengesha, 2012). Fifty percent of the eggs produced are eaten or sold by the farmers. The sale of eggs is done at the farm gate or the local market. Growing pullets and cocks of between four to six months that weigh 1.5kg to 2.0kg are sold at the farm gate or at the local market. The names used to describe the common phenotypes of indigenous chicken in Kenya are frizzled feathered, naked neck, barred feathered, feathered shanks, bearded and dwarf sized and vary in body size, conformation, plumage colour and performance (Nyaga, 2007). They get most of their feed from scavenging and may occasionally benefit from kitchen and other household wastes. Eggs and meat from the chicken contribute to the protein nutrition of the rural population thus alleviating malnutrition (King'ori *et al*., 2010).

Indigenous poultry farmers rarely purchase commercial feed or seek veterinary services for their birds, unless the situation warrants it (Omiti & Okuthe, 2009). In most cases, they in are kept in a separate house that is located near the main house for security purposes. In some communities, chickens are kept in the family house during the night. In some instances, chicken houses are elevated above the ground. Such houses usually have wooden floors and walls with either grass thatched or iron sheet roofs. In other cases, houses are usually not elevated and have earthen floors, mud walls and thatched roofs. Indigenous birds are usually transported to the market on foot, by bicycle or by motor vehicles. Indigenous poultry meat and eggs are increasingly gaining popularity in major urban centres in Kenya due to changing consumer preferences associated with desirable health characteristics, such as lower saturated animal fats and lower cholesterol levels (Omiti & Okuthe, 2009).

The introduction of Improved Indigenous Chicken farming in Kenya by the Kenya Agricultural Research Institute (KARI) now Kenya Agricultural and Livestock Research Organisation (KALRO) in the year 2011 has given a new face to Poultry Farming in Kenya. The objectives of KALRO were to increase indigenous chicken productivity, generate income and ultimately enhance food security among rural households (Okitoi *et al*., 2008). There are several improved indigenous chicken in Kenya which have higher productivity than the local kienyeji chicken: Rainbow Rooster, KARI improved Kienyeji, Sasso F1, Kuroiler and Kenbro.

Rainbow rooster: This is a slow growing broiler, low input, multi-coloured chicken from India. It was bred by Indbro Research and Breeding Farms in Southern India. Rainbow Roosters have been distributed to Kenya through Partnerships with Indbro Research (Mulwa *et al*., 2017).

KARI improved kienyeji: This was bred from a range of indigenous chickens in Kenya by KARI (now KALRO) Naivasha. The breed develops faster, is highly resistant to diseases and has high productivity. It achieves a weight of 1.5kg in five months while the rest of the indigenous breeds take up to seven months or more (Mulwa *et al*., 2017).

Kuroiler: This breed was introduced in Kenya from Uganda but originated from India. It is a slow growing broiler. It is a cross of either White Leghorn roosters or coloured broiler roosters with Rhode Island Red hens. It has gained popularity among chicken farmers in Kenya (Ngeno, 2015).

Kenbro: This is a crossbreed developed by Kenchic-(broiler X indigenous chicken) that is robust and resistant to disease and possesses a well-rounded conformation. These birds can be used for sustainable or commercial farming for meat or eggs. Kenbro lays more eggs than indigenous chicken and has lean, soft, high quality meat that is popular with consumers (Gikunda, 2015).

2.3 Production systems

The main production systems used in rearing indigenous chicken are free range (extensive) system, semi-intensive (improved) system and intensive system. Choice of production system depends on the households' land availability and the objective attached to the enterprise (Magothe *et al*., 2012).

2.3.1 Free range system

This chicken production system is practiced in most rural areas of the country and objectives of production are for household consumption and as a source of additional income. In this system, the chickens are set free to move around and search for feeds as well as promote their natural behaviour (Larsen *et al*., 2017). This is common in low population density rural areas and is based entirely on low input-low output management (Magothe *et al*., 2012). Small flocks of less than 30 chickens per household are kept most being indigenous breeds and dependant on locally available feed material as supplement, with low health services and other management practices. There is also long broody periods and risk of exposure to diseases and predators (Magothe *et al*., 2012). The chicken leave their night shelters in the morning and are left to source any available feed resources around the homestead and are self-reliant. Free-range feed resources usually include grass, insects, earthworms and various seeds.

Indigenous chicken are known to be alert to predators, protective of their young, have high hatching ability, possess excellent foraging ability and long legs which are suitable for fast running. All these adaptation are necessary in a free range production system. Chicken are sometimes confined and supplemented with maize, kitchen leftovers and any other available feed resource. Free-ranging chickens can fulfil their nutritional requirements for proteins, energy, vitamins and minerals by scavenging due to good foraging ability, and the ability to utilise high fibre diets. Night shelters include rudimentary coops, kitchens, stores and human habitats (Birech, 2002). Chicken brooding and rearing is only the care they obtain from their mother/hen (Mengesha, 2012). There is little or no labour input. The amount of rubbish is reduced in a productive way and the direct costs of the system are low. In this system, it is difficult to control and manage the chicken especially young chicks that are easily exposed to predators and unfavourable weather conditions. Most poultry kept in free-range scavenging systems are infected with various sorts of endo- and ecto-parasites. A large percentage of the eggs can be lost since the laying hens are not provided with laying nests. There is high diseases transmission and mortality rate (Mengesha, 2012).

2.3.2 Semi-intensive production system

In this system, birds are left to free range (to source any available feed resources) during the day around the homestead or in fenced runs and confined to shelters during the night (Magothe *et al*., 2012). They are supplemented with grains, kitchen leftovers and commercial feeds. Water and veterinary care are provided (Kingori *et al*., 2010). Chickens reared in this system are more productive than in free range system. This system contains flock sizes of 50-200 birds/chicken per household comprised of improved breeds. In this system, there is complete control over operation, record keeping throughout the year, economic use of land and there is better protection during the cold season. The cost of fencing is high, there is also the danger of over stocking (high spread of diseases) if enough space is not available, and exposure to different diseases if the compound is not clean and dry (Omiti & Okuthe, 2009).

2.3.3 Intensive production system

In this system, more inputs are used than in free range and semi-intensive systems. It is market oriented and focuses on profit maximization. Chickens are genetically selected for economically important traits of fast growth, high production and reproduction. Commercial feeds are used and the chickens are vaccinated (Menge *et al*., 2005). The type of housing used includes; use of cages and deep litter system of housing. The intensive system is rare in rural areas and but common in urban and peri-urban areas and it is mostly practiced by financially stable individuals who want to invest in poultry farming (Magothe *et al*., 2012). The number of chickens involved are relatively high (more than 200 chicken). Breeds used are specialized improved breeds (layer or broiler).

Deep litter housing system

It involves rearing of chickens on a floor littered by 5-10 cm thickness litter. The litter can be made from locally available material such as dry hay, straw, coffee pulp and sawdust. Thickness should not be less than 5cm (Yakubu *et al*., 2007). The litter should be dry at all time otherwise it can cause diseases. In addition to provision of comfort for the chicken, the litter absorbs any waste material excreted from the chicken and make the house dry (Ovwigho *et al*., 2009). Feeders and drinkers are placed on the litter or hanged as the age of the chicken increases. For layers, laying nests are also placed in the house. Litter is sprayed with disinfectant.

Deep litter system is recommended for both meat birds and layers. The existing litter should be removed totally when the existing stock is culled. Before introduction of new stock, the house is cleaned thoroughly and left free at least for two weeks. This prevents problem of cannibalism and diseases outbreak (Sosnówka *et al.,* 2010). The deep-litter system is inappropriate for very humid areas (80–90% humidity) damp litter spreads diseases (Yakubu *et al.,* 2007).

Battery/Cage housing system

The system is best for layers whereby the hens live in cages where feeds and water are provided. For controlled-environment housing of layers, multi-tier cage systems are common. Most large-scale commercial farms use controlled-environment systems to provide the ideal thermal environment for the chicken. Achieving the ideal environment for birds depends on appropriate management of the poultry house (Yakubu *et al.,* 2007). In this system, there is proper accommodation, prompt culling of unproductive birds, proper control of diseases and predators, good record keeping and high egg production (Ovwigho *et al.,* 2009). Land requirement is minimum, easy and economic management, scientific feeding and management, high degree of supervision, minimum labour and automation is possible and manure value is increased.

2.4 Overview of livestock feed industry in Kenya

The feed industry in Kenya largely relies on imported feed ingredients such as maize, maize germ/bran, wheat bran and pollard, soybean and its derivatives, sunflower cake, cotton seed cake, fish meal, and micro-ingredients (usually additives) from the East African Community (EAC), Southern Africa Development Community (SADC) regional market and other international markets (Oloo, 2010). There has been an upward trend in the cost of ingredients used in feed manufacturing. This trend is a result of increases in demand for the ingredients, and competition between animal and human food requirements, and other industrial needs such as bio-fuel production in the world market (Said & Mbugua, 2005). Over-dependence on rain-fed agriculture exacerbates susceptibility of the agricultural sector to climate change thereby causing instability in the supply chain whenever there is inadequate rainfall (Oloo, 2010). In the Kenya feed industry, competitive rivalry is real because the feed industry has low entry barriers that allow mediocre manufacturers with little equipment investment and animal nutrition knowledge. Successful feed manufacturers/suppliers require nutrition knowledge supported by scientific data from laboratories that determine real time product chemical composition and quality control that meets target animal requirements. Due to importation of alliterated raw materials, it is difficult to make feed products that distinguish each feed miller's brand. In the absence of strong regulatory framework, counterfeiting and repackaging remains a major threat.

2.5 Nutritional requirements of poultry

There are many inconsistencies documented in the nutrient requirements for indigenous scavenging chicken. Crude protein (CP) requirements for growing indigenous chickens are 20, 16 and 14% and 17, 14 and 12% for the heavy (1.66-2.14 kg) and light birds (1-1.65 kg) respectively, during week 5-8, 8-14 and 14-21. Energy requirements during the same growth period are approximately 3000, 2600 and 2400 kcal/kg ME, respectively. Indigenous chicken during the 14-21-week growth phase require a CP concentration of approximately 160g/kg (Kingori *et al*., 2010).

Table 2.2 ***Nutrient Requirements of Indigenous Chicken Per Week Growth Phase***

Nutrient	Week 5-8	Week 8-14	Week 14-21
Energy ME kcal/kg	3000	2600	2400
Crude protein % (Heavy)	20	16	14
Crude protein % (Light)	17	14	12

Source: Kingori *et al*. (2010)

Table 2.3 ***Nutrient Requirements of Indigenous Chicken***

Nutrient	Chicks	Growers	Layers
Energy ME MJ/kg	11.9	11.2	11.2
Crude protein %	19-20	13-14	15-17
Crude fibre (min) %	3.0	4.0	4.5
Calcium %	1.0-1.1	1.3-3.0	3.3-3.7
Phosphorus %	0.6	0.5	0.5
Lysine %	1.0	0.6	0.7
Methionine %	0.4	0.3	0.4

Source: Birech (2002)

2.6 Plant protein sources and their impact on egg quality

The need for protein is high in indigenous chicken than in improved breeds. This is due to the fact that sulphur amino acids deficiency affects the quality of egg contents. The main plant proteins available for poultry feeds are: soybean meal, sunflower and rapeseed. At the moment, soybean has been the main protein source for poultry feeds. This is due to its high content of amino acid (AA), quality and composition (Panigrahi, 1989). Crude protein in mulberry leaves varies from 15-35% depending on the variety, age of leaves and growing conditions and it is considered to be similar to most legume forages, for example, lupins, chickpea and alfalfa (Sánchez, 2002).

