EFFECT OF PARTIAL SUBSTITUTION OF NOUG SEED CAKE WITH STINGING NETTLE (*Urtica simensis S.*) LEAF MEAL ON EGG QUALITY PARAMETERS OF COMMERCIAL LAYER HENS

MSc THESIS

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Effect of Partial Substitution of Noug Seed Cake With Stinging Nettle (*Urtica simensis* S.) Leaf Meal on Egg Quality Parameters of Commercial Layer Hens

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DEDICATION

This Thesis is dedicated to my beloved wife Simenesh Gezahagn and to my elder son Bereket Galaye next to my great God.

STATEMENT OF THE AUTHOR

I declare that this thesis is my original work and that all sources materials that are used for this thesis have been duly acknowledged. This thesis has been submitted to partial fulfillment of the requirements for Msc degree at Hawassa University and is deposited at university library to be made available to users under rules of University. I also declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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ACRONYMS

ANOVA	Analysis of variance
CF	Crude fiber
CP	Crude protein
CSA	Central Statistic Agency
DBWG	Daily body weight gain
DMI	Dry matter intake
DM	Dry matter
EE	Ether Extract
FI	Feed Intake
FBW	Final body weight
HU	Haugh unit
ISA	Institute of standard De Animalia
Kcal	Kilo calorie
ME	Metabolizable energy
OM	Organic matter
RIR	Rhod Island Red
WBWG	Weekly body weight gain
SEM	Standard error of means
SNNPR	South Nation, Nationalities and People region
SPSS	Stastical Package for Social Science
SNLM	Stinging nettle leaf meal

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EFFECT OF PARTIAL SUBSTITUTION OF NOUG SEED CAKE FOR STINGING NETTLE (*URTICA SIMENSIS* S.) LEAF MEAL ON EGG PRODUCTION AND EGG QUALITY PARAMETERS OF COMMERCIAL LAYER HENS

By

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ABSTRACT

The effect of various levels of Stinging nettle leaf meal (SNLM) on the egg production and egg quality of commercial birds was investigated using 150 Isa Brown layer hens. Five iso-nitrogenous and iso-caloric layer diets were formulated to contain SNLM at 0% (treatment 1 = T1), 3% (treatment 2 = T2), 6% (treatment 3 = T3), 9% (treatment 4 = T4) and 12% (treatment5=T5) by partially substituting Nuge seed cake. The results indicated that the total and daily feed intake did not differ among hens reared in different treatment diets. The individual final body weight (g) of hens reared in T1, T2, T3, T4 and T5 was 1786,1804,1804, 1819, 1858, respectively, being significantly higher for T5 than those of other treatments. The average egg weight (g) of hens reared in T1, T2, T3, T4 and T5 was 45.0, 47.2, 48.9, 53.5 and 55.5, respectively, being significantly different for T4 and T5 as compared with other treatments. The average hen-housed egg production (%) for hens fed with T1, T2, T3, T4 and T5 diets was 44.3, 49.5, 59.4, 70.9 and 80.5%, respectively, being significantly different from each other. Similarly, the respective individual daily egg mass output (g) in hens reared in T1, T2, T3, T4 and T5 diets was 20.0, 23.4, 29.1, 37.9, and 44.7, being ($p<0.05$) different from each other. The average feed conversation ratio (kg feed/kg egg mass) was 5.20, 4.18, 3.55, 2.83 and 2.40 for hens reared in T1, T2, T3, T4 and T5, respectively and was lower ($p<0.05$) for those of T4 and T5. The results further indicated that the experimental diet did not affect most of the external egg qualities at 23 weeks of age. However, at the same age, there was a significant difference between treatments in yolk colour being significantly higher for hens reared in T5 than those of T1 and T2. On the contrary, the Haugh unit was significantly lower in hens raised in T5 diet than those fed on other treatment diets at 23 weeks of age. At the age of 28 weeks, significant differences were observed between treatments for all external egg qualities. Accordingly, hens reared in T3, T4 and T5 diets had significantly higher shell thickness values than those of T1 and T2. However, hens reared in T2 showed better egg shape index as compared to those of T5. Hens reared in T3, T4 and T5 had significantly higher shell thickness values than those of T1 and T2. The yolk index at the age of 28 weeks was significantly higher in hens reared in T5 than that of T1 and T3. At the same age, the HU was significantly higher for hens reared in T2 (79.5) than those of T3 (77.1) and T5 (76.0). In conclusion, the substitution of Noug seed cake with increasing levels of SNLM improved the egg production and egg quality parameters of hens. 9% and 12% substitution of Noug seed cake with SNLM has been thus recommended for better results by reducing the cost of the Noug seed cake for commercial layer hens.

Keywords: *Stinging nettle leaf; Noug seed cake; Isa Brown layer hens; Egg production; mass; Egg qualities*

1. INTRODUCTION

With steadily growing population of Ethiopia (current census indicate over 90 million), there is an increasing demand in recent years for animal products (personal observation). However, the livestock sector could not be able to meet this growing demand mainly due to limited availability and/or expensive of animal feed resources. This has led to shortage of high quality protein from animal products to the fast growing population by widening the gap between estimated protein requirement and actual protein consumption.

It is therefore imperative that some efforts must be undertaken towards improving the per capita animal protein consumption of the country through the incorporation of relatively less expensive locally available feed resources (Aberra *et al*., 2009, 2012). Among those locally available unconventional protein feed resources, the Samma (*Urtica simensis* S.) leaves, which is endemic to Ethiopia, might be used as one of alternative feed resources to monogastric nutrition. The plant grows all year round in the mid and highlands of Ethiopia and therefore can be harvested whenever there is a need. Recent studies have revealed that Samma leaves are excellent and easily available source of protein as well as minerals. The CP content of Samma leaves ranged from 263-318 g/kg DM (Eskedar *et al.*, 2013; Aberra *et al*. [unpublished data]). The Samma leaves were also found to be rich in Calcium (58.6 g/kg DM), Iron (1186 mg/kg DM) and Manganese (433 mg/kg DM; Aberra *et al*. [unpublished data]). Most of essential amino acids are also available in appreciable amounts (Aberra *et al.* [unpublished data]). Moreover, it contains negligible amounts of total phenols (below 0.72 %) and there were no condensed tannins detected; and its crude fiber content was below 9% (Aberra *et al*., unpublished data).This makes the Stinging nettle leaves as alternative suitable feed resources for feeding monogastric animals such as chickens. Previous works have revealed that adding dried powder of the common nettle (*Urtica dioica*) into laying hens diets significantly increased egg production, proved the modulating effects of the immune parameters and lowered the total cholesterol and triglycerides concentration (Mansoub, 2011).

In Ethiopia, few research works are available on Samma and are limited to medicinal uses. There is no information available regarding the utilization of Samma leaves in layer hens' diet. Moreover, both Noug seed cake and Samma leaves contain comparable values of crude protein (Noug seed cake 28% and Stinging nettle leaves 31.8%) on dry matter basis but differ in their crude fiber content considerably being highest in Noug seed cake (24.5%) compared to that of Samma leaves (9.12%). Moreover, Samma leaves are very rich in Calcium (5.86% of DM) compared to that of Noug seed cake (0.5%). Noug seed cake is considered in Ethiopia as one of the most common protein sources in poultry nutrition. However, the cost of Noug cake has been consistently rising over the last many years making it inaccessible to smallholder poultry producers. Moreover, due to its high content of crude fiber, feeding high levels of the Noug seed cake poses problems on the feed consumption and subsequently on the performance of chickens. Thus, substituting the Noug seed cake with other suitable cheap protein and calcium sources becomes justifiable in monogastric nutrition such as poultry, particularly for layer hens.

1.1. Objectives of the study

1.1.1. Main Objective

To evaluate the nutritional potential of leaf meal prepared from Stinging nettle (*Urtica simenses* S.) as alternative protein and mineral sources on egg production and egg quality parameters of the laying ISA Brown hens.

1.1.2. Specific objectives

- To assess the feed consumption of chickens to Stinging nettle leaf;
- To evaluate the feeding value of Stinging nettle leaf on the egg production performance of layer hens by partially substituting Noug seed cake of the ration and
- To investigate the effect of Stinging nettle leaf supplementation on internal and external egg quality parameters by replacing lime stone.

2. LITRETURE REVIEW

2.1. Poultry production in Ethiopia

The term poultry applies to a wide variety of birds of several species including; chicken, guinea fowls, pigeons, ducks, geese, turkeys, swans, peafowl, ostriches, pheasants, quails and other game birds. Chickens originated in South-East Asia and introduced to the rest of the world by sailors and traders. Estimate on livestock in Africa shows that chicken population was the highest (Sonaiya *et al.*, 1998).