An insufficient amount of protein in indigenous chicken feeds leads to poor egg quality. It is important to note that an extra protein is required to enhance egg quality. A study by Burbaugh *et al*. (2006) showed that dietary protein has an impact on the feed intake. With low protein level in the feed, chickens tend to consume more so as to meet their nutritional requirement. Fourteen percent protein content in feed has the ability to optimize egg production though this cannot help in body weight maintenance. Thus, a laying hen requires about 16 to 18% of protein with optimum being 15% (Noy & Sklan, 1995). Egg production has been observed to be optimum in hens which have a diet containing 16% crude protein compared to hens on diet containing 12% crude protein (Hall & Mckay, 1993).

The intake of forage depends on the motivation to forage. Hens which are in a restricted environment have a higher motivation to forage than those in an open environment. Hens exhibit foraging behaviour even when feed is freely available in feed troughs, demonstrating an instinctive motivation to forage for feed (Schütz *et al*., 2001). Although there is some hydrolysis of starch in the crop the main aim of the crop is softening the ingested feed before being transported to the gizzard and the proventriculus for enzymatic digestion. Hens which have been trained to forage when young have a high crop capacity since it increases with increase in intake. This is due to fact that poultry are animals of habit. These are habits acquired early in their life and acts as an important factor in foliage feeding (Jacob *et al*., 1996). If the hens are introduced to forage at an early age, they are able to enhance their ability to forage.

Eggs which are produced from foraging are considered to be an excellent source of animal protein since they contain more vitamin A and E and access to pastures and/or insects may contribute to the flavor (Burbaugh *et al*., 2006). They are also considered to have an additional source of nutrients. According to Karsten *et al*. (2010) eggs from hens raised on legume pasture have more vitamin A and E and more omega-3 fatty acids than eggs from chickens with no access to forage. Karsten *et al*. (2010) concluded that eggs from hens consuming legumes and grasses contained more omega-3 fatty acids and vitamins than eggs from hens foraging on grass alone. Legumes contain more unsaturated fatty acids and the leafier the plant, the more omega-3 fatty acids it contains (Burbaugh *et al*., 2006). Therefore, different types of forages have varying content of nutrition which affects the egg's omega 3 fatty acids and vitamins.

Feeds which have high essential amino acids are vital as protein supplements. Several types of forage such as kales and calliandra leaves have high content of amino acids. Effect of alfalfa meal in diets of laying quails on performance and egg quality was researched by Kocaoğlu *et al*. (2004). The research showed that addition of 9% alfalfa meal into the laying quail diet improved eggshell quality and reduced egg yolk cholesterol without any adverse effect on performance. According to Karunajeewa *et al*. (1987), increasing the level of sunflower seed meal which contained unknown factors in layer rations led to a reduction in albumen height and Haugh unit.

Lopez-Bote *et al*. (1998) compared the diet and eggs of hens fed on a commercial mixed feed for laying hens in Spain, to the eggs of 'free-range' hens fed on foliage. Free-range hens were fed with natural grassland, which was dominated by the Italian ryegrass. Eggs from layers on free-range had a higher concentration of α-tocopherol than those of hens maintained in cages and fed the commercial diet. Moreover, the ratio of omega-6 to omega-3 was also significantly lower in the forage fed hens compared to those fed only commercial feed. It has been proven that plants have the highest concentrations of unsaturated fats when they have a high leaf to stem ratio compared to stages of development when plants have a high proportion of stem tissue ratio (Ebenebe *et al*., 2013). Research has also found that vegetative legumes have a higher content of linolenic acid compared to grass species. Indigenous chicken eggs are highly responsive to dietary changes of vitamins A and E. Moreover, they are responsive to unsaturated fats, linoleic and linolenic acids. Moving pastured hens to forage grass or common legume (alfalfa, red and white clover) pastures influence the egg composition as reported by Abdel (2015). Legumes have been found to have an impact on the amount of nutrition in an egg since they impact more omega-3 fatty acids and vitamins.

It is possible to determine the effect of low intake of crude protein during rearing on yield and the impact on quality of eggs (Karunajeewa *et al*. 1987). Based on his study, number and overall quality of eggs were not affected by the low protein diet, given to pullets between the ages of 13 and 20 weeks. A research by Nassiri *et al*. (2012) showed a decrease in egg weight and albumin weight when protein inclusion was reduced. Grela *et al*. (2014) researched the effect of dietary protein level on the reproductive performance of pullet and egg quality. They reported that shell thickness increased in eggs laid by chicken given diet composed of 15.4% crude protein but there were no significant differences in egg weight with the different dietary protein levels.

2.7 Soybean meal in poultry nutrition

Soybean meal (SBM) is often the major source of dietary protein in meat chicken diets due to its favorable 44-48 % crude protein composition. Soybean meal is an animal feed supplement that is rich in energy content and has an ideal amino acid profile (Dilger & Adeola, 2006). In comparison to other plant protein sources, SBM has significantly higher protein content. Soybeans can be fed to poultry whole or as soybean meal, a by-product of oil extraction (Waldroup & Smith, 2018). Pressure is applied to the soybeans in order to extract the oil using methods such as mechanical extraction, or by solvent extraction, even though mechanically extracted soybean meal is used in organic poultry diets.

Whole soybeans contain an anti-nutritional factor, trypsin inhibitor, which is heat sensitive. Roasting whole soybeans can destroy this anti-nutritional factor. It is also destroyed during the production of soybean meal (Chianu et al., 2008). Fifty five to 60% of the total phosphorous found in soybeans is bound to phytate. Poultry possess insufficient endogenous phytase to liberate substantial quantities of phosphorous from the phytate compound. The high phytate content of soybean meal requires supplementation with inorganic sources of phosphorus in monogastric animals. Dietary phosphorous in excess of animal requirements is excreted into the environment and becomes an environmental pollutant (Dilger & Adeola, 2006).

Globally, the United States dominates soybean production followed by Brazil and Argentina. In Kenya, local soybean demand is high, while most of it is met by external imports (Chianu *et al*., 2008). There is possibility of the staple protein source for poultry to become unaffordable in the future especially in low-income African countries, due to the unending increase of the price of Soybean in poultry diets on the international market (Mulwa *et al*., 2017).

2.8 Use of forage legumes in layer diets

There are numerous studies on use of other leaves and legumes as source of forage and their impact on egg quality. Foraging feeds have a positive impact on the development of poultry intestinal system and the micro flora (Kimiaeitalab, 2017). Moreover, they have an impact on the chicken egg quality since they constitute a large percentage of intakes for the chicken. Research shows that some of the egg qualities are affected by foraging. A study done on the Leghorn hens kept under free range showed an increase in the egg yolk based on α-tocopherol and α-linolenic acid dietary levels (Karsten *et al*., 2010).

Forages have high content of several bioactive compounds such as polyunsaturated fatty acids, vitamins and pigments. This is what impacts the egg quality. A study done by Abdel (2015) showed that using foraging feeds can improve and modify the eggs qualitative characteristics and nutritional quality. The nutritional qualities of the eggs improved through foraging feeds were tocopherol, carotenoids, n-3 fatty acids content of the eggs produced. The findings by this study showed the importance of foraging feeds to hens based on the egg quality (Burbaugh *et al*., 2006).

Chicken feeds help to give necessary elements required for body functions which include growth, egg production and meat (Ondwasy *et al*., 2006). Thus, it is always important to meet the nutritional requirements to attain high quality eggs. For the chicken, there is need to ensure that the forage feeds have adequate supplements. A study done showed that use of forage legumes or mixed grasses had an impact on the omega-3 fatty acids and the level of concentration for Vitamins A and E (Burbaugh *et al*., 2006). Compared to the eggs from the commercial feeds, hens fed on foliage had twice as much of the vitamin E and long chain of omega-3 fatty acids. They also had more than double the total omega -3 fatty acids. The study found out that the level of vitamin A was higher by 38% compared to chicken feeding on commercial feeds. Chicken have a short digestive tract hence can easily assimilate dietary nutrients found on the highly digestible forage feeds. Thus, the fat-soluble vitamins which might be in the forage diets can be easily transferred to the liver and then to the egg yolk (Woods & Fearon, 2009). Thus, it is possible to prove that the egg-nutritional content is based on the dietary change. Use of supplements such as mash rather than forage was associated with a reduction in omega-3 fatty acids and vitamin A and E in the eggs (Karsten *et al*., 2010). Use of forage leads to higher concentration of omega -3 fatty acids and fat-soluble vitamins in the eggs.

Poultry have the ability to consume up to 30% of their bodyweight in forage when provided (Burbaugh *et al*., 2006). This is through use of indirect measurements which relate to the measurements of forage consumption and egg production. Forage contains high amount of calcium which is important for eggshell formation (Karsten *et al*., 2010). According to NRC (1994) calcium requirement for the brown egg indigenous chicken were 4g daily per hen. For the non-restricted hens, the amount of calcium was estimated to be between 5.3 to 6.7g per hen per day (Burbaugh *et al*., 2006).

During the pre-industrial period, poultry production through use of forage was of high interest. This initially ran out of interest until the current times where forage is seen as an alternative source of feed due to the nutritional benefits associated with forage feeds (Burbaugh *et al*., 2006). An increase in forage intake for the layers has the ability to increase the biological cycles in the farming system. Forage feeds help in provision of a balanced diet. Studies have shown that inclusion of fiber diets helps in organ development such as gizzard, increases HCl, bile acid and the enzyme secretion (Kimiaeitalab, 2017).

Different types of poultry have varying foraging behavior and consumption. Moreover, genetics have been associated with the ability of poultry to each forage feeds. Research also points out that feeding times have an impact on the forage consumption. Poultry tend to be most active during the morning and evening hours. Laying hens with access to forage feeds tends to feed more before sunset (Spencer, 2013). Shade used in the shelter also has an impact on the amount of forage feeds consumed. When there is a shade protection, the amount of forage consumed by the poultry increases which impacts the egg quality. Laying hens which feed on more forage feeds produce eggs with higher shell weight and darker yolk colour due to high carotenoid levels found in the forage feeds and higher synthesis of Vitamin D due to exposure to sunlight while foraging (Mugnai *et al*., 2009). Foraging feeds increase albumin Haugh unit as indicated by a study done by Al-kirshi *et al*. (2010) and Mugnai *et al*. (2009). Mulberry leaves can be fed to poultry to improve egg quality and reduce cholesterol content of the egg (Panja, 2013).

2.9 Mulberry plant

Figure 1. Mulberry plant

Source: Tuigong *et al.* (2015)

Mulberry belongs to the family *Moraceae* and comprises of 10–16 species of deciduous trees, growing wild and under cultivation in many temperate world regions. It has been established as functional food (Srivastava *et al*., 2006). Mulberry is a multi-functional plant and an excellent source of nutrients and phytochemicals which include flavonoids which have antioxidant activity and phenolic constituents with major active components of biological activities (Rebai *et al*., 2017). The leaves possess various bioactive compounds (phenolic acids, flavonoids, alkaloids, and γ-aminobutyric acid) with antioxidant and anti-inflammatory function. Major antioxidant compounds such as chlorogenic acid, isoquercitrin, and astragalin are also present (Hassan *et al*., 2020). They have also been proven to own pharmacological and biological properties which include antibacterial, antiviral, antitussive, hypoglycemic, hypotensive, antiatherogenic, diuretic and antioxidant (Rebai *et al*., 2017).

Mulberries are fast-growing when young, but become slow-growing and rarely exceed 10–15 m tall. The leaves are alternately arranged, simple, and often lobed and serrated on the margin. Lobes are more common on juvenile shoots than on mature trees (Tuigong *et al*., 2015). Mulberry can be established through stakes or seeds. Yields depend on variety and location. A quarter an acre can accommodate 3556 plants with a spacing of 5 x 2ft. The plantation is raised and in block formation with a spacing of 1.8 by 1.8 m, or 2.4 by 2.4 m, as plant-to-plant and row-to-row distances. The plants are usually pruned once a year to a height of 1.5–1.8 m and allowed to grow with a maximum of 8–10 shoots at the crown (Sánchez, 2002).

Mulberry leaves are commonly used as feed for silkworm (Saddul *et al*., 2004). The leaves are highly palatable and digestible for herbivores and also monogastric animals. There are large variations in leaf production and quality in terms of protein content among the varieties (Sánchez, 2002). The leaves are harvested three or four times a year by leaf-picking method or cutting whole branches or stems. Harvesting should be done in the mid-morning and stored in the leaf chamber. The leaves should be covered to preserve freshness. Leaf production depends on the variety, location and agronomic practices. The yield of harvested fresh leaves is about 40 tonnes/ha/year, which is approximately 10 tonnes of dry matter (Sánchez, 2002). In Kenya, a mulberry tree on attaining maturity in the 3rd year, it should yield 2kg per tree, giving a total yield of 20 tons/ha/season of leaf. Depending on the rainfall pattern, 3-5 crops can be realized in a year.

2.9.1 Varieties of mulberry

There are about 68 species of the genus morus in the world, majority of which occur in Asia. In Kenya, the dominant species is *Morus alba*. Some varieties do well under rain fed conditions while others require irrigation (Tuigong *et al*., 2015). Leaf production and leaf quality varies in terms of protein content among the numerous varieties. The varieties and cultivars also vary due to being grown at different locations under a wide range of soil and environmental conditions. There are various varieties in Kenya which incorporates the following:

Morus alba - *Ex-Embu* variety is characterized by short internodes; purplish coloured bark prominent at the shoot tips. The variety has many small leaves and is drought resistant. This variety is more susceptible to leaf spot than other varieties but can be controlled by timely harvesting of leaves.

Morus alba - *Ex-Thika* is characterized by large light green slightly drooping leaves, has long internodes and whitish bark. It is fairly drought tolerant. The young shoot is weak and may need support to avoid falling or bending.

Morus alba - *Ex Limuru* is characterized by small finger shaped deeply serrated leaves, very thin shoots with short internodes. It is a high berry producer and is thus not recommended for silkworm rearing due to low leaf harvest but recommended for berry production.

Morus alba *Ex-Ithanga* is characterized by medium heart shaped and smooth light green leaves. It may sometimes produce a few lobed leaves. Roots easily and is fairly drought tolerant. It is suitable for both silkworm rearing and berry production (Wangari *et al*., 2013).

2.9.2 Nutritive value of mulberry leaves

Mulberry leaves contain significant levels of protein with good amino acid profile, carbohydrates, fats, minerals, fibers, metabolizable energy and vitamins such as - carotene and ascorbic acid (Sánchez, 2002). Fibre fractions are lower in mulberry leaves as compared to other forages (Sánchez, 2002). According to Ustundag and Ozdogan (2015) mulberry leaves supplementation up to 10% does not affect the productive performance and egg quality. Also, mulberry leaves decrease yolk cholesterol and increase pigmentation of egg yolk. Table 2.4 shows the nutrient composition of fresh and dry mulberry leaves.

Table 2.4 *Nutrient Composition of Mulberry Leaves*

Nutrient	**Composition**	
	Fresh mulberry leaves	**Dry mulberry leaves**
Moisture %	71-75	5.11-10.75
Crude protein %	5-10	15.31-35.0
Crude fat %	0.64-1.5	2.09 – 6.90
Total ash %	4.5	8.91 – 11.81
Crude fibre %	9.9 – 13.85	9.9 – 13.85
Carbohydrates %	8-13	9.70 – 39.70
Energy Kcal/100g	69-86	113-224
Acid detergent fibre (ADF)%	18.3-18.7	17.33-28.00
Neutral detergent fibre (NDF) %	8-11	19.38 – 35.77
Acid detergent lignin (ADL) %	3.4 – 8.10	17.33 – 28.00
Hemicellulose %	2.5 – 12.80	2.5 – 12.80
Ascorbic acid, mg/100g	160-280	100 – 200
β-carotene, mg/100g	10.000-14.688	8.44-13.13
Iron mg/100g	4.70-1040	19.00-35.72
Zinc mg/100g	0.22-1.12	0.72-3.65
Calcium mg/100g	380-786	786-2226
Magnesium mg/100g	533.24	720
Anti-nutritive factors		
Oxalates mg/100g	59.44-113.05	183
Phytates mg/100g	0.20-1.05	156
Tannic acid %	0.04-0.08	0.13-0.36

Source: Srivastava *et al*. (2006), Ustundag and Ozdoga (2015)

2.9.3 Use of mulberry leaves in poultry nutrition

Digestibility of mulberry leaves is high in ruminants but low in poultry due to high crude fibre content. Despite poor utilization of fibre, crude protein and ether extract are highly digested by poultry (Al-Kirshi *et al*., 2013). Poultry raised from mulberry gardens showed an increase in Vitamin K1 in the yolk (Machii, 2002). Inclusion of mulberry leaves reduces odour in the manure due to their ability to inhibit ammonium emission. Mulberry leaves have been reported to have many biological benefits, such as antioxidants, antimicrobial, antifungal, anti-allergic and hypoglycemic activities (Hajati & Ahmadian, 2014).

According to a study done by Andallu *et al*. (2014) mulberry leaves exhibited antioxidant properties postulated to be as a result of the synergistic action of free radical scavenging compounds such as carotenoids, flavonoids, moracins and others present in the leaves. Mulberry leaves improve FCR and egg mass due to antioxidant activities of mulberry leaf meal, which provides healthy uterine and ovarian environments (Lin *et al*., 2017). This also indicates that there is a beneficial effect during oviposition and enhanced conversion of digested food into eggs. They have been reported to significantly increase yolk weight, shell weight, shell strength, shell thickness, Haugh unit, and yolk color (Lokaewmanee *et al*., (2009).

Mulberry has an expansive range of secondary metabolites in its edible biomass (Al-Kirshi *et al*., 2013). Due to co-evolution with herbivorous organism, some of these compounds have arisen. Others are synthesized in certain physiological stages of the plant in the regulation of the metabolic processes as defence mechanisms against pests and diseases and as reserves of specific organic chains (Has et al 2013). Mulberry has an anti-nutritive compound called 1-deoxy nojirimycin (DNJ). Energy source absorption can be affected by this anti-nutritive compound, therefore resulting to prevention of polysaccharide hydrolysis and decreasing metabolic energy. DNJ from mulberry can block α-glycosidase activity which hydrolyzes polysaccharide into plain molecule (Has *et al*., 2013).

In poultry, mulberry leaves dry matter digestibility is poor as a result of high neutral detergent fiber (NDF) content (Ustundag & Ozdoga 2015). The leaves contain high crude fiber and deoxynojirimycin. In human, deoxynojirimycin becomes active compound for diabetes, but in poultry feed serve as anti-nutrition preventing carbohydrate digestibility; therefore its limited use as feed. In mulberry leaves the ratio of calcium to phosphorous unbalanced 10:1. Providing high levels of MLM in the diet will require supplementation of P due to the low content of phosphorous.

2.9.4 Effect of feeding mulberry leaf meal on egg cholesterol content

There are two main forms of lipoproteins that carry cholesterol to and from body cells in the blood in human beings, low density lipoprotein (LDL) and high-density lipoprotein (HDL). Low density lipoprotein cholesterol is often referred to as "bad cholesterol" because too much is unhealthy since it causes buildup of cholesterol in the arteries hence risk of heart attack (Berger *et al.,* 2015). High density lipoprotein is often referred to as “good cholesterol” because it protects the body against narrowing of blood vessels.

Blood cholesterol is measured in millimoles per litre (mmol/L) of blood. Total cholesterol levels should be 5mmol/L or less for healthy adults and 4mmol/L or less for those at high risk. Low density lipoprotein levels should be 3mmol/L or less for healthy adults and 2mmol/L or less for those at high risk. An ideal level of HDL is above 1mmol/L. A lower level of HDL can increase risk of heart disease (Berger *et al*., 2015).

Cholesterol deposition in the egg yolk can be affected by nutrition (Faitarone *et al*., 2013). In laying hens, it is biosynthesized in the liver and secreted into the plasma in the form of very low-density lipoproteins (VLDL) which transfers to the ovary and form high cholesterol containing yolk (Kamruzzaman *et al*., 2014). Egg-yolk cholesterol has been shown to vary with species of bird, breed or strain as well as age of fowl. Egg-yolk cholesterol contents can be altered by genetic selection and diet alteration. Mulberry leaf also contains phytosterols which are structurally similar to cholesterol that act in the intestine to lower cholesterol absorption and helps in reduction of cholesterol in the blood vessels (Panja, 2013).

The liver plays a major role in the regulation of deposition of the lipids and phospholipids in the egg yolk. The liver and serum cholesterol are reduced through the supplementation of the Mulberry leaves which leads to a reduction in the egg yolk cholesterol. Fibre binds bile acids in the intestines and causes more acid to be excreted in the faeces. This reduces the amount of bile acids returning to the liver and forces the liver to produce more bile acids to replace those lost in the faeces. In order to produce more bile acids, the liver converts more cholesterol into bile acids (Mayes & Botham, 2003) which lowers egg yolk and plasma cholesterol levels.

Mulberry leaves have phytosterol which is associated with lower cholesterol absorption by the liver in egg yolk when the yolk is being synthesized (Islam *et al.*, 2014). This could be the cause of reduction of cholesterol in the egg yolk (Shahryar *et al.*, 2010). Mulberry leaves inhibit the oxidation of LDL-cholesterol (Panja, 2013). Free radicals cause oxidation, a type of chemical destabilization of molecules such as LDL cholesterol. The oxidized LDL becomes more reactive with the surrounding tissues, which can produce inflammation that leads to disease and organ damage. Once LDL becomes oxidized, it inhabits the endothelium of the arteries in the body, such as the carotid arteries, the coronary arteries, or the arteries that supply legs and arms with blood. The inflammation in the arteries produced by oxidized LDL is dangerous since these blood vessels carry blood to all of organs and tissues. Mulberry leaves decrease lipid peroxide content in the yolk (Machii, 2002). The average cholesterol intake in humans from one egg is 217 mg, but it may vary between 153 to 264 mg (Vorlova *et al.*, 2001).

2.10 Egg quality

Egg quality is a vital parameter and plays a major role on the income gained from the egg production. Most important characteristics in egg quality are the shell quality, nutritional composition, egg size, and egg shell integrity (Gerber, 2006). The main egg quality aspects considered by egg producers are egg weight and eggshell quality, whereas consumers are interested in shelf life, external appearance, and sensorial qualities, such as eggshell and yolk color. On the other hand, processors take into account easy eggshell removal and separation of the yolk from the albumen, as well as egg functional properties (Faitarone *et al.*, 2016). Poor egg shell quality is a huge hidden cost to the egg producer since the eggs break easily before reaching the market and hence lead to losses. Most egg producers often accredit their egg loss to poor shell quality and shell strength causing breakages during transportation. Egg contains approximately 76% water, 12% protein, 10% lipids and the rest vitamins, minerals and carbohydrates but composition varies according to the diet (Naber, 1979). It is comprised of 32-35% yolk, 52-58% albumen and 9-14% shell.