Ethiopia is one of the few African countries with a significantly large population of chickens (Fikre, 2001). In sub-Saharan Africa, 85% of all households keep chicken under free range system, with women owning 70% of it, providing scarce animal protein in the form of meat and eggs as well as being a reliable source of cash income (Guéye, 1998; Sonaiya *et al.*, 2004; Bagnol, 2000; Ambali, 2007and Aklilu *et al.*, 2007).

According to Sonaiya (1990), Kitalyi (1998) and Reddy (1991) there are three chicken management systems in the world namely: intensive, semi-intensive and extensive, which are differentiated on the basis of flock sizes and input-output relationships. Alternatively, Bessei (1987) reported that family chicken were kept under a wide range of conditions, which could be classified into four broad production systems: free-range extensive, backyard extensive, semi-intensive and Intensive systems. According to Alam (1997) family chicken meat & eggs were estimated to contribute 20–30% of the total animal protein supply in low-income and food-deficit countries. Both chicken meat and eggs were affordable sources of protein and contribute to a well balanced diet to satisfy human needs.

Village chicken could be particularly important in improving the diet of young children in Sub-Saharan Africa (Alam, 1997) Chicken provide major opportunities for increased protein production and income for smallholder farmers because of presence of small generation interval, high rate of productivity, the ease with which its products can be supplied to different areas, the ease with which its products can be sold due to their relatively low economic values, its minimal association of with religious taboos and its complementary role play in relation to other crop-livestock activities (Muchenje *et al.*, 2000).

According to Halima (2007) a substantial amount of phenotypic diversity for various traits in the indigenous chicken genetic resources of Ethiopia was expected because of presence of diverse agro-ecology, ethnic groups and socio-economic, religious and cultural considerations.

In many developing countries the local gene pool still provides the basis for the poultry sector (Yakubu *et al.*, 2008).In many developing countries; chicken production is based mainly on traditional extensive production systems with local chicken ecotypes and low purchased-inputs (Gueye, 1998; Gueye, 2000 and Garcia, 2007).

The extensive chicken production system in Africa, where birds are kept on free range, is different from the more recent extensive free range system coming up in developed countries, due to the hot chicken welfare issues (Thear, 1997).In most part of Ethiopia, village chicken represents a significant component of the rural household livelihood as a source of cash income and nutrition. The birds scavenge in the vicinity of the homestead during daytime where they may be given cereal grains, cereal bran, broken grains and other house waste products as supplementary feed (Aklilu *et al.*, 2007).

The number of chicken flocks per household of most Ethiopian rural community is small in number and containing birds from each age group with an average of 7-10 mature birds, consisting of 2-4 adult hens, a male bird (cock) and a number of growers of various ages (Tadelle and Ogle, 1996).

The egg production potential of local chicken is 30-60 eggs/year/hen with an average of 38g egg weight under village management conditions, while exotic breeds produce around 250 eggs/year/hen with around 60gm egg weight (Alganesh *et al.*, 2003) in Ethiopia. According to Alganesh *et al. (*2003) and Negussie *et al.* (2003), the low productivity of the local scavenging hens is not only because they are low producers of small sized eggs and slow growers but also the system is characterized by high chick mortality before they reach around 8 weeks of age. Moreover, the local chickens are the results of uncontrolled breeding between various local chicken ecotypes, which have not been selected by systematic breeding methods.

There are many factors that can adversely affect egg production. Egg production can be affected by feed consumption (quality and quantity), water intake, intensity and duration of light received, parasite infestation, diseases, management and environmental factors (Jacob *et al.*, 1998).

With the aim of improving poultry productivity, there has been a substantial effort to introduce improved hybrid layer chickens particularly Isa Brown (IB), Bovan Brown (BB) and dual purpose hybrid Potchefstroom Koekoek (PK) to smallholder farmers under backyard management (Desalew *et al.*, 2013).In the layer reference for more than 30 years every wherein the world, IB is the most efficient layer in the poultry industry producing many high quality eggs and adapts itself to all climates and environments (ISA, 2010).

2.2. Description of the stinging nettle

Stinging nettle (*Urtica simensis*) is one of species of Nettle and endemic in Ethiopia. It is dark green perennial wild plant and the leaves are used as a food eaten as pot herbs in some areas of Ethiopia. Ecologically *Urtica simensis* found in Upland grassland areas most common in disturbed localities, often plentiful near houses. It could fill a valuable place in the production of food in rural areas where the climate is not conducive to the production of vegetables and at a time when cultivated green leafy vegetables are not ready to use. Although in few areas of Ethiopia, Stinging nettles have traditionally been used for food and as medicinal plants. Its potential contribution to food security, nutrition, health, and income generation for the well-being of mankind is still largely underexploited.

The potential of spreading its use across regions and cultural groups is, likewise not yet `looked in to. In general, the potential of wild vegetable species in food and nutrition security in Ethiopia, health and income generation should be increased in the face of the growing environmental and socioeconomic changes.

An important remedy for food security problems is food items diversification. Stinging nettle can assist the majority of our society, provided the society acknowledges it and produce it in line with the other crops (Eskedar, *et al.* 2013)

Nettles include two families of the herb i.e., stinging nettle (*Utica dioica, Urtica simensis*) and Nettle leaf (*Urtica urens)*. Stinging nettle leaf is one of the less used herbal Galactogogue among the community participant in Africa. Though stinging nettle has a longstanding reputation for enriching milk (Gladstar, 1993; Weed, 1986; Yarnell, 1998). The herb is believed to be completely non-toxic (Yarnell, 1998). Nettle is a well known galactogogue and best female tonic herb. It is rich in trace minerals like iron, calcium and Vitamins like A, C and K including phosphorus, potassium, sulphur and vitamin-D, and promotes milk production (Weed, 1986; Lieberman, 1995). It also contains B-complex vitamins and appreciable amounts of magnesium. It contains up to 20% mineral salts, mainly calcium, potassium, silicon and niyrates (Blumenthal *et al.* 2000).

Nettle extracts contain all of the essential amino acids. Nettle is believed to support lactation by providing essential nutrients (Weed, 1986). It has no medicinal action apart from being mildly diuretic and haemostatic (Bradley, 1992). Dried nettle mixed into cattle fodder are known to boost milk production in cows (Grieve, 1971; Phillips and Fov, 1990). Nettle is used to support lactation and nettle leaves are typically brewed as a tea, often in combination with raspberry leaf. Sometimes it forms a component of mother's milk tea and can also be used as nettle tea or as a tincture. Nettle tea can be made by pouring one cup boiling water over two tea spoons of herb and allowing it to simmer for two minutes.

2.3. Preventive and medicinal use of Stinging nettle (*Urtica simensis S.*) for layer hens

Preventing diseases is overall a much better option than fighting against them. Prevention of animal preventing an infection from entering the farm and spreading, but includes good nutrition, diseases is therefore a focal point on many farms. Prevention comprises not only of suitable housing, good management and appropriate rearing. This way, the general resistance to diseases of the animals is improved (Maria *et al.* 2011).Resistance and animal welfare go hand in hand, also in the case of poultry. To increase disease resistance, the animals should experience as little stress as possible.

Natural products can play a useful role in improving the condition and general resistance of poultry; resistance to specific diseases can be increased using vaccinations. Vaccination is a well known preventive measure. Blood can be examined for antibodies to check whether the vaccination has worked. The effectiveness of other preventive management measures is often less easy to prove than the effectiveness of curative (healing) products. It is for example hard to prove that an animal does not become ill or gets better quicker by using certain feed components.

By improving the general disease resistance, fewer animals will get ill, or the illness will be less severe in case of infections. Also, animals will recover more quickly when they have better resistance. It is therefore always a good idea to work on the general disease resistance of animals, even if no disease has (yet) been identified. Animals will heal under their own steam, as much as possible, using for instance certain herbs that can temporarily be added to the feed (Maria *et al.*2011). Nettle (*Urtica dioica),* as a medicinal plant, belongs to the family of *Urtica ceae* which is a perennial herb of tropical regions.

The main active components of nettle include phenolic compounds (like carvacrol and thymol), mono terpenoid components (like terpenediols, terpenediols glucosides and alphatocopherol) and flavono lglycosides (like kaempherol 3 Orutinoside, isorhamnetin 3 – O-glucosides and quercetin-3-O-rutinoside) (Gülçin *et al.*, 2004). Today nettle is recognized as having astringent, expectorant, galactagogue, tonic, anti-inflammatory, homeostatic, and diuretic properties, and is recommended for treating bone and joint conditions, inflammation and irritation, of the urinary tract and for preventing urinary system grave.

Stinging Nettle has traditionally been used as a detoxifying spring tonic, so it’s interesting that it contains secret in, a substance that helps the bowels to eliminate mucus. It has also been used to treat arthritis; the ant proliferative effect of stinging nettle roots observed in both an *invivo* model and in an invitro system clearly indicates a biologically relevant effect of compounds present in the extract. Yet another study found that nettles have significant anti mutagenic effects, so consumption of nettles may also be beneficial for people at risk for prostate cancer and other types of cancer as well (Karakaya and Kavas, 1999). Akbay *et al.*(2003) reported that the major flavonoid glycosides have anti-inflammatory, antioxidant and antiallergenic and anti-carcinogenic activities.

2.4. Nutritional Value of Stinging Nettle (*Urtica simensis)* Leaf meal for poultry

The most prominent member of feed resource is the genus stinging nettle (*Urtica simenesis*) leaves, which is native to Europe, Africa, Asia and North America (Hegi, 1981) and is one of a well-known galactogogue native to Ethiopia. Stinging nettle is perenial plant that is widely known for its unpleasant stinging hairs located under the stems and lower leaf surface. It reproduces by wind-dispersed seeds and creeping underground rhizomes and grows in dense clumps, often forming large colonies. It is an erect non branched, wild-growing nettle plant that grows in the highlands. The plant grows wild as a weed in uncultivated fields and pasture land. The herb usually used as emergency famine food in northern Ethiopia specially around Gonder, Gojam, Kofole and inmost high lands of Sidama Zone in southern region (Tsegaye, 2008).

The genus of it contains a number of other species with similar properties and it is highly variable in morphological characteristics and probably represents a number of subspecies. However, a large number of species included with in this genus in the older literature are now recognized as synonyms of *Urtica dioica.* Some of these taxa are still recognized as subspecies [htt://www.vortexhealth.net/stinging nettle.html]. The true nettles belong to the*Urtica ceae,* also known as the nettle Family.

The nettle family is found worldwide and consists of about 45 genera and 700-1000 species. Most of the species are tropical and herbaceous (Walters and Keil, 1996). Stinging nettle (*Urtica simenesis S.*) is a perennial plant of 1-2 m tall. Nettle leaves are reported to be excellent and easily available source of protein as well as vitamins. The leaves contain on the average about 25% protein on DM basis. Amino acids in nettle leaf meal are nutritionally superior to those of alfalfa meal. This makes the leaves suitable for feeding monogastric animals. It also contains 4% fats, 37% non-nitrogen extracts and 9-21% fiber. It is rich in vitamins A, C, Fe, K, Mn and Ca (Radford *et al*., 1988).

In Ethiopia, few research works are available on stinging nettle and are limited to medicinal uses. In this regard some research works revealed that stinging nettle is traditionally used to treat some diseases. There are only few literatures regarding the utilization of nettle leaves meal in chickens' diet and almost no information available. The two minerals interact with each other, both before and after their absorption from the digestive tract. An over-supply of either mineral can interfere with the utilization of the other. During egg production, calcium needs are more than doubled. Poultry's calcium and phosphorus requirements are influenced by the amount of vitamin D in the diet, increasing as the level of vitamin D decreases and vice versa. For growing birds, the ratio of Ca: P should be between 1:1 and 2:1. However, laying birds need a ratio of up to 6:1, and they need about 4.0 g of calcium per day for egg shell formation.

Supplement like steamed bone-meal is added to chicks and growers diets to provide extra calcium and phosphorus. For laying birds, the extra calcium requirement is provided by oyster-shell grit fed separately or by limestone flour added to the diet. The main criteria for determining laying hens requirements of calcium and phosphorus are egg production and shell thickness. Calcium requirements should be specified in terms of amount of calcium per day rather than percentage in diets (van *et al.* 2006).

Although animals avoid fresh nettles, the dried plants lose their sting and are a valuable for animal feed. Nettle hay is actually too rich in protein (about 25% dry weight) to be used alone, so is added to other feed as a nutritional supplement. It is said to increase the disease resistance, weight and general health of cows, poultry and most other animals. Dried nettles have been widely used for farm animals (Tozer, 2007). In laying hens the use of nettle extract via Lymphocyte proliferation can stimulate the innate cell mediated immune response (Sandru *et al.,* 2007). Finally, young nettles can simply be boiled and eaten like spinach. As mentioned above, nettles are an extremely nutritious vegetable. When nettles are dried, they are eaten by cows resulting in an excellent food that helps to increase the quantity and d quality of their milk. It also makes their coats shine. Used as fodder for cows, they will increase milk production. Nettle leaves are excellent for feeding poultry; and especially in the winter.

When boiled and eaten the stinging nettles promote the laying of eggs right throughout the winter. Added to chicken feed, they will increase egg production (Khosravi *et al.,* 2008). When cut and dried, nettles also provide excellent, nutritious feed for livestock. Powdered dried nettle leaves are actually as rich in protein as cottonseed meal. Many animal feeds are now supplemented with antibiotics to increase the growth rate of animals. It seems kind of unbelievable that a plant that provides so much-fiber, food, medicine, animal feed-could possibly be scorned as a worthless and annoying weed. Moreover, nettles contain high levels of minerals (especially iron), vitamin C and pro-vitamin A, that is simply digested in the host's small intestine (Toldy *et al.,* 2005).

3. MATERIALS AND METHODS

3.1. Description of the study area

The experiment was carried out at poultry farm of School of Animal and Range Sciences, College of Agriculture, Hawassa University, which is situated 275 km south of Addis Ababa. Geographically it is situated at 7°4'N latitude and 38°31'E longitude at an altitude of 1650 m above sea level. Rainfall is bi-modal and ranges between 674 and 1365 mm. The mean temperature ranges between 12 ^{0}C and 27 ^{0}C.

3.2. Stinging nettle leaf preparation

Fresh Stinging nettle leaves were collected from farmers residing in Boditti town areas of Wolaita Zone that grow the plant materials abundantly. After collecting, the leaves were dried under the shade and transported to the research site. During the drying process, leaves were removed from the stem dried under the shade. Moreover, regular turning of leaves was done to prevent the possible growth of moulds. The dried leaves were then processed into Stinging nettle leaf meal through grinding which is referred hereafter as SNLM.

3.3. Ingredients of the experimental diets

The dietary ingredients used in this experiment were maize (*Zea mays)*, roasted soybean seed (*Glysine max*), wheat bran, Noug seed (*Guizotia abyssinica)* cake, meat and bone meal, limestone flour, poultry premix and salt. All ingredients were purchased from the local market. The soybean seed was roasted for 5 minutes until the beans were brown to deactivate trypsin inhibitor (Newkirk, 2010; Negesse and Tera, 2010) and milled in sieve size of 5 mm separately. All other feed ingredients were also milled with similar sieve size and mixed at the feed processing plant located at the College of Agriculture

(Hawassa University).Moreover, representative samples were taken for the determination chemical and mineral compositions.

3.4. Experimental design

After having results on chemical and mineral analysis of each feed ingredients, five iso-nitrogenous and iso-caloric experimental diets were formulated. Following this, the dietary treatments were prepared to contain 0% of SNLM (control diet, T1) and diets containing SNLM at the levels of 30 g/kg (T2), 60 g/kg (T3), 90 g/kg (T4) and 120 g/kg (T5) by replacing the Noug seed (*Guizotia abyssinica)* cake in the control diet. At the age of 19 weeks (at start of egg lay), 150 pullets were randomly assigned to each of the three replicates of the five dietary treatments in a completely randomized design (Table 1). The experiment lasted 60 days exclusive of the adaptation period.

Table 1: Experimental design of the feeding trial with layer hens

Treatment Diets	Inclusion rate of SNLM (%)	Number of Replicates	Hens per replicate	Hens per treatment diets
T1	0	3	10	30
T2	3	3	10	30
T3	6	3	10	30
T4	9	3	10	30
T5	12	3	10	30
Total (N)				150

SNLM = Stinging nettle leaf meal

3.5. Management of chickens

Initially 161 (12 weeks of age) ISA-brown pullets were purchased from Alema Poultry Farm Limited, Campany that is located at Debrezeit town, Ethiopia. The chickens were vaccinated against major poultry viral and bacterial diseases including Marek's disease, Newcastle, infectious bursal disease (Gumboro), fowl typhoid and fowl pox diseases according by the company before they were purchased. After arrival, pullets were allowed to adapt to the new set of environments. After the end of the adaptation period, matured pullets were then leg-tagged, weighed and transferred to the already prepared experimental pens based. As shown in Tables 2, 3 and 4, three rations namely, pullet ration, pre-lay ration and layer rations were formulated for optimum management. Feed were offered twice a day early in the morning (between 7.00 and 8.00 am) and late afternoon (between 4.00 and 5.00 pm) and refusals were always collected every day in the morning before feed is offered.