Eggs are a major source of human dietary protein with high biological value and excellent protein efficiency ratio. They are rich in these essential amino acids and high in lutein which lowers the risk of cataracts and macular degeneration. Egg quality is a product of feed composition and the way in which the birds are fed. The contents of egg components maybe changed by the diet, and the inclusion of specific ingredients in layer feeds have been used to change the yolk lipid profile and to improve yolk quality (Faitarone *et al.*, 2016). On the other hand, their egg shell percentage in comparison to the egg size reduces as the chicken gets older. The eggs become bigger but their egg shell percentage reduces while remaining strong. The total amount of calcium which is exported through the egg increases as the poultry ages. The poor calcification of egg shell in old eggs appears to be due to some dysfunction of the shell gland, associated with reduced synthesis of 1,25-dihydroxycholecalciferol by the kidney (Joyner *et al*., 1987), therefore higher calcium requirement as the chicken ages. Calcium deficiency is associated with a reduction in eggshell weight and strength (King'ori, 2012).

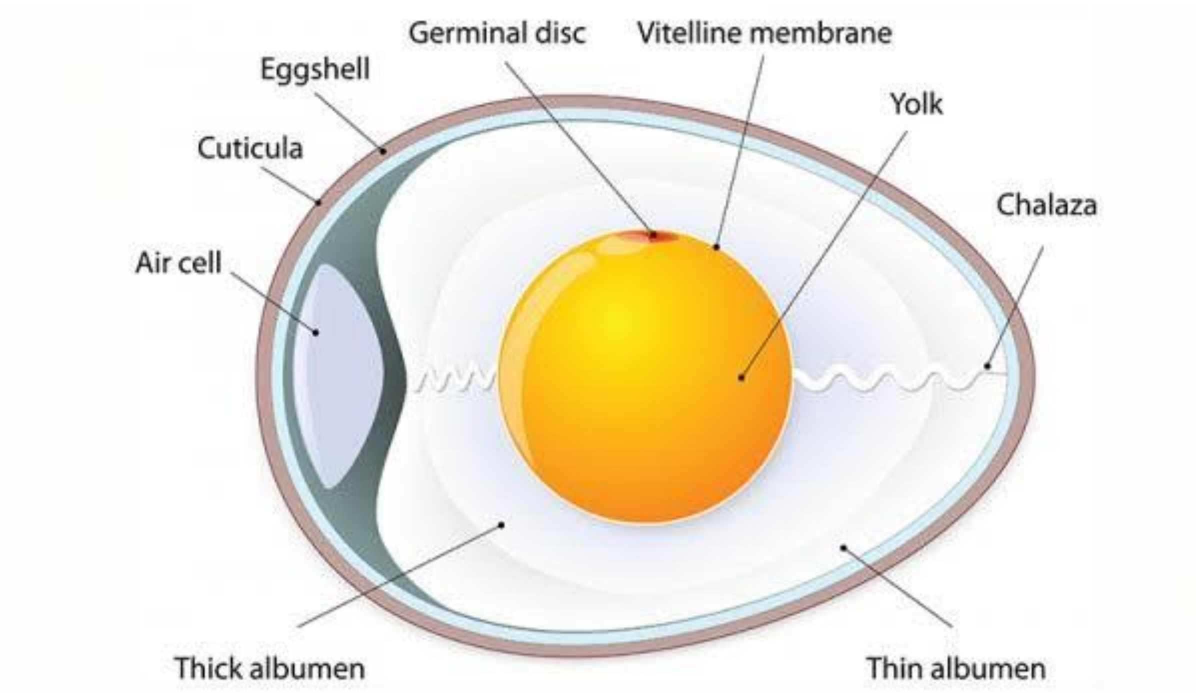

Figure 2. Anatomy of the egg

Source: Tamiru *et al*. (2019)

2.10.1 Albumen

Egg albumen consists chiefly of proteins, including ovalbumin, ovotransferrin, ovomucoid, ovoglobulin, lysozyme and ovomucin. The various roles of these proteins include inhibition of protein break down, and maintenance of viscosity of the thick albumen. The function of the albumen is to protect the embryo from attack by microorganisms and also to serve as a source of water, protein and minerals for the embryo (Ahmadi & Rahimi, 2011). There are four distinct layers of albumen that surround the yolk. The innermost layer is known as the chalaziferous layer (attached to the yolk) comprising 2.7% of the total albumen, followed by the inner thin layer (16% total albumen), the middle thick layer (50% total albumen) and the outer thin layer (25% total albumen). The majority of the modifications to the albumen occur during the first 6-8 hours (2-3 hours in modern layers), after which the developing egg enters the shell gland and undergoes a process called plumping. During this process a uterine secretion, which is mostly water with some minerals such as sodium, calcium and potassium, is pumped into the egg (Karsten *et al.,* 2010).

Although the albumen represents the largest proportion (approximately 58.5%) of the whole egg, it also includes the highest proportion (approximately 88.5%) of water. While much attention has been paid to the egg yolk in terms of chemical composition, the most important characteristic of albumen is largely physical. This is because the physical form of the albumen is largely the chemical composition of the entire egg, once it has been removed from the egg shell. Normal albumin is transparent, with a slightly yellow green colour. Discoloration of the albumin may occur if the eggs are stored for an extended time period in poor conditions, with the albumin becoming much yellower (Beyer, 2005).

Since 1937 when Haugh first described the Haugh Unit, it has become almost the universal method of measuring the height of the albumen (Wells & Belyavin, 1987). The Haugh unit indicates egg quality. The height of the thick albumen surrounding the yolk, combined with the egg weight, determines the Haugh unit score (Ahmadi & Rahimi, 2011). The higher the unit score (72-100), the better the egg quality. The Haugh unit score declines rapidly with the passage of storage time; therefore, it can sensitively quantify the egg deterioration at the early stage after being laid. However, the yolk index score declines slowly with storage period, enabling the detection of quality differences even among degraded eggs (Tamiru *et al*., 2019). A minimum measurement in HU for eggs reaching the consumer is 60. Most eggs leaving the farm should be between 75 and 85 HU (Gerber, 2006).

Albumin consistency is influenced by: Age of the hen, HU will decrease with increasing bird age value, genetics whereby some strains of hens consistently produce eggs with thin albumin. Age and storage of the egg also influence albumin consistency (Gerber, 2006). As the egg ages and carbon dioxide (CO2) is lost through the shell, the contents of the egg become more alkaline, causing the albumin to become transparent and increasingly watery. Eggs stored at ambient temperatures and humidity lower than 70 % will lose 10 – 15 HU in a few days from point of lay. Diseases such as New Castle disease can cause a decrease in albumin consistency (Beyer, 2005).

2.10.2 The yolk

The yolk provides the lipids and proteins that are required for embryonic growth. It is comprised of 33% lipid, 17% protein, and small amounts of minerals, vitamins and carbohydrates and the lipid composition consists of various lipids and fatty acids, of which the triglycerides represent more than two third of the total lipid content (Ahmadi & Rahimi, 2011). Most of egg lipids are in the form of yolk lipoproteins (4 g of its average weight), with the lipids associated with the proteins vitelline and vitellinin. The lipid profile of the egg yolk is influenced by genetics, egg size, feed composition, and type of fat added to feed. The main yolk fatty acids are oleic acid (38%), palmitic acid (23%), and linoleic acid (16%). Chicken eggs contain 33.84% saturated fatty acids and 45.26% monounsaturated fatty acids, and 17.63% and 2.34% polyunsaturated fatty acids of the series omega 6 and omega 3 (Lin *et al*., 2017). Omega 3 and Omega 6 fatty acids gotten from natural sources such as fish are highly unsaturated and susceptible to peroxidation when there is excessive level of consumption with insufficient amount of antioxidants (Shahryar *et al*., 2010). The oxidative stability of shell eggs during storage and food processing is essential. During oxidation, several compounds may form and adversely influence the overall quality of eggs, including their flavour, taste, and nutritional value; moreover, oxidation can produce toxic compounds in eggs (Lin *et al*., 2017). Antioxidants, such as tocopherol, may be added to layer diets to protect fatty acids from oxidation and to enrich eggs with vitamin E (Faitarone *et al*., 2016).

Yolk quality is determined by the colour, texture, firmness and smell of the yolk. Carotenoids impart a yellow coloration to the egg yolk which is mostly preferred by consumers (Sajilata *et al*., 2008). The majority of this past research, however, involved the feeding of natural ingredients such as yellow corn, corn gluten meal, and alfalfa meal, etc., rich in xanthophylls or crude concentrates made from these natural sources. Carotenoids present in egg yolk depend partly on the carotenoids fed in the diet (Beardsworth & Hernandez, 2004). Yolk colour intensity is determined by the incorporation of xanthophylls present in the diet, particularly of lutein and zeaxanthin, and depends on the inclusion levels (Faitarone *et al*., 2016). The group, zeaxanthin and lutein, are present in far greater quantity than the group cryptoxanthin and carotene. Xanthophylls (class term, meaning hydroxyl carotenoids e.g., zeaxanthin and lutein) from green feed are deposited in the yolk to the extent of about 15% (Ahmadi & Rahimi, 2011). Pale yolks can result from any factor which alters or prevents the absorption of pigments from the diet or the deposition of these pigments in the yolk. These factors include: endo-parasites and any factor which inhibits liver function and coccidiosis (Gerber, 2006).

Measurement of egg yolk however is of considerable importance. Consumers have specific preferences for specific yolk colours, and this varies in different parts of the world. Yolk colour is extremely sensitive to the presence of carotenoids or their precursors in the diet (Sajilata *et al*., 2008), and must therefore be critically examined by those involved in the development of feeding programs. Most assessment of egg yolk colour has been made by means of visual comparisons with prepared standards. Optical evaluation of extracted pigments and light reflectance has also been used.

For many years Heiman-Carver Colour Rotor was used as industry standards. This consisted of 24 painted watch glasses ranging in colour from light creamy white through yellow and orange to orange red (Wells & Belyavin, 1987). The Roche Yolk Colour Fan (RYCF) is widely accepted throughout the food chain as the standard for measuring yolk colour on a routine and reliable basis. Each fan blade contains a colour that has been measured objectively and can thus be reproduced in the yolk. By using the fan to define the desired yolk colour and by formulating the hens' feed accordingly, the target yolk colour can not only be achieved but also reproduced consistently (Beardsworth & Hernandez, 2004).

2.10.3 Egg shell

Exterior egg quality is judged on the basis of texture, colour, shape, soundness and cleanliness according to USDA (2000) standards. The shell of each egg should be smooth, clean and free of cracks. The eggs should be uniform in colour, size and shape. The five major types of shell problems in the egg industry include: cracks due to excess pressure, cracks due to thin shells, body-checks, pimpled or toe holes and shell-less eggs. To maintain consistently good shell quality throughout the life of the hen, it is necessary to implement a total quality management programme throughout the egg production cycle (Tamiru *et al*., 2019).