Table 2: Feed ingredients of the pullets ration (12-16 wks of age**)**

Ingredients (%)	T1	T2	T3	T4	T5	Required
Maize	51	51	51	51	51	-
Roasted soya bean	20	20	20	20	20	-
Noug seed cake*	12	12	12	12	12	-
Wheat bran	12	12	12	12	12	-
Meat & bone meal	2	2	2	2	2	-
Lime stone flour	1.5	1.5	1.5	1.5	1.5	-
Min/vit premix	1	1	1	1	1	-
Salt	0.5	0.5	0.5	0.5	0.5	-
Total	100	100	100	100	100	
Supplied by the ration (%)						
ME (kcal/kg)	2800	2800	2800	2800	2800	2750
Crude protein	17.7	17.7	17.7	17.7	17.7	16-17
Crude fiber	7.37	7.37	7.37	7.37	7.37	3-7
Fat	3.56	3.56	3.56	3.56	3.56	3-4
Calcium	0.88	0.88	0.88	0.88	0.88	0.80
Phosphorous	0.64	0.64	0.64	0.64	0.64	0.70

*Guizotia abyssinica

Table 3: Feed ingredients of the pre-layer ration (17-20 wks of age**)**

Ingredients (%)	T1	T2	T3	T4	T5	Required
Maize	50	50	50	50	50	-
Roasted soya bean	19	19	19	19	19	-
Noug seed cake	10.8	10.5	10.5	10.5	9.9	-
Wheat bran	11.7	12	12	12.3	12.6	-
Meat & bone meal	2	2	2	2	2	-
Lime stone flour	5	5	5	5	5	-
Min/vit premix	1	1	1	1	1	-
Salt	0.5	0.5	0.5	0.5	0.5	-
Total	100	100	100	100	100	
Supplied by the ration (%)						
ME (kcal/kg)	2770	2765	2765	2765	2760	2750
Crude protein%	17.0	17.0	17.0	16.9	16.9	16-16.5
Crude fiber%	7.15	7.10	7.10	7.15	7.15	3-7
Fat	3.45	3.45	3.45	3.45	3.45	3-4
Calcium	2.16	2.16	2.16	2.16	2.16	2-2.2
Phosphorous	0.61	0.61	0.61	0.61	0.61	0.70

Table 4:Feed ingredients of the layer ration (21-30 wks of age)

Ingredients (%)	T1	T2	T3	T4	T5	Required
Maize	48	46.7	47.2	47.7	48	-
Roasted soybean	20	20	19	19	19	-
Wheat bran	10	11.3	12	11.5	11.2	-
Meat & bone meal	2	2	1.8	1.8	1.8	-
Noug seed cake	12	9	6	3	0	
Samma leaf meal	0	3	6	9	12	
Lime stone flour	6.5	6.5	6.5	6.5	6.5	-
Min/vit premix	1	1	1	1	1	-
Salt	0.5	0.5	0.5	0.5	0.5	-
Total	100	100	100	100	100	
Supplied by the ration (%)						
ME (kcal/kg)	2780	2780	2770	2760	2750	2750
Crude protein%	17.5	17.5	17.3	17.3	17.2	16-17
Crude fiber%	6.64	6.26	5.78	5.29	4.86	3-7
Fat	3.45	3.45	3.40	3.40	3.42	3-8
Calcium	2.72	2.88	3.02	3.18	3.15	2.5-3.7
Phosphorous	0.63	0.63	0.62	0.61	0.60	0.6-1.0

3.6. Data collection protocols

3.6.1. Feed intake and egg production

Chickens were fed on replicate basis and each day a measured amount of feed was offered twice a day (morning and afternoon). The feed refusal was collected the next morning before providing the feed. The collected feed refusal was then air dried and weighed and subtracted from the weight of the feed provided initially to determine the feed intake on group basis. Eggs were collected on daily basis and egg production rate was calculated on hen-housed basis by considering the number of hens that were initially housed. Egg weight was determined on weekly basis and average egg weight was calculated. Total egg mass was calculated as a factor of the average egg weight and total number of eggs produced. Daily egg mass per hens was computed by dividing the total egg mass by the number of hens that were initially housed and total number of days in which the hens were in lay. Feed conversion ratio (feed consumed/egg mass) was then calculated as grams of feed: grams of egg mass output (Aberra, 2000; Aberra *et al*., 2005). Body weight was taken at the beginning and end of the experimental period and then weight gain was calculated by subtracting the initial from the final body weight.

3.6.2. Egg quality parameters

Internal and external egg quality parameters were assessed in all birds on weekly basis. To this effect, two eggs from each hen was collected over two consecutive days and the following parameters was determined: egg weight, egg length and breadth, egg specific gravity, shell thickness, albumen and yolk heights, yolk diameter and colour. Eggs were weighed using triple beam balance. Egg length and width and yolk diameter was measured using electronic digital caliper. A tripod micrometer was used to measure the heights of albumen and yolk. Yolk colour was measured by using the Roche Colour Fan. Egg shell thickness was measured according to Aberra *et al.* (2010). Individual Haugh unit was calculated according to the equation of Haugh (1937).

The two eggs from each replicates were collected attentively over two consecutive days and various parameters were carefully taken to analyze the quality of eggs of ISA brown commercial birds; some of these parameters were external egg quality parameters; egg weight, and shell thickness, Egg length, Egg width and shape index. Internal egg quality parameters egg yolk index, yolk color, Hough unit, and albumen height and yolk height and yolk width. Eggs were weighed using triple beam balance. Egg length and width and yolk diameter was measured using electronic digital caliper. A tripod micrometer was used to measure the heights of albumen and Yolk. Yolk colour was measured by using the Roche Colour Fan. Egg shell thickness was measured according to Aberra *et al.* (2010). Individual Haugh unit was calculated according to the equation of Hough (1937).The external egg qualities including; average weight of eggs, shell thickness, egg length, egg width, shape index, and internal egg qualities; albumen height, yolk height and width, color and Haugh unit measurements were seriously taken.

3.6.3. Chemical analysis

Analyses of proximate nutrients were performed as outlined by AOAC (1995). Samples of Samma leaf meal and feeds offered were analyzed for dry matter (DM, method 950.46), ether extract (EE, method 920.39), crude fibre (CF, method 962.09) and ash (method 942.05). The crude protein (CP) was assessed using Kjeldahl procedure (AOAC, 1995, method 954.01) and the nitrogen content was multiplied by 6.25 to obtain the crude protein. Calcium was determined by atomic absorption spectrophotometer and phosphorus by calorimetrically methods as described by AOAC (1995). The metabolizable energy (ME) of diets was estimated based feed composition tables of tropical feeds for poultry. All the samples were analyzed in duplicates at Animal Nutrition Laboratory of Hawassa University.

3.6.4. Statistical analysis

Data obtained on average feed intake (g), body weight, feed conversion ratio, egg production and egg quality parameters were subjected to one-way ANOVA using the General Linear Model (GLM) procedures of SAS ver. 9.4 (SAS, 2012). Treatment means were compared using Tukey's Student zed Range (HSD) test. Comparisons with $p<0.05$ were considered significant and all statements of statistical differences were based on this level unless noted otherwise. The following statistical models were used to analyze the data:

$Y_{ijk} = \mu + Ai + Dj/A_i + e_{ijk}$, where:

Yijk= observed values of the dependent variables (feed intake, body weight, egg production, egg quality) for k^{th} hen

μ = overall mean of the response variable

Ai = the effect of the i^{th}Samma leaf meal level (i = 1, 2, 3, 4, 5) on the dependant variable

Dj/Ai = the effect of the j^{th} replicate (j =1, 2, 3) within the level of the i^{th}Samma leaf meal

e_{ijk}= residual error

4. RESULTS

4.1. Nutrients content of the experimental diets

Nutrient (% DM basis) compositions and Metabolizable energy (ME, kcal/ kg DM) contents of Stinging nettle leaves meal and experimental diets fed to layer ISA browns Presented in Table 5. The levels of dry matter (DM), nitrogen free extract (NFE) and phosphorous (P) did not show variation between treatment diets. However, crude protein (CP) in T4 and T5 and ash levels showed a slight increase as the inclusion rate of SNLM increased. In addition, the level of calcium (Ca) and crude fiber (CF) showed a slight increase with increasing levels of SNLM in the diet. Among the dietary treatments, T3, T4 and T5 had more percentage of CF than T1 and T2 diets. The birds fed on T4 and T5 diets) showed increased feed intake. The nutrient contents of SNLM indicated that it is rich in CP (29.4%), P (0.72%) and Ca (2.9%) contents.

Table 5: Nutrient compositions (% DM basis) and Metabolizable energy (kcal/ kg DM) contents of Stinging nettle leaves meal and experimental diets fed to layer ISA browns.

Treatment diets	DM %	CP	EE	CF	Ash	NFE	Ca	P	ME
T1	91.6	19.1	9.7	7.75	8.99	40.9	2.52	0.35	2655
T2	91.5	19.4	9.32	7.80	9.10	41.2	2.76	0.39	2670
T3	91.8	19.2	9.35	7.67	9.22	41 .34	3.05	0.41	2662
T4	91.9	19.7	9.32	7.95	8.97	41.04	3.20	0.42	2663
T5	91.5	20.8	9.01	8.24	10.2	41.03	3.30	0.46	2672
SNLM	95.5	29.4	6.20	8.71	11.7	39.8	2.99	0.72	2991

NFE = nitrogen free extract [DM - (CP + EE + CF + Ash]; DM = dry matter; CP= crude protein's; CF = crude fiber; NFE = nitrogen free extract; Ca = calcium p; ME = metabolizable energy; CP = crude protein; EE = crude fat; SNLM = stinging nettle leaf meal.

4.2. Feed intake

The effect of feeding different levels of stinging nettle leaf meal eight weeks experimental periods of ISA Brown layer hens was indicated in Table 6. The feed intake has progressively increased from one up to eight experimental weeks. The feed intake at week 4 and 5 were comparable. As the results indicated, there was significant difference ($p<0.05$) in feed intake during the experimental period of 8 weeks. During the week 3, week 4, week 5, week 6, week7 and week 8 of experimental periods, chickens fed on T1 and T2 diets showed comparable intake as well as those fed on the T3 and T4 indicated comparable intake during the Week 3 and week 4. After second week, chickens fed on T5 of experimental diet showed low feed intake. However, starting from the Week 3 up to the Week 8, chickens fed on the T4 dietary treatment showed relatively higher intake than the rest of the group.

Table 6: Weekly mean daily feed intake (g/hen/day (Mean±SE) of ISA brown fed with different treatment diets

Period	T1	T2	T3	T4	T5
Week 1	97±3.6^{b}	97.2±2.3^{ab}	97.5±1.8^{b}	97.4±1.3^{a}	97.24±1.4^{a}
Week 2	100±0.79^{b}	101±4.47^{ab}	100±2.98^{b}	101±1.81^{a}	101± 16^{a}
Week 3	106±0.29^{b}	107±1.59^{b}	105±2.85^{ab}	106±0.32^{a}	104±9.99^{a}
Week 4	110±0.12	110±0.63	109±14.21	110±9.44	110±4.2
Week 5	111±0.12^{b}	111±0.16^{ab}	110±4.73^{b}	111±0.13^{a}	111±0.2^{a}
Week 6	111 ±0.17	111±0.25	111 ±0.31	111±0.33	111±0.2
Week 7	111±0.87	111±0.18	111±0.38	111±0.46	111±0.2
Week 8	112±0.12^{b}	112±0.25^{ab}	112±0.00^{b}	111.7±0.36^{a}	111.7±0.8^{a}

$^{a,\ b}$ means with different superscript letters across the raw are significantly different (P < 0.05)

4.3. Nutrient intake of hens

The effects of different levels of SNLM on mean daily nutrient intakes of growing layers during experimental period are presented in Table 7. The results indicated that the average DM intake were not significantly different in hens fed on the experimental feeds (T1, T2, T3, T4 and T5). The average CP intake was higher (P<0.05) for the hens receiving T4 and T5 diets than those fed on other treatment diets. The CF intake did not show significant difference across treatment diets. However, the Ca intake of hens fed with T5 diet were significantly (P<0.05) higher than those of T1, T2, T3, T4 diets. The P and ME intakes were similar across treatment diets.

Table 7: Nutrient (g/bird/day) and metabolizable energy (kcal/bird/day) intakes of ISA brown fed diets with various levels of Stinging nettle leaf meal [mean±SE].

Nutrients	T1	T2	T3	T4	T5	Overall mean	SEM
Dry matter	107	108	107	107	107	107	0.58
Crude protein	20.48[c]	20.95[c]	20.54[b]	21.07[a]	22.26[a]	31.5	0.045
Crude fat	2.92	3.7	3.63	3.65	3.67	3.51	0.055
Crude fiber	5.21[b]	5.68[b]	6.17[a]	6.74[a]	7.62[a]	6.2	0.001
Calcium	2.92[c]	3.09[c]	3.22[b]	3.24[b]	3.41[a]	3.18	0.002
Phosphorus	0.64	0.65	0.63	0.62	0.61	0.63	0.035
ME	286	287	284	287	286	286	0.03

[a,,b,c] means with different superscript letters across treatment diets are significantly different (p<005) ME = Metabolizable energy; SEM =Standard error of the mean

4.4. Growth performances and Feed intake

The effect of feeding SNLM on the body weight gain of ISA brown layer hens across treatments is presented in Table 8.At the beginning of the experiment, the initial average body weight recorded per bird did not show any significant difference. However, the average final weight gain of layer hens reared in T5 were significantly (P<0.05) higher than that of T1, T2, T3, and T4.The mean individual body weight gain was significantly different (p<0.05) across the treatment diets up to the 8thweeks of experimental period. However, hens fed on T4 and T5 diets had significantly (P<0.05) higher weight gain than those reared in the other treatment diets. Those hens fed on the T2 and T3 diets showed comparable weight gain throughout experimental periods. Birds fed with T5 (12% SNLM) showed significantly (P<0.05) higher weight gain than the rest of treatments after 20th week of experimental period. In general, the mean body weight gain across all treatment groups increased from 20 to 28 weeks of experimental period. The mean body weight gain across all treatment groups also consistently increased from the 1st to the 8th weeks of experimental period. The feed intake of birds did not differ significantly among treatment diets although hens reared in T2 consumed less feed as compared to other groups. A high mortality rate (10%) was observed in T2 while no mortality occurred in hens reared in T4 and T5 diets.

Table 8: The effect of feeding Stinging nettle leaves meal on body weight (g/hen) and feed intake (g/hen/d) of ISA brown layer hens [mean±SD]

Parameters	T1	T2	T3	T4	T5
Initial weight	1312±107	1309±104	1341±76.7	1308±75.5	1319±88.8
Final weight	1786 ± 26.2^{d}	1804 ± 16.4^{c}	1804 ± 76^{c}	1819 ± 27.1^{b}	1858 ± 2.6^{a}
Weight gain	470 ± 105^{b}	500 ± 105^{b}	465 ± 80.5^{b}	511 ± 85^{ab}	538 ± 90^{a}
Feed intake	104±5.8	97±10.5	103±6.08	107±0.6	107±0.25
Mortality rate	3.33	10.0	3.33	-	-

^{a,b,c}means with different superscript letters across treatment diets are significantly different(P < 0.05)

4.5. The effect of experimental feed on egg production of ISA Brown layer hens

The total egg number significantly (p<0.001) differed among treatment diets being higher in hens reared in T5 than those of other treatments (Table 9). Hens fed with T4 were significantly better in these parameters than those of T3, T2 and T1 and so on. The rate of hen-housed egg production also showed similar trend as that of total egg number being higher (p<0.001) in hens fed on T5 as compared to those reared in the other treatment diets. The individual average egg weight from hens reared on T4 and T5 diets was higher (P<0.05) than those of T1, T2, and T3. Hens fed with T3 diet produced heavier (p<0.01) eggs than those reared in T1. No significance difference was observed in egg weight between chickens fed with T3 and T2 diets as well as between those reared in T1 and T2. The highest total egg mass output was obtained from chickens reared in T5 followed by T4, T3, T2 and T1 being different (p<0.001) from each other. Similar result was observed in the individual daily egg mass production. No difference was obtained in total feed intake among treatment diets although hens reared in T2 consumed the least. The lowest feed conversion ratio (FCR) was observed from hens reared in T5 and differed (p<0.05) from those fed with T1, T2 and T3. Hens fed with T5 had the best FCR which differed significantly from the rest of the treatment diets.

Table 9: The effect of experimental feed on feed intake, egg production, and feed conversion ratio of ISA Brown layer hens

Parameters	T1	T2	T3	T4	T5
Total egg number	239±8.0^{e}	267±12.6^{d}	321.0±12.2^{c}	383±11.7^{b}	435±6.12^{a}
Hen-housed egg production (%)	44.3±1.45^{e}	49.5±2.36^{d}	59.4±2.23^{c}	70.9±2.13^{b}	80.5±1.11^{a}
Average egg weight (g)	45.0±0.43^{c}	47.2±1.90^{bc}	48.9±1.93^{b}	53.5±0.31^{a}	55.5±0.72^{a}
Total egg mass output (kg)	10.8±0.45^{e}	12.6±1.07^{d}	15.7±1.21^{c}	20.5±0.73^{b}	24.1±0.67^{a}
Egg mass production/hen/d (g)	20.0±0.85^{e}	23.4±1.93^{d}	29.1±2.25^{c}	37.9± 1.41^{b}	44.7±1.17^{a}
Total feed intake (kg)	56.0±3.29	52.2±5.75	55.6±3.07	58.0±0.17	57.9±0.15
FCR (kg feed/kg egg mass)	5.20±0.40^{a}	4.18±0.80^{b}	3.55±0.26^{bc}	2.83±0.01^{cd}	2.40±0.07^{d}

^{a,b,c} means with different superscript letters across treatment diets are significantly different (P < 0.05)

4.6. The effect of experimental feed on external and internal egg qualities

4.6.1. External and internal egg qualities at 23 weeks of age

The effect of experimental feed on external egg qualities is presented in Table 10. The first egg laying started at the age of 22 weeks. The average egg width of birds fed on T2 was higher (P<0.05) than that of T3. However, there was no significant difference between treatment diets for shell thickness, egg length and shape index.