The shell accounts for about 12 percent of the weight of a large egg. The eggshell consists predominantly of a matrix of interwoven protein fibres and calcium carbonate crystals, and the cuticle which covers the surface of the shell (Nakano *et al*., 2003). The matrix consists of the mamillary matrix and the spongy matrix. The mamillary matrix region is interconnected to the protein fibres of the outer shell membrane and calcite crystals are oriented randomly within each mamillary tip to form a cone (Ahmadi & Rahimi, 2011). The spongy matrix has fine fibres that run parallel to the shell surface and the crystals within have their long axes oriented toward the shell surface. Calcium carbonate, magnesium and phosphate, make up the composition of the eggshell. The water-insoluble cuticle consists of approximately 90% protein and forms a protective coating on the surface of the shell (Mann *et al*., 2007).

An eggshell's strength is naturally influenced by the vitamins and minerals in a hen's diet, especially vitamin D, calcium, phosphorus and manganese (Karsten *et al*., 2010). Selenium is important for cellular protection of the reproductive tract, copper is used for egg shell development, pigment and collagen formation. Manganese is important for the eggshell membrane and helps with the formation of bone and organic matrix of the shells. Zinc is used for bone and eggshell calcification, keratin shell membrane formation and eggshell carbonate production (Nakano *et al*., 2003).

Shell strength is also influenced by a hen's age, older hens lay larger eggs with thinner, weaker shells. It also gets strength from its shape as well as from its composition. The curved surface is designed to distribute pressure evenly, provided the pressure is applied at the ends of the egg, not at the middle (Nakano *et al*., 2003). The middle of a shell must be weak enough to allow an emerging chick to peck all around and break out of an incubated egg. By contrast the ends of an egg must be quite strong so a newly laid egg won't crack when it plops into a nest, blunt end down (Ahmadi & Rahimi, 2011).

Shell quality defects include the following:

Pale shelled eggs: caused by high stress, old age or infectious bronchitis.

Shell-less egg: caused by immature shell gland, avian influenza, infectious bronchitis, or deficiency in calcium, phosphorus, manganese, or vitamin D3.

Soft shell eggs: caused by excessive phosphorus consumption, heat stress, old age, saline water or mycotoxins

Corrugated eggs: caused by heat stress, saline water, old age, deficiency in calcium and vitamin D3 or mycotoxins.

White or brown speckled egg: caused by defective shell gland, disturbances during calcification or excess calcium in the diet.

Wrinkled egg: caused by stress, infectious bronchitis, defective shell gland or overcrowding.

Pimpled egg: caused by bird age, strain of bird or inadequate nutrition.

White banded egg: caused by stress or changes in lighting.

Calcium coated eggs: caused by defective shell gland, disturbances during calcification or excess calcium in the diet (Ahmadi & Rahimi, 2011).

2.10.4 Factors affecting egg quality

Several factors affect egg quality; both internal and external qualities, namely:

Nutrition and feed additives.

The provision of adequate dietary minerals and vitamins is essential for good eggshell quality. The role of drinking water in mineral and trace element supply should not be overlooked (Gerber, 2006). Egg quality mainly depends upon Calcium, Phosphorus and Vitamin D3 and their interaction. Calcium required is 4-6 times higher than that of non-laying hen during laying period. Intestine absorption of Ca is about 40% when the shell gland is inactive, but reaches up to 75% during active period. Feeding of calcium levels above the requirement of the bird for production has not been shown to improve shell quality. Indeed, feeding hens high levels of calcium may interfere with the availability of other minerals (NRC, 1994) and can have a negative impact on the ability of the bird to utilize calcium, particularly if the levels in the diet are subsequently decreased (Ahmadi & Rahimi, 2011; Beyer, 2005).

High levels of phosphorus inhibit the Ca homeostasis. The required ratio of calcium: phosphorus in feed will be about 12.12:1. Vitamin D3 is essential for Ca absorption. Rations having less than 15% proteins give smaller size egg (Beyer, 2005). High phytate, chlorine, low dietary cationic-anaionic balance, nonstarch polysaccharides feed can reduce the quality of egg shell. Vitamin K plays an important role in blood clotting. Vitamin K deficiency can result in an increased occurrence of blood spots. Vanadium (minerals) when fed to the poultry as little as 6ppm it may reduce the albumin quality. Gossypol from cotton seed meal causes mottling yolks (Beyer, 2005). Vitamin D also plays an important role in the proper utilization of calcium and phosphorous and sufficient amounts of this vitamin should be included in the feed (Gerber, 2006).

Age and genetics.

Older birds tend to lay bigger eggs and have a higher egg output, which impacts on shell strength. Young birds with immature shell glands may produce shell-less eggs or eggs with very thin shells. During old age, hens cannot mobilize Ca efficiently from bone hence shell quality decreases, it may decrease up to 50%. Intensity of pigmentation decreases as age increases (Gerber, 2006). Albumen quality of fresh eggs is also affected by the age of the hen. As the hen becomes older, albumen quality decreases (Tamiru *et al*., 2019).

Strain of hen has also been shown to play a role in albumin consistency, with some strains consistently producing eggs with thin albumin. High producing hens tend to lay eggs with relatively lower amounts of thick albumin (Gerber, 2006). Although this can be influenced by selective breeding whereby some strain of hens can deposit Ca at faster rate than others

Stress.

Stress or disturbance to a flock of laying hens is enough to de-synchronise the process of egg formation for several days, during which time, a number of different egg quality faults may be seen. It may be due to heat, over stocking density, loud noise, poor transportation, rough handling and fear. Secretion of cell lying the oviduct or uterus becomes acidic and the cell can be damaged or destroyed. Epinephrine, a stress hormone, will cause a delay in oviposition and cessation of shell gland cuticle formation, which can cause pale shelled eggs (Gerber, 2006).

Temperature and light.

High temperatures above 25^{o}C may affect the feed and therefore calcium intake of the bird, thus resulting in a decreased availability of calcium for shell deposition (Gerber, 2006). High temperature causes pH to become alkaline and Ca availability decreases. Laying hens overcome heat stress by panting. However, this causes a decrease in the amount of carbon dioxide (CO_2) in the hens' blood, a condition known as respiratory alkalosis. As egg shells are made up of 95% calcium carbonate ($CaCO_3$), this decrease in blood CO_2 levels, combined with an increase in blood pH and a subsequent decrease in Ca^{2+} ions for shell formation leads to an increase in the number of thin or soft-shelled eggs produced (Beyer, 2005). Feed intake is reduced with increase in temperature.

Poultry are long-day breeders and they need at least 12 hours light a day for egg production. Below that there is no activation of the hypothalamus and pineal gland and this affects other activities like vision and feed intake capacity. That will stop the production of eggs (Gerber, 2006).

Diseases.

There are many diseases responsible for production of faulty eggs or no production of egg. Some of these diseases that are associated with poor quality egg are: Viral diseases, e.g. Newcastle disease, bacterial diseases, e.g. Colibacillosis, Fungal mycotoxin and parasites (Beyer, 2005). According to Gerber (2006), Infectious bronchitis (IB), a viral disease caused by a coronavirus which attacks the mucus membranes of the respiratory and reproductive tracts may result in egg defects. These include pale shelled eggs, and eggs with poor shell structure and integrity. Similarly, birds affected by egg drop syndrome (EDS), caused by an adenovirus, initially produce pale eggs, quickly followed by thin soft-shelled or shell-less eggs (Tamiru *et al*., 2019). Prolonged use of sulphur drugs inhibits the activity of carbonic anhydrase (CA) enzyme which is responsible for calcium carbonate deposition on shell. Nicarbazin drug in the feed cause mottling of yolks (Beyer, 2005).

Housing systems and management practices.

The type of production system may influence egg shell quality. Eggs from hens in non-cage systems may result in contamination of shells with microorganisms since more eggs tend to be laid outside nest boxes and the interactions between active hens and bedding material increases dust in the atmosphere which is a carrier of microbes (Tamiru *et al*., 2019).

Good management practices ensure the health status of poultry and encourage them to produce quality egg. Lack of proper management makes poultry susceptible to injuries and diseases that reduce the quality of egg (Gerber, 2006). Some of the poor management practices that may damage the egg quality are; poor water quality, improper ventilation, overcrowding, changes in lighting program, biosecurity and methods of sterilization. Good management practices help in reducing the number of dirty eggs. These practices include frequent collection of eggs, regular replacement of litter material in nest boxes, regular maintenance and cleaning of cage floors and roll out trays (Tamiru *et al*., 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

3.1.1 Location

The study was conducted at the Poultry Research Unit, Tatton Agriculture Park (TAP) at Egerton University. The university is in Nakuru County, Njoro Sub-County (S 0˚22'11.0", E 35˚55'58.0), Kenya. It is 1,800 m above sea level with average temperatures between 17 - 22 ˚C but can drop to 11 ˚C during cold season. Average annual rainfall in the area is 1,200±100 mm (Egerton University Weather Station, 2018) in two seasons, short and long rains. The long rains start in March and end in May while the short rains start in October and end in December.

Figure 3. Map of Kenya showing Nakuru County

Source: Wikipedia. Retrieved on 2nd December 2020 from https://en.wikipedia.org/wiki/Nakuru_County#/media/File:Nakuru_County_in_Kenya.svg

3.2 Collection of mulberry leaves and preparation of experimental diets

Mulberry leaves were harvested by plucking whole branches from National Sericulture Research Centre (NSRC). The Centre is located in Gatanga Sub-County, Murang'a County, S 1˚0'9.894", E 37˚4'42.661". The branches were dried at room temperature in a drying chamber at constant moisture content and then the leaves were crushed to separate from the branches at NSRC and transported to Egerton University. They were ground to pass through 1mm screen. After milling, mulberry leaf meal (MLM) was stored in bags. Proximate analysis was conducted at Animal Science Nutrition laboratory following the procedures of AOAC (AOAC, 2006 Version 3).

Figure 4. Map of Kenya showing Murang'a County

Source: Wikipedia. Retrieved on 2nd December 2020 from https://en.wikipedia.org/wiki/Muranga_County#/media/File:muranga_County_in_Kenya.svg

3.3 Experimental diets

There were 4 experimental diets (Table 3.1).

Table 3.1 *Composition of Experimental Diets (Kg)*

Ingredients	T1	T2	T3	T4
Maize meal	66	65	62	60
Soybean meal	23	20	17	14
Fish meal	2	2	2	2
Mulberry leaf meal	0	5	10	15
DCP	2	2	2	2
Limestone	6	6	6	6
Iodized salt	0.5	0.5	0.5	0.5
Premix (layer)	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**
Calculated CP	16.9	16.7	16.5	16.3
Calculated ME	2836	2808	2777	2745
Calculated CF	2.4	2.6	2.9	3.2

Experimental diets were formulated to meet nutritional requirements of the layer chicken (NRC, 1994). The leaf meal was included at 0, 5, 10, and 15% to substitute soybean meal protein in diets formulated to contain 16% crude protein. The inclusion levels of MLM and maize meal were determined by the calculated crude protein and metabolizable energy. This was done to achieve a standard content of crude protein and metabolizable energy. A layer premix was added to supply minerals and vitamins.