Table 10: The effect of experimental feed on external egg qualities at 23 weeks of age [mean±SD]

Parameters	T1	T2	T3	T4	T5
Shell thickness (mm)	0.25±0.04	0.26±0.02	0.26±0.03	0.27±0.02	0.28±0.02
Egg length (mm)	53.2±3	52.92±2.4	52.65±2.4	54.52±2.6	53.45±1.0
Egg width (mm)	41.0±1.5[ab]	41.6±1.5[a]	40.3±1.4[b]	41.5±0.9[a]	41.3±0.9[ab]
Egg shape index	0.77±0.03	0.79±0.04	0.77±0.03	0.76±0.03	0.77±0.03

[a, b] means with different superscript letters across treatment diets are significantly different ($P < 0.05$)

As presented in Table 11, no significance difference was noted in egg weight among hens fed with various levels of SNLM. The mean yolk index, the yolk width, and yolk height recorded from birds fed on T1, T2, T3, T4 and T5 diets did not differ significantly. The results further revealed that the average yolk color observed in birds fed on T5 was higher ($P<0.05$) than those fed with T2 diets. On the other hand, the average albumen height and Haugh unit of eggs recorded from hens reared in T5was lower ($p>0.05$) than those fed with of T1, T2, and T4 diets.

Table 11: The effect of experimental feed on internal egg qualities at 23 weeks of age [mean±SD]

Internal egg quality	T1	T2	T3	T4	T5
Egg weight (g)	49.1±5.4	50.4±4.4	48.9±5.61	52.1±4.68	51.1±4.21
Albumen height (mm)	7.31±1.34[a]	6.89±1.9[a]	6.21±1.1[ab]	6.68±1.5[a]	4.95±1.1[b]
Haugh unit	88.1.±8.0[a]	85.±12.0[a]	81.6±6.6[ab]	83.04±9.5[a]	71.0±9.8[b]
Yolk height(mm)	17.4±1.5	17.3±1.4	17.1±1.1	17.3±1	16.7±0.9
Yolk width (mm)	34.7±1.4	36.2±1.02	37±1.63	36.1±2.12	34.8±6.2
Yolk index	0.50±0.04	0.48±0.04	0.47±0.03	0.48±0.03	0.50±0.14
Yolk color	6.3±2.3[ab]	5.9±1.6[b]	7.8±1.4[ab]	7.29±1.6[ab]	8.6±2.4[a]

[a,b,c] means with different superscript letters across treatment diets are significantly different(P < 0.05)

Correlation of internal and external egg qualities at 23 weeks of age

As shown in Table 12,egg weight of eggs collected at 23 weeks age was positively (P<0.01) correlated with egg length and egg width. On the other hand, egg weight was negatively correlated with shape index (-0.3), yolk index (-0.02), yolk color (-0.03) and Haugh unit (-0.03). Egg length was significantly correlated with egg width and albumen height; but was negatively correlated with shape index (-0.74). Egg width was positively (p<0.05) correlated with shell index (0.3), albumen height (0.34) and yolk height (0.3) Albumen height was positively correlated with yolk height (0.6), Haugh unit (0.97); but negatively correlated with yolk color (-0.13) and shell thickness (-0.1). Yolk height was positively (p<0.05) correlated with Haugh unit (0.5) and Yolk width (0.83). Yolk index was negatively correlated with Haugh unit (-0.03) and shell thickness (-0.04). Albumen height was strongly correlated with Haugh unit (0.97) and yolk height

(0.6). Yolk index was negatively correlated with Haugh unit (-0.03) and shell thickness (-0.04). Yolk color was negatively correlated with Haugh unit (-0.12).

Table 12: Correlation of internal and external egg qualities at 23 weeks of age

Traits	Ew	El	Ew	SI	AH	YH	YW	YI	YC	HU	ST
Ew		0.74**	0.7**	-0.3*	0.16	0.26*	0.23	-0.02	-0.03	-.03	0.6**
El			0.4**	-0.7**	0.48**	0.03	0.06	0.18	-0.09	0.1	-0.09
Ew				0.3**	0.34**	0.3**	0.3*	0.12	0.07	- 0.1	0.13
SI					-0.26*	0.17	0.13	-0.1	0.15	-0.2	0.20
AH						0.6**	0.17	0.04	-0.13	1.**	- 0.1
YH							0.32*	0.2	0.12	0.5**	0.02
YW								0.8**	0.03	0.2	0.13
YI									0.09	-0.03	-0.04
YC										-0.12	0.07
HU											-0.20
ST											

EW=egg weight, EL= egg length,EW=egg width,SI=shape index,AH=albumen height,YH=yolk hight,YW= yolk width=yolk index,YC=yolk color,HU= Haugh unit,ST=shell thickness

4.6.2. External and internal egg qualities at 28 weeks of age

The effect of experimental feed on external egg qualities has been presented in Table 13. The average shell thickness from hens reared on T3, T4and T5 were significantly (P<0.05) higher than those of T1. The average egg length from hens reared on T5 was the highest of all other treatments. The average egg length from hens reared on T1, T2, and T3 were significantly (P>0.05) lower than that of T4 and T5. The average egg width of hens reared on T4 and T2 was comparable. However, the average egg width from T5 was (P<0.05) higher than that of T1, T2, and T3. The average shape index values from hens reared in T2 was similar to those of T1, T3 and T4 diets while it was higher ($P<0.05$) than those reared in T5.

Table 13: The effect of experimental feed on external egg qualities at 28 weeks of age [mean±SD]

Parameters	T1	T2	T3	T4	T5
Shell thickness (mm)	0.32 ± 0.07^{b}	0.36 ± 0.06^{ab}	0.37 ± 0.06^{a}	0.38 ± 0.06^{a}	0.40 ± 0.04^{a}
Egg length (mm)	53.0 ± 1.8^{c}	53.1 ± 1.41^{c}	54.2 ± 1.2^{bc}	55.2 ± 1.31^{ab}	56.7 ± 1.76^{a}
Egg width (mm)	41.1 ± 1.14^{c}	42.2 ± 2.77^{ab}	41.8 ± 0.97^{bc}	42.8 ± 0.82^{ab}	43.1 ± 0.91^{a}
Egg shape index	0.78 ± 0.02^{ab}	0.79 ± 0.05^{a}	0.77 ± 0.02^{ab}	0.77 ± 0.02^{ab}	0.76 ± 0.02^{b}

^{a,b,c,d} means with different superscript letters across treatment diets are significantly different ($P < 0.05$)

As shown in Table 14, the average egg weight from hens fed with T5 was heavier ($p<0.05$) than that of other treatments. Hens from T4 also produced heavier ($p<0.05$) eggs than fed with T1, T2 and T3 diets. However, hens reared in T2, T4 and T3 diets did not differ statistically. The highest mean yolk height and its index was scored in hens reared in T5 while the lowest from those of T1 being significantly different from each other.The average value of albumen height,HU and yolk width did not vary among treatment diets. The highest mean value of yolk color was recorded in hens reared in T4 and T2 diets while the lowest from those fed on T1 and differed significantly. However, the average value of yolk color for hens reared in T1, T3 and T5 was similar.