3.4 Experimental chicken

Sixty, 29-week-old indigenous chicken were used in this experiment, randomly sampled from a population of 200 chickens. They were purchased from Mary Poultry Farm located in Rhonda ward in the western side of Nakuru town. The chickens were in good health and of uniform weight and had been vaccinated against Marek's disease, Gumboro, Fowl pox, Fowl typhoid and Newcastle Disease. Dewormers were administered to the chicken prior to the experiment. Physical indicators of a good layer were used to select the chickens that were transported to Tatton Agriculture Park (TAP), Egerton University. Care was taken when transporting the hens to minimize stress associated with overcrowding. The hens ware allowed one week of adaptation before the feeding trial commenced. They were randomly distributed to four treatments which were replicated thrice. The hens were housed into individual cages measuring 1m by 1m by 1m, each had five hens in a completely randomized design (CRD). The cages were equipped with drinkers and open feed trough. The hens were given 150g/chicken of feed. Left-over feed was weighed to determine intake. Each cage was the experimental unit. Biosecurity was maintained in Mary's farm and also during data collection period at Egerton University. Data collection was conducted for 8 weeks.

3.5 Feed analysis

Feed samples were analyzed for proximate composition following the procedures of AOAC (2006). Proximate analysis: Crude fiber (CF) was analyzed according to the AOAC, Method 978.10 Crude protein (CP) by Method 984.13, and Moisture by Method 934.01, 2006.

3.6 Experimental design

The completely randomized design (CRD) was used. Sixty, 29-week-old, laying indigenous chicken were used in this study. There were four treatments (Diet 1, 2, 3, and 4) which were replicated thrice. Each replicate had 5 hens and hence a total 15 hens per treatment.

3.7 Data analysis

Data was analyzed using GLM (SAS, 2009). Mean separation was done using the least significance difference (LSD) at 5% level.

The model was as follows:

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

where;

Y_{ij} = observation on the j^{th} egg of the i^{th} treatment

μ = overall population mean

α_i = effect due to the i^{th} treatment (Diet 1, 2, 3 and 4)

e_{ij} = random error associated with Y_{ij}

3.8 Objective one: To determine the effect of inclusion of mulberry leaf meal in indigenous chicken layer diets on performance

Data collection

Average daily feed intake, feed conversion ratio and egg production were determined as described below:

Feed intake per cage was determined by subtracting feed offered (g) from feed leftover (g) before the next feeding.

ADFI (average daily feed intake) = feed intake per cage (g)/number of birds in the cage for each day.

FCR (per kg egg mass) = g of feed consumed/ g of egg produced. A value of 2.2 or less is advantageous to the farm.

Hen day production = Total number of eggs produced in a day/ Total number of hens present on that day x 100.

3.9 Objective Two: To determine the effect of inclusion of mulberry leaf meal in indigenous chicken layer diets on external egg quality

Data collection

Eggs laid were collected twice daily at 9.00 hr. and at 18.00 hr., labeled per week, treatment(T) and day(D) of data collection (e.g., week 1:T1D1) and placed in egg trays. Five eggs per treatment were used to determine the following characteristics on a weekly basis.
External egg quality assessed included egg weight, shell thickness, shape index and egg shell ratio. Individual eggs were weighed on a digital balance to the nearest of 0.01 g accuracy (Şekeroğlu & Altuntaş, 2009). Shape index was calculated as the ratio of breadth: length, multiplied by 100. Egg height and width were measured with a digital vernier caliper ruler from the bottom (pointed end) to the top and diameter of the egg respectively. Shape index affects the appearance and those with unusual shapes do not fit well in egg trays and therefore, they are much more likely to be broken during the transportation than the eggs of normal shape (Altuntaş & Şekeroğlu, 2008). All the shells were wiped dry with a paper towel and weighed with a digital balance. The shell weight was divided by the egg weight to get the shell ratio and multiplied by 100. The thickness of four pieces of shells, one each from the two ends (broad and narrow end) and two from the body of the egg shell were measured with digital vernier caliper to the nearest of 0.01 mm and the measurements averaged.
Shell thickness: This was measured using a digital vernier caliper

Figure 5. Digital vernier caliper
Source: Disen Tools China

Egg weight: Eggs were weighed on a digital balance model number SF- 400 equipped with high precision strain gauge sensor.

3.10 Objective three: To determine the effect of inclusion of mulberry leaf meal in indigenous chicken layer diets on internal egg quality

Data collection

Measurement of internal egg quality

The internal quality assessed included yolk index, haugh unit, egg: yolk ratio, yolk colour and yolk: albumin ratio. The length and width of the albumen and yolk were measured (in mm) with a digital vernier caliper. The quality of the albumen was determined by the height of the albumen at a distance of one centimeter from the edge of the yolk. Yolk ratio was determined as follows: (yolk width/egg weight) X 100. Yolk-albumin ratio was calculated as follows: (yolk width/albumin width) X 100.

Yolk quality was determined by the colour of the yolk and strength of the peri-vitelline membrane. If the peri-vitelline membrane is weak, the yolk will break more easily. Yolk index was calculated as follows: (yolk height/yolk diameter) X 100.
Haugh unit score was calculated as follows:

$$H.U. = 100 \text{ LOG } [H - 1.7W^{0.37} + 7.6]$$

Where;
HU = Haugh units
H = Albumen height in mm
W = weight of whole egg in grams

Yolk colour was determined using Roche scale

Figure 6. Roche yolk colour fan

Source: Bovšková *et al*. (2014)

Determination of cholesterol

Cholesterol was determined using the procedure of AOAC, Official Method, 941.09 (AOAC, 1996).

CHAPTER FOUR

RESULTS

4.1 Chemical composition of mulberry leaves

The results of the chemical composition of mulberry leaves are shown in Table 4.1.

Table 4.1 ***Chemical Composition of Mulberry Leaf Meal, Soybean Meal And Fishmeal***

Nutrient	Mulberry	SBM	FM
CP (%)	23.9	44.0-48.0	60.0-70.0
CF (%)	19.8	4.9-11.2	0-1.9
Moisture (%)	9.9	8.0-9.0	8.0-10.0
Av. Ash (%)	11.6	5.8-10.0	16.8-18.0

CP= Crude protein, CF= Crude fibre

4.2 Experimental diets

The proximate analysis results are shown in Table 4.2.

Table 4.2 ***Analyzed Chemical Composition of the Diets***

Nutrients	Diets			
	T1	**T2**	**T3**	**T4**
CP (%)	16.03	16.34	16.60	16.91
CF (%)	4.97	5.46	7.83	9.38
Moisture (%)	10.36	10.58	10.82	10.44
Av. ash (%)	11.90	11.56	11.18	10.36

CP= Crude protein, CF= Crude fibre

4.3 Objective 1: Effects of inclusion of mulberry leaf meal in indigenous chicken layer diets on performance

Results of average daily feed intake, feed conversion ratio and egg production are shown in Table 4.3.

Table 4.3 ***Effects of Mulberry Leaf Meal on Performance***

Parameters	T1	T2	T3	T4	SEM	*p* Value
ADFI /hen (g)	114.13^{a}	135.87^{b}	144.39^{c}	145.03^{c}	1.14	0.0001
FCR	10.90^{b}	6.89^{a}	6.55^{a}	6.87^{a}	0.62	0.0001
Hen day production (%)	27.98^{a}	42.74^{b}	40.59^{b}	39.27^{b}	1.83	0.0001

FCR= Feed conversion ratio ADFI= Average daily feed intake, ^{a,b,c} Means in the same row with different superscripts differ ($p < 0.05$)

Average daily feed intake

Treatments had a significant effect ($p < 0.05$) on average daily feed intake per hen from T1 to T2. Treatment 4 recorded the highest average daily feed intake 11per hen compared to T2 and T1. However, ADFI did not differ significantly between T3 and T4.

Feed conversion ratio

Feed conversion ratio of T1 differed significantly with that of T2, T3, and T4 ($p < 0.05$). However, FCR of T2, T3, and T4 did not differ.

Hen day production

Hen day production differed ($p < 0.05$) between T1 and T2, but was similar from T2 - T4.

4.4 Objective 2: Effects of inclusion of mulberry leaf meal in indigenous chicken layer diets on external egg qualities

The results for the effects of inclusion of MLM on external qualities of the egg are shown in Table 4.4.

Table 4.4 ***Effects of Mulberry Leaf Meal on External Egg Quality***

Parameters	T1	T2	T3	T4	SEM	*p* Value
Shape index (%)	76.05	76.14	74.66	74.06	0.63	0.0604
Shell thickness (mm)	0.34^{b}	0.33^{b}	0.32^{b}	0.29^{a}	0.01	0.0001
Egg: shell ratio (%)	12.96	13.01	16.15	12.90	0.99	0.4439
Shell weight	7.96	7.92	7.76	7.88	0.61	0.8833

^{a,b} Means in the same row with different superscripts differ ($p < 0.05$)

Treatments had no significant effect ($p > 0.05$) on shape index, egg shell ratio and shell weight. Shell thickness was similar in T1 to T3 but deferred ($p < 0.05$) in T4.

4.5 Objective 3: Effect of inclusion of mulberry leaf meal in indigenous chicken layer diets on internal egg qualities

Table 4.5 shows the effect of inclusion of MLM on internal egg quality.

Table 4.5 ***Effects of Mulberry Leaf Meal on Internal Egg Quality***

Parameters	T1	T2	T3	T4	SEM	*P* value
Yolk index (%)	32.16	34.04	35.34	30.84	1.79	0.3093
Haugh unit	76.96	76.97	75.5	78.90	1.11	0.2035
Egg: yolk ratio (%)	27.35	31.20	29.65	28.42	1.45	0.2871
Yolk colour	1.57^{a}	2.95^{b}	5.40^{c}	7.13^{d}	0.20	0.0001
Yolk: albumin ratio (%)	49.82	57.15	53.74	51.75	2.77	0.2971

^{a,b,c,d} Means in the same row with different superscripts differ ($p < 0.05$)

Treatments had no significant effect ($p > 0.05$) on yolk index, haugh unit, yolk ratio and albumin ratio.

Yolk colour

Treatments had a significant effect ($p < 0.05$) on yolk colour. The yolk colour increased significantly from T1 - T4.

Yolk cholesterol content

Table 4.6 shows the effects of incorporation of MLM on cholesterol content.

Table 4.6 ***Effects of Mulberry Leaf Meal on Egg Cholesterol Content***

Parameters	T1	T2	T3	T4	SEM	*p* value
Cholesterol (mg/100g)	218.6	248.0	267.3	252.7	20.67	0.4112

Treatments had no significant effect ($p > 0.05$) on cholesterol content.

CHAPTER FIVE

DISCUSSION

5.1 General discussion

The indigenous chicken population continues to increase with time thereby leading to the need for feed ingredients that are more available and of less competition with human beings. The overall goal of this study was to contribute to sustainable egg production and quality through improved nutrition by use of mulberry leaf meal (MLM) in layer diet. Increasing the percentage of mulberry leaf meal in the diet as replacement for soybean meal led to an increase in the proportion of crude fiber in the diet which, on the basis of most nutritional studies with poultry (De Vries, 2015), was expected to lead to depressed egg production. The fact that egg production increased in the early stage of MLM substitution for soybean implied that, despite the increase in percentage of fiber in the diet, the birds produced more eggs.

5.2 Effects of inclusion of mulberry leaf meal in indigenous chicken layer diets on performance

Treatment 3 (10% MLM) and 4 (15% MLM) had the highest feed intake, probably due to the hens trying to meet their nutritional requirements due to depressed feed digestibility as a result of the high fibre content. High fibre content in the diet increase viscosity of the intestinal content, resulting in a decrease in the bioavailability of vitamin A and utilization of dietary fats, hence nutrient availability is compromised (De Vries, 2015). Viscosity of ingesta reduces the passage rate of the feed, slows down digestion processes and encapsulates nutrients. This makes them inaccessible to digestive enzymes hence the high feed intake in order for the hens to meet nutritional requirements. Proteins and carbohydrates contained in diets high in CF are not effectively digested due to interfered digesta mixing and reduced digestibility and absorption of nutrients in the GIT (Jiménez-Moreno *et al*., 2013). In this study however, T3 and T4 had a high feed intake which could have been due to the ability of IC to utilize feeds with high fibre content. The results agree with the findings of Kondra *et al*. (1974) who reported that that egg and meat chicken are capable of enlarging the length and weight of the digestive system during the growing period, in accordance with the increased fibre content so that required nutrients may be obtained.

Feed conversion ratio decreased with increase of MLM up to 5% inclusion in diet but was similar from 5-15% inclusion. Egg production increased with inclusion of MLM. Increasing the MLM level also decreased FCR. The significant decrease in the FCR and the increase in laying percent suggested that the MLM provided available nutrients for egg production.

These results are in agreement with the findings of Lin *et al*. (2017) who reported that feeding mulberry leaves improved FCR and egg mass due to the antioxidant activities of MLM, which provided healthy uterine and ovarian environments. Mulberry leaf meal contains components such as ∞-tocopherol, ascorbic acid, β-carotene, and glutathione with antioxidant abilities (Roy *et al*., 2010). According to a study done by Iqbal *et al*. (2012) *Morus alba* leaves were found to have high antioxidant capacity in terms of its ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) state. Iron has a very specific function as a component of the protein heme found in the red blood cell's protein hemoglobin and in the muscle cell's protein myoglobin. Iron has a rapid turnover rate in the chicken – 10 times per day. Morck & Austic (1981) study proved that iron supplementation to 55ppm increased hatchability. In this study egg production increased and FCR reduced probably due to high antioxidant capacity of MLM in terms of its ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) state.

5.3 Effects of inclusion of mulberry leaf meal in indigenous chicken layer diets on external egg qualities

Shell thickness decreased in T4 (15% MLM). This could be due to high fibre content in the diet (9.38%) which led to depressed nutrient (minerals) digestibility. Hens require a crude fibre content of below 7%. High fibre content increases viscosity of the intestinal content and decreases utilization of nutrients (De Vries, 2015). The results are in agreement with studies conducted by Al-Kirshi *et al*. (2010) which showed that shell weight decreased with increase of MLM in groups fed 15 and 20% due to insufficient Ca intake in the groups.

Shell thickness contributes to shell strength. According to Yan *et al*. (2013) thicker shells do not guarantee stronger egg. Increased shell strength means more mineral consumption from feed. In their study, eggs with thin but uniform shells were stronger than those with thick but less uniform shells. There is a positive correlation between eggshell strength and egg shape index. Shape index and shell thickness affect the risk of cracked eggs. Larger and rounder eggshells have the higher resistance to breaking forces (Ketta & Tůmová, 2018). In this study however, there was no significant difference in shape index.

5.4 Effects of inclusion of mulberry leaf meal in indigenous chicken layer diets on internal egg qualities

Egg yellow yolk colour increased intensity as the level of MLM in the diet increased. The results agree with a study by Teguia (2000) which reported that hens fed on forages had darker yellow colour than those fed a diet without forages due to supply of xanthophyll.

Some xanthophylls are precursors of Vitamin A (Oke *et al*., 2014) which is of nutritional benefit to consumers. Vitamin A is fat-soluble and is good for healthy vision, skin, bones and other tissues such as soft tissue and mucus membrane in the human body. It often works as an antioxidant, fighting cell damage hence healthy uterine and ovarian environments. Therefore, the increase in yolk colour is an indication of an improvement in internal egg quality.

The egg yolk colour (deep yellow) is an indicator of a well-balanced, healthy chicken diet and an egg with excellent nutritional value rich in beta carotene, higher levels of Vitamins A, E, and beneficial fatty acids (Karsten *et al*., 2010). Consumers prefer the dark yellow colour as indicated by Ayim-Akonor and Akonor (2014) and Lokaewmanee *et al*. (2009). According to Senbeta *et al.* (2015) most consumers desire yellow colored egg yolks and believe that they are more delicious, have a high nutritional value and are attractive from a visual perspective. The inclusion of MLM to the diets resulted in a higher intensity of yellowness of the yolk. This observation is in agreement with the results of Lokaewmanee *et al*. (2009) who observed that the yolks from eggs laid by hens fed on mulberry leaves had a deeper yellow yolk colour. The significant difference observed in the results of egg yolk colour indicated that the MLM inclusion improved the yolk quality due to supply of xanthophyll. The MLM based diets showed a high yolk quality than those in control group.

Mulberry leaf meal supplementation did not affect Haugh unit. This observation is in agreement with the results of Lokaewmanee *et al*. (2009) who observed that there was no significant difference in HU after feeding mulberry leaves. The HU levels were within the normal range of between 75 and 85 HU which is an indication of freshness in eggs, i.e., ability of albumen to remain viscous (Gerber, 2006). Haugh unit score is determined by the height of the thick albumen surrounding the yolk combined with the egg weight. Supplementation of hens' diets with a natural source of antioxidant significantly improves egg HU by slowing down or stopping lipid peroxidation and preserve product freshness (Al-Harthi, 2014). The eggs produced by hens supplemented with mulberry leaves appear fresh at 21 days of storage (Lin *et al*., 2017). The yolk index values fell within the normal range of 0.33-0.50. The yolk index is an indicator of the spherical nature of the egg yolk, which can be used to reflect freshness (Torrico *et al*., 2014). During the course of storage of an egg, the yolk index decreases as a result of a progressive weakening of vitelline membranes due to yolk absorbing water from albumen, reduction of the total solids and a progressive transition of egg yolk properties.

The results of cholesterol content are in agreement with the findings of Machii (2002) who reported that there was no significant difference in cholesterol content. According to Faitarone *et al*. (2013), manipulating diets to reduce cholesterol is not effective since chickens are able to maintain the egg cholesterol levels that are considered essential for egg composition and ensure embryo development. Mulberry leaves contain phytosterols (β-sitosterol, campesterol, stigmasterol, isofucosterol) which act in the intestine to lower cholesterol absorption and helps in reduction (Panja, 2013). In cases of incorrect energy balance, excessive energy intake beyond maintenance and production requirements increases body weight and cholesterol synthesis and therefore, excess cholesterol is transferred to the egg yolk (Faitarone *et al*., 2013). In this study, cholesterol content levels were within the normal range of between 153 to 264 mg (Vorlova *et al*., 2001).

The results showed that the amount of MLM that can be incorporated in the diet of IC layers is up to 10%. This will not negatively affect the egg production, yolk colour, FCR and cholesterol level, but will reduce shell thickness slightly. This implies that MLM can be used as a source of β-carotene and protein. These results agree with the findings of Al-Kirshi *et al*. (2010) who reported that there was both negative and positive impact in internal and external egg qualities with MLM inclusion above 10%.

It is therefore possible to increase egg production and quality through incorporating MLM in chicken feed as a protein source. Mulberry leaves meal is therefore a locally available alternative protein source to supplement the commonly used sources such as soybean and fish meal used in the poultry industry. This will help to lower feed costs without having a negative impact on egg production and quality. It will also create employment in the mulberry production value chain and diversify sources of farm incomes.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Inclusion of MLM in IC layer diets up to 10% significantly increased feed intake egg production and decreased FCR

ii. Inclusion of MLM in IC layer diets had no significant effect on external (shape index, egg shell ratio and shell weight) but significantly decreased shell thickness.

iii. Inclusion of MLM in IC layer diets had no significant effect on internal egg quality (yolk index, yolk: albumin ratio) but significantly increased the yolk colour to deep yellow.

6.2 Recommendations

i. MLM can be included in IC layer diets up to 10% due to its beneficial effect of improving egg production and decreasing FCR.

ii. Feed manufacturers can include MLM in IC layer diets instead of using synthetic β-carotene to enhance egg yolk colour.

iii. Addition of calcium to improve shell strength for 10% or more MLM inclusion is recommended.

6.3 Areas for further research

i. Conduct a study to evaluate the effect of inclusion of different varieties of mulberry leaf meals in Kenya in IC layer diets on FI, egg production and quality.

ii. Conduct a study on the effect of incorporating a cellulolytic enzyme in MLM- based diets on FI, egg production and quality of IC layer chicken.

iii. Conduct a study to determine the economic benefit of MLM inclusion in IC layer diets.

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APPENDICES

Appendix A: National Commission for Science, Technology and Innovation Research Permit

REPUBLIC OF KENYA

NACOSTI

Ref No: **895341** Date of Issue: **10/October/2019**

RESEARCH LICENSE

This is to Certify that Miss LILIAN MWAI of Egerton University, has been licensed to conduct research in Nakuru on the topic: Evaluation of Mulberry leaf meal in indigenous chicken layer diet in egg production and quality for the period ending:

10/October/2020.

License No: **NACOSTI/P/19/1973**

895341

Applicant Identification Number

Director General

NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY & INNOVATION

Verification QR Code

Appendix B: Egerton University Research Ethic Permit

EGERTON **UNIVERSITY**

Tel: (051) 2217808

FAX: 051-2217942

P. O. BOX 536

EGERTON

EGERTON UNIVERSITY RESEARCH ETHICS COMMITTEE

EGERTON EU/RE/DVC/009

Approval No. EUREC/APP/102/2020 **11 September,2020**

Lilian Muthoni Mwai

Egerton University

P.O. Box 536-20115 Egerton

Telephone: 0721293290

E-mail: mulimwa@gmail.com

Dear Lilian,

RE: ETHICAL CLEARANCE APPROVAL;

EVALUATION OF INCLUSION OF MULBERRY LEAFMEAL ON INDIGENOUS CHICKEN LAYER DIET ON EGG PRODUCTION

This is to inform you that **Egerton University Research Ethics Committee** has reviewed and approved your above research proposal. Your application approval number is **EUREC/APP/102/2020**.The approval period is **11th September, 2020 –12th September, 2021**.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.

ii. You are required to adhere Institutional Experimental Animals use and Care policy.

iii. All changes including (amendments, deviations, and violations) are submitted for review and approval by Egerton University Research Ethics Committee.

iv. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to Egerton University Research Ethics Committee within 72 hours of notification

v. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to Egerton University Research Ethics Committee within 72 hours

vi. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.

vii. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

viii. Submission of an executive summary report within 90 days upon completion of the study to Egerton University Research Ethics Committee.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.keand also obtain other clearances needed.

Yours sincerely.

Prof. J. Kipkemboi.

11/09/2020

CHAIRMAN, EGERTON UNIVERSITY RESEARCH ETHICS CTTEE

JKK/BK/BK

Appendix C: Research pictorial

Mulberry tree

Morus alba Ex-Limuru

Morus alba Ex-Embu

Mulberry fruits

Experimental set up

Eggs arranged in trays

Contrast in yolk colour

ANOVA TABLES

Appendix D: Data analysis on feed intake, FCR and laying percent

Average daily feed intake

The SAS System 13:29 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 224

The SAS System 13:29 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	35020.59567	11673.53189	133.92	<.0001
Error	220	19176.49931	87.16591		
Corrected Total	223	54197.09498			

R-Square	Coeff Var	Root MSE	Yi Mean
0.646171	6.923128	9.336268	134.8562

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	35020.59567	11673.53189	133.92	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	35020.59567	11673.53189	133.92	<.0001

The SAS System 13:29 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison wise error rate, not the objective wise error rate.