Table 14: The effect of experimental feed on internal egg qualities at 28 weeks of age [mean±SD]

Parameters	T1	T2	T3	T4	T5
Egg weight (g)	50.3±4.03^{d}	52.3±2.87^{cd}	53.3±2.92^{c}	56.9±2.50^{b}	59.6±3.00^{a}
Albumen height (mm)	4.97±1.70	5.32±1.76	5.03±1.63	5.11±1.91	5.68±1.72
Haugh unit	70.3±14.5	72.4±13.4	69.9±12.2	67.8±18.9	72.3±14.1
Yolk height (mm)	16.5±1.35^{c}	17.5±1.5^{ab}	16.6±1.88^{bc}	17.6±1.29^{ab}	18.1±0.68^{a}
Yolk width (mm)	37.7±2.09	37.3±1.52	37.3±1.80	37.8±1.29	37.6±0.96
Yolk index	0.44±0.05^{c}	0.47±0.04^{ab}	0.45±0.05^{bc}	0.47±0.04^{ab}	0.48±0.02^{a}
Yolk color	6.53±2.32^{b}	8.12±1.90^{a}	7.82±2.16^{ab}	8.17±2.20^{a}	7.94±2.14^{ab}

^{a,b,c} means with different superscript letters across treatment diets are significantly different(P< 0.05)

Correlation of internal and external egg qualities at 28 weeks of age

As indicated in Table 15, the egg weight of those eggs collected at 28 weeks of age was positively correlated (p<0.05) with egg length, egg width, and yolk height and shell thickness. Egg length significantly correlated with egg width. Egg width was positively (p<0.05) correlated with shape index, albumen height and yolk height (0.4). Shape index was negatively correlated (p<0.05) with shell thickness (-0.04). Albumen height was positively correlated (p<0.01) with yolk height (0.6), yolk index (0.62) and Haugh unit (0.97).Yolk height was positively correlated (p<0.01) with yolk index (0.9), Haugh unit (0.55) and shell thickness (0.28).Yolk index was positively significantly correlated with Haugh unit (0.6) and shell thickness (0.3).

Table 15: Correlation of internal and external egg qualities at 28 weeks of age

Traits	Ew	EL	EW	SI	AH	YH	YW	YI	YC	HU	ST
Ewt		.80**	0.7**	0.1	0.19	.35**	0.23*	0.21*	0.2	0.02	0.21*
EL			0.4**	-0.5*	0.11	0.25	0.19	0.14	-0.04	-0.02	0.24*
EW				0.6**	0.3**	0.4**	0.20	0.23*	0.15	0.14	0.19
SI					0.16	0.12	0.02	0.09	0.16	0.15	-0.04
AH						0.6**	-0.12	0.62**	0.05	0.97**	0.22*
YH							0.07	0.9**	0.05	0.55**	0.28**
YW								-0.4**	0.20	-0.17	-0.07
YI									0.05	0.6**	0.3**
YC										0.01	0.14
HU											0.18
ST											

EW=egg weight,EL= egg length,EW=egg width,SI=shape index,AH=albumen height,YH=yolk height, YW= yolk width, YI =yolk index,YC=yolk color,HU= Haugh unit,ST=shell thickness

5. DISCUSSION

5.1. Nutrient content of Stinging nettle meal leaves

The CP content of SNLM found in the current study was comparable with values reported by Dereje *et al.* (2016). For dried stinging nettle leaves and Stinging nettle (*Urtica Simensis*) leaf samples collected from SNNPR, Wolaita zone, Bodditi area had more or less similar protein contents with a mean value of the values reported by Liu (2007) for the nettle hay (29.40%).

Also CP values found in stinging nettle in the current study were comparable to those reported for *Moringa setnopetala* and *Moringa oleifera* leaves (Aberra *et al.*, 2009; 2012a).The nutrient contents of SNLM indicated that it is rich in crude protein (29.4%), phosphorous (0.72%) and Ca (2.9%) contents. The protein content of raw Stinging nettle (*Urtica simensis*) was also found to be higher compared to commonly consumed vegetables in Ethiopia such Lettuce (*Lactuca sativa*) (15.5 %), Swiss chard (*Beta vulgaris*) (12.2 %), Kale (*Brassica carinata*) (8.0 %) and Spinach (*Spinacea oleracea*) (18.6 %) (EHNRI, 1997) .The CP values of experimental feeds were above16% which was dietary CP level recommended by Shewangizaw *et al.* (2011). This indicates that the leaves of Stinging nettle (*Urtica simensis*) might be another cheap source of plant protein for marginal resource communities of Ethiopia.

The crude fat composition of SNLM (5.8%) obtained from this study was comparable with the values reported by Odeyinka *et al*. (2008) and Asaolu *et al.* (2010) respectively for *M. oleifera* leaves (5.5-6.68%) and within the ranges reported by Aberra *et al*.(2009,2011, 2012a) for the *Moringa oleifera* and *Moringa stenopetal* leaves (4.73-

8.4%). The fat content was found to be higher than that of Malabar Spinach *(Basella rubra*) (0.86%),Bonongwe, mowa (Amaranthus hybridus) (Bhardwaj, R., *et al.* 2009).

The crude fiber content of SNLM (8.71%) obtained from the study result was less than the values reported by (Bangu *et al.,* 2015) for the same species. The variation could be location, maturity of the leaves and as the methods of processing the leaf meals. The crude fiber content of Stinging nettle (*Urtica simensis)* leaves was higher than those cultivated green leafy vegetables consumed in Ethiopia such as spinach(*Spinacea oleracea*) (4.60 %), lettuce (*Lactuca sativa)* (3.7%), Swiss chard (Beta vulgaris) (6.10%) and Kale (*Brassica carinata*) (7.50 %) Muchuweti M., *et al.*, 2009.

As protein and Calcium are important nutrients for growth, skeletal development, and egg production, the high Protein and Calcium level of SNLM made it a potentially good alternative feed resource to substitute the very expensive Nuge seed cake for layers.

The composition of calcium linearly increased as the level of stinging nettle leaf meal in the diet increases. This might be explained due to the fact that stinging nettle leaf meal contains relatively high calcium content. The phosphorous content was comparable in all treatment diets. However, all the treatment diets were in accordance with the recommended levels of calcium and phosphorous in terms of quantity and proportion (2:1 ratio) in practical grower birds diets and (6:1) layer hens under tropical conditions (Smith, 1990). In laying hens the use of nettle, extract via Lymphocyte proliferation can stimulate the innate cell mediated immune response (Sandru *et al.*, 2007).

5.2. Nutrient and Feed intake of response of layer hens

Voluntary feed intake could be defined as the amount of feed consumed by an animal or group of animals in a given period of time during which they have free access to it. It is important parameter to evaluate the nutritive value of animal’s feed.

The feed intake of experimental chickens result showed that substitution of Nuge seed cake with various levels of Stinging nettle (*Urtica simensis*) demonstrated an improvement trend in the feed intake of ISA Brown layer hens. The DM intake of layer birds improved in all Stinging nettle) leaf fed treatments. That means the feed intake per day perhen were not significantly different.This finding is in accordance with that of Allardice, (1993) who reported that nettles are a very nutritious food that is easily digested and is high in minerals (especially iron), vitamin C and pro-vitamin A). The body weight gain did not vary across the experimental diets up to the eight (8) weeks of experimental periods. The findings are in good agreement with those of Windisch *et al.* (2008) who reported that spices influence the gastrointestinal ecosystem mostly through growth inhibition of pathogenic microorganism's growth. So, it might be possible that the increase of digestion and absorption of essential nutrients due to increasing the enzyme activity and/or inhibition of pathogenic microorganism's growth could be the main reason of pennyroyal medicine plant to accelerate the performance. On the contrary to current studies, Esonu *et al*. (2006) also reported that higher dietary inclusion levels of*Azadirachta indica* leaf meal resulted in decreased weight gain of egg laying hens. This might be attributed to the effects of nutrient imbalance and poor metabolism by monogastric animals when fed high levels of unconventional feed ingredients. The current study showed that the inclusion rate of SNLM at 12% were significantly increased production. Feeding of different levels of SNLM to experimental birds showed significant improvements in final body weight, total body weight gain, weekly and daily body weight gain however, the average initial weight of birds in (T1, T2, T3, T4 and T5) were similar. Nettles are a very nutritious food that can be easily digested and contain minerals (especially Calcium), vitamin C and provitamin A (Allardice, 1993). Amino acids in dehydrated nettle meal are nutritionally superior to those of alfalfa meal (Hojnik *et al.*, 2007) and this could be also possible reasons for increased performances of ISA Brown layer hens fed with diets containing stinging nettle leaf meal. In this study, the inclusion of SNLM in the birds' diet up to the level 12% had a positive effect egg production than the control diet (T1).

The values of Tannins were very low compared to other indigenous wild vegetables. In fact this low concentration of condensed tannin was an advantage for not lowering the bioavailability of other nutrients. The feed conversion ratio was not significant throughout the experimental diets. However, birds fed with T4 diets showed slightly more fed conversion than the rest of the treatment groups.

There were no adverse effects of SNLM observed on the birds' health during the experimental period. In agreement with current observation, Kwiecien and Mieczan (2009) reported that supplementing the diet with plant material that is rich in active substances might have beneficial effects on the immune system and can be used as an alternative to antibiotic growth promoters.