Alpha	0.05
Error Degrees of Freedom	220
Error Mean Square	87.16591
Critical Value of t	1.97081
Least Significant Difference	3.4773

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	145.035	56	4
A			
A	144.393	56	3
B	135.872	56	2
C	114.125	56	1

Feed Intake

The SAS System 14:18 Saturday, October 2, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 224

The SAS System 14:18 Saturday, October 2, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	8890539.73	2963513.24	111.79	<.0001
Error	220	5832381.25	26510.82		
Corrected Total	223	14722920.98			

R-Square	Coeff Var	Root MSE	Yi Mean
0.603857	8.261865	162.8214	1970.759

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	8890539.732	2963513.244	111.79	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	8890539.732	2963513.244	111.79	<.0001

The SAS System 14:18 Saturday, October 2, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	220
Error Mean Square	26510.82
Critical Value of t	1.97081
Least Significant Difference	60.642

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	2165.89	56	3
A			
A	2163.30	56	4

B	1841.96	56	2
C	1711.88	56	1

Feed Conversion Ratio

The SAS System 13:43 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 224

The SAS System 13:43 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	709.602476	236.534159	8.92	<.0001
Error	220	5830.926854	26.504213		
Corrected Total	223	6540.529330			

R-Square	Coeff Var	Root MSE	Yi Mean
0.108493	65.77064	5.148224	7.827542

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	709.6024757	236.5341586	8.92	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	709.6024757	236.5341586	8.92	<.0001

The SAS System 13:43 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	220
Error Mean Square	26.50421
Critical Value of t	1.97081
Least Significant Difference	1.9174

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	10.8981	56	1
B	6.9843	56	2
B			
B	6.8740	56	4
B			
B	6.5538	56	3

Laying Percent

The SAS System 13:47 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 224

The SAS System 13:47 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7322.14006	2440.71335	12.81	<.0001
Error	220	41906.16050	190.48255		
Corrected Total	223	49228.30056			

R-Square	Coeff Var	Root MSE	Yi Mean
0.148738	36.66310	13.80154	37.64423

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	7322.140063	2440.713354	12.81	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	7322.140063	2440.713354	12.81	<.0001

The SAS System 13:47 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	220
Error Mean Square	190.4825
Critical Value of t	1.97081
Least Significant Difference	5.1403

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	42.737	56	2
A			
A	40.595	56	3

A			
A	39.269	56	4
B	27.976	56	1

Appendix E: Data analysis on external and internal egg qualities

External qualities

Shape index

The SAS System 15:00 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 160

The SAS System 15:00 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	128.027289	42.675763	2.52	0.0604
Error	156	2646.792713	16.966620		
Corrected Total	159	2774.820003			

R-Square	Coeff Var	Root MSE	Yi Mean
0.046139	5.475739	4.119056	75.22374

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	128.0272894	42.6757631	2.52	0.0604

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	128.0272894	42.6757631	2.52	0.0604

The SAS System 15:00 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	156
Error Mean Square	16.96662
Critical Value of t	1.97529
Least Significant Difference	1.8193

Means with the same letter are not significantly different.

t Grouping		Mean	N	trt
	A	76.1360	40	2
	A			
	A	76.0486	40	1
	A			
B	A	74.6545	40	3
B				
B		74.0558	40	4

The SAS System 14:33 Sunday, January 10, 2020 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 100

Shell thickness

The SAS System 14:51 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 160

The SAS System 14:51 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.05987187	0.01995729	9.72	<.0001
Error	156	0.32041250	0.00205393		
Corrected Total	159	0.38028438			

R-Square	Coeff Var	Root MSE	Yi Mean
0.157440	14.14877	0.045320	0.320313

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	0.05987187	0.01995729	9.72	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	0.05987187	0.01995729	9.72	<.0001

The SAS System 14:51 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	156
Error Mean Square	0.002054
Critical Value of t	1.97529
Least Significant Difference	0.02

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	0.33575	40	1
A			
A	0.33475	40	2
A			
A	0.32275	40	3
B	0.28800	40	4

Egg-shell ratio

The SAS System 15:09 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 160

The SAS System 15:09 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	306.40352	102.13451	0.90	0.4439
Error	156	17750.43595	113.78485		
Corrected Total	159	18056.83946			

R-Square	Coeff Var	Root MSE	Yi Mean
0.016969	77.54710	10.66700	13.75551

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	306.4035172	102.1345057	0.90	0.4439

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	306.4035172	102.1345057	0.90	0.4439

The SAS System 15:09 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	156
Error Mean Square	113.7848
Critical Value of t	1.97529
Least Significant Difference	4.7115

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	16.151	40	3
A			
A	13.011	40	2
A			

A	12.957	40	1
A			
A	12.903	40	4

Shell weight

The SAS System 14:52 Saturday, January 9, 2020 7

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 100

The SAS System 14:52 Saturday, January 9, 2020 8

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.56000000	0.18666667	0.22	0.8833
Error	96	82.00000000	0.85416667		
Corrected Total	99	82.56000000			

R-Square	Coeff Var	Root MSE	Yi Mean
0.006783	11.72857	0.924211	7.880000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	0.56000000	0.18666667	0.22	0.8833

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	0.56000000	0.18666667	0.22	0.8833

The SAS System 14:52 Saturday, January 9, 2020 9

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison wise error rate, not the objective wise error rate.

Alpha	0.05
Error Degrees of Freedom	96
Error Mean Square	0.854167
Critical Value of t	1.98498
Least Significant Difference	0.5189

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	7.9600	25	1
A			
A	7.9200	25	2
A			
A	7.8800	25	4
A			
A	7.7600	25	3

Internal qualities

Yolk index

The SAS System 15:23 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 160

The SAS System 15:23 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	475.95672	158.65224	1.21	0.3093
Error	156	20514.06481	131.50042		
Corrected Total	159	20990.02154			

R-Square	Co eff Var	Root MSE	Yi Mean
0.022675	34.64968	11.46736	33.09515

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	475.9567239	158.6522413	1.21	0.3093

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	475.9567239	158.6522413	1.21	0.3093

The SAS System 15:23 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	156
Error Mean Square	131.5004
Critical Value of t	1.97529
Least Significant Difference	5.065

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	35.340	40	3
A			

A	34.042	40	2
A			
A	32.159	40	1
A			
A	30.840	40	4

Haugh Unit

The SAS System 14:33 Sunday, January 10, 2020 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	145.201753	48.400584	1.56	0.2035
Error	96	2973.274220	30.971606		
Corrected Total	99	3118.475973			

R-Square	Coeff Var	Root MSE	Yi Mean
0.046562	7.219422	5.565214	77.08669

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	145.2017530	48.4005843	1.56	0.2035

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	145.2017530	48.4005843	1.56	0.2035

The SAS System 14:33 Sunday, January 10, 2020 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison wise error rate, not the objective wise error rate.

Alpha	0.05
Error Degrees of Freedom	96
Error Mean Square	30.97161

Critical Value of t 1.98498
Least Significant Difference 3.1245

Means with the same letter are not significantly different.

t Grouping		Mean	N	trt
	A	78.903	25	4
	A			
B	A	76.972	25	2
B	A			
B	A	76.959	25	1
B				
B		75.512	25	3

Yolk ratio

The SAS System 15:13 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 160

The SAS System 15:13 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	328.70393	109.56798	1.27	0.2871
Error	156	13473.37739	86.36780		
Corrected Total	159	13802.08132			

R-Square Coeff Var Root MSE Yi Mean

0.023816	31.87475	9.293428	29.15608

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	328.7039253	109.5679751	1.27	0.2871

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	328.7039253	109.5679751	1.27	0.2871

The SAS System 15:13 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	156
Error Mean Square	86.3678
Critical Value of t	1.97529
Least Significant Difference	4.1048

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	31.201	40	2
A			
A	29.648	40	3
A			
A	28.425	40	4
A			
A	27.351	40	1

Yolk colour

The SAS System 13:11 Sunday, October 24, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 160

The SAS System 13:11 Sunday, October 24, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	737.3250000	245.7750000	147.66	<.0001
Error	156	259.6500000	1.6644231		
Corrected Total	159	996.9750000			

R-Square	Coeff Var	Root MSE	Yi Mean
0.739562	30.26687	1.290125	4.262500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	737.3250000	245.7750000	147.66	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	737.3250000	245.7750000	147.66	<.0001

The SAS System 13:11 Sunday, October 24, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	156
Error Mean Square	1.664423
Critical Value of t	1.97529
Least Significant Difference	0.5698

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	7.1250	40	4
B	5.4000	40	3
C	2.9500	40	2
D	1.5750	40	1

Yolk-albumin ratio

The SAS System 15:31 Friday, September 10, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 160

The SAS System 15:31 Friday, September 10, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1178.41367	392.80456	1.24	0.2971
Error	156	49403.22222	316.68732		
Corrected Total	159	50581.63589			

R-Square	Coeff Var	Root MSE	Yi Mean
0.023297	33.50415	17.79571	53.11495

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	1178.413671	392.804557	1.24	0.2971

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	1178.413671	392.804557	1.24	0.2971

The SAS System 15:31 Friday, September 10, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	156
Error Mean Square	316.6873
Critical Value of t	1.97529
Least Significant Difference	7.8601

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	57.155	40	2
A			
A	53.739	40	3
A			
A	51.750	40	4
A			
A	49.816	40	1

Data analysis on yolk cholesterol

The SAS System 14:52 Friday, September 17, 2019 1

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	4	1 2 3 4

Number of observations 96

The SAS System 14:52 Friday, September 17, 2019 2

The GLM Procedure

Dependent Variable: Yi

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	29935.3491	9978.4497	0.97	0.4112
Error	92	947995.1066	10304.2946		
Corrected Total	95	977930.4557			

R-Square	Coeff Var	Root MSE	Yi Mean
0.030611	41.14854	101.5101	246.6918

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	3	29935.34911	9978.44970	0.97	0.4112

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	3	29935.34911	9978.44970	0.97	0.4112

The SAS System 14:52 Friday, September 17, 2019 3

The GLM Procedure

t Tests (LSD) for Yi

NOTE: This test controls the Type I comparison-wise error rate, not the objective-wise error rate.

Alpha	0.05
Error Degrees of Freedom	92
Error Mean Square	10304.29
Critical Value of t	1.98609
Least Significant Difference	58.199

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	267.29	24	3
A			
A	252.77	24	4
A			
A	248.02	24	2
A			
A	218.69	24	1

A 218.69 24 1

PUBLICATIONS

Appendix F: Publication abstract

Livestock Research for Rural Development 32 (6) 2020 LRRD Search LRRD Mission Guide for preparation of papers LRRD Newsletter Citation of this paper

Mulberry (*Morus alba*) leaf meal as partial replacement for soybean meal in indigenous chicken layer diets

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Abstract

This study aimed to determine the effects of inclusion of mulberry leaf meal as a protein source in the diet of indigenous layer chicken. Sixty, 29-week old indigenous chicken were allocated to diets with 0, 5, 10 and 15% mulberry leaf meal partially substituting soybean meal.

Feed intake was increased by 27%, with a curvilinear trend 15% of mulberry leaf meal replaced 9% of soybean meal in the diet. The increase in feed intake was reflected in better feed conversion up to the 10% level of mulberry leaf meal in the diet beyond which the feed conversion deteriorated. Egg production followed the same trend as feed conversion with maximum production being reached at 10% mulberry leaf meal in the diet.

Keywords: *β-glucan, ethnomedicine, phenols, prebiotics*