Debersac *et al.* (2001) reported that a plant extract from rosemary enhanced hepatic metabolism and hence, increased relative liver weights in rats. Although, as observed by Born *et al. (*2006) that the increase in gizzard size is related to the volume of feed, increased time spent on grinding the feed and increased frequency of gizzard contraction which is needed the large particles for further digestion in the distal parts of the intestine, and small increases in the level of dietary fiber are needed to stimulate gizzard development. This was in line with observations suggested by Platel and Srinivasan (2005) and Suresh and Srinivasan (2007) that extracts from herbs and spices accelerated the digestion and shorten the time of feed passage through the digestive tract.

5.3. The effect of Stinging nettle leaf meal on Egg production and egg qualities of Layer hens

The egg production performance of (5) five different treatments of chickens were recorded. Average egg production per week per treatment was 55.50±14.68gm for hens of eggs in T5 significantly better than (T1, T2, T3, andT4) at 23 weeks of production period. It was significantly higher than local chickens, which lay 55 to 80 eggs/year (Dessie and Ogle, 2001). However, significantly lower than the layers reared on slat flooring with manure pit and deep litter had over 90 % egg laying capacity from26to 61 weeks of age (Gerzilov *et al.*, 2012).Since these were egg production at 23 weeks. The average HHEP was 79.29 forT5 were significantly (P<0.05) higher than 58.57 and 69.58 for T3 andT4, respectively and difference were significantly (P<0.05) higher than that of chickens receiving diets (T1, T2 and T3). This could be attributed difference in inclusion rate of 9% and 12% of SNLM used.

The eggs weight influences the weight of components of eggs especially egg albumen and yolk (Zhang *et al.*, 2005.), Egg weight is one of the important phenotypic traits that influence egg quality and reproductive fitness of the chicken parents (Islam *et al,* 2001, .Farooq *et al*.2001).The result also revealed that there was significant difference (P<0.05) in average weight of eggs collected from different treatments. The result of (first round) average experimental eggs weight in T5 was significantly (P<0.05) higher than reported by Hallima (2007) for RIR chicken breed eggs. However, the average egg weights obtained in this study at 28 weeks hens reared inT1 (60gm) were in line with (Olugbemi *et al.* (2009). The average shape index of all treatments was agreed with the finding of (Ebenebe *et al.* 2013) the same breed fed on Moringa Leaf Meal.

Ledvinka *et al.* (2000) concluded that the shell quality was one of the most important parameters for the technology of further egg manipulation. The result of shell thickness obtained in the current study *was* lower than the reported by Halima (2007) for eggs collected from intensively managed local chicken ecotypes of Northwest Amhara and RIR chicken breeds, respectively.

Haugh unit (HU) is calculated from the height of the inner thick albumen and the weight of an egg and it is considered to be a typical measure of albumen quality. Accordingly, however the average Hough unit of eggs of hens collected from the different treatments were good in quality based on the obtained average Haugh unit value (>70). However, the Haugh unit of eggs collected from T5 was significantly lower (P>0.05) than the report of (Ledvinka *et al,* 2010). This might be attributed to feed effects and genetic efficiency of ISA brown as well as freshness of eggs. Since the H*a*ugh unit value is highly correlated with storage condition and duration of eggs.

A statistically significant negative correlation was obtained between egg weight and H*au*gh unit and between egg weight and egg index (Table 12). Similarly, Ozcelik (2002), Iposu *et al*. and Shawkat (2002) reported significant negative correlations between HU and egg weight. On the contrary eggs collected at 28 weeks (Table14) indicated eggs weight was strongly positively correlated with external egg qualities like egg length egg width and shell thickness. Positive correlation was observed in between egg weight and other internal egg quality traits including; albumen height yolk height and yolk width Similarly, Silversides (1995) & Zhang *et al.* (2005) reported statistically positive correlation between egg weight and albumen height. The current study of average yolk heights of T4and T5 as well as yolk width of T4 and T5 (yolk height of T4and T5 and albumin height were significantly higher (P<0.05)than the report of Desalew *et al. (*2013).

The other most important internal egg quality traits considered in this study was yolk color and it was estimated using roach color fun (ranging 1-15). The mean yolk color result T4(8.06)and T5(7.86) from this experiment of second round egg collected was significantly higher($P< 0.05$) than the reported 3.48 and 4.0 by Halima (2007) for eggs collected from intensively managed local hens of North-West Amhara and RIR chicken breed hens, respectively. The higher yolk color value obtained from the experiment was due to SNLM which were rich in xanthophylls, some of which are precursors of vitamin A. The effect of different levels of stinging nettle leaf meal on egg production and egg quality was significantly different ($P < 0.05$) across treatment groups.

The significant improvement in weight gain, egg weight, Haugh unit and shell thickness were observed with inclusion level of SNLM. The result of the current study implies that SNLM up to 9%, 12% of inclusion level had significant effect in the health of birds. On the other hand, birds fed diets containing different levels of stinging nettle leaf meal were significantly superior in weight gain and quality egg production compared to the control diet. Birds fed diets with SNLM showed yellow coloration of shank, beak and skin than those fed control diet. According to (Fasuyi *et al.*, 2005; Aberra *et al.*, 2013).the stinging nettle not only serve as source of protein but also provide some necessary vitamins, minerals and oxycaretenoids which cause yellow color of shank and egg yolk. The current finding disagrees with the findings of Ozcelik (2002), Iposu *et al.* (1994) and Shawkat (2002) who reported significant negative correlations between HU and egg weight.

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

As stinging nettle (*Urtica Simensis*) abundant and cheap plants in high lands and mid high lands of Ethiopia, it is feasible using this plant for ration formulation of poultry diets. So, the substitution of Nuge seed cake with stinging nettle leaf meal (SNLM) up to 12% in the diet of egg layers had significant impacts by providing Protein and Calcium. The result indicated that appreciable total body weight gain, final body weight gain and daily weight gain in the whole experimental periods. Also the egg production and egg quality in terms of HHEP, egg mass, egg weight, egg length, egg width, shell thickness, yolk height, yolk width, albumen height, and yolk color were significantly increased at the inclusion rate of 9% and 12% of SNLM. The general improvement in the performance of chickens especially those kept under 9% and 12% levels together with their physical appearance provide sights to conclude that SNLM have supplied better nutritional benefits. Thus, the inclusion of stinging nettle leaf meal 12% in layers diet could be an alternative feeding strategy for substituting other expensive protein sources such as Nuge seed cake in areas where Stinging nettle is available.

6.2. Recommendation

The following recommendations are suggested based on the result of the current study:

Currently the demand of chickens and chicken products are increasing Ethiopia, commercialization of poultry production should be given an emphasis.

The Stinging nettle leaf meal inclusion level in the diets of layer hens at 12% is better to healthy of birds, egg production and egg quality.

Provision of proper trainings to chicken producers on how to formulate supplementary rations to village birds, using locally available feeds ingredients, could be important.

Further studies to determine the nutrient composition and amount of inclusion of the locally available feed stuffs especially, stinging nettle and quantify the economic importance of supplementation needs to be carried out.

As ISA brown birds are more productive and diseases resistance, provision of successive trainings on modern chicken husbandry practices to small scale producers, farmers any Enterprises who participate in poultry production would be essential for the improvement of chicken production and productivity.

The suitability of Stinging nettle (*Urtrica Simenesis)* inclusion rate about 12% and above for layer hens needs to be investigated, in commercial and breeding area for Hatchability and fertility.

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8. APPENDIX

Appendix Table 1: ANOVA result regarding initial body weight of ISA brown layer hens fed on different levels of stinging nettle *(Urtirica Seminesis S*) leaves meal.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	20283.316	5070.829	0.61	0.6536
Error	144	1190308.966	8266.034		
Corrected total	148	1210592.282			

Appendix Table 2:ANOVA result regarding final body weight of ISA brown layer hens feed on different levels of stinging nettle (*Urtirica Seminesis S*) leaves meal

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	86916.78	21729.19	39.59	<0.001
Error	139	76294.16	548.88		
Corrected Total	143	163210.94			

Appendix Table 3:ANOVA result regarding net body weight gain of ISA brown layer hens feed on different levels of stinging nettle leaves meal

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	108046.29	27011.57	3.13	0.017
Error	139	1197803.01	8617.29		
Corrected total	143	1305849.31			

Appendix Table 4:ANOVA result regarding total egg number

Source	DF	SumSquares	Mean Square	F Value	Pr > F
Model	4	78036.933	19509.23	178.98	<.0001
Error	10	1090.	109.		
Corrected total	14	79126.93			

R-Square	Coeff Var	Root MSE	TEP Mean
0.986	3.174	10.44	328.93

Appendix Table 5:ANOVA result regarding egg production per hen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	780.369	195.09	178.98	<.0001
Error	10	10.90	1.090		
Corrected total	14	791.27			

R-Square	CoeffVar	Root MSE	EPpHen Mean
0.986	3.174	1.044	32.893

Appendix Table 6:ANOVA result regarding hen housed egg production rate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2676.817	669.2	181.26	<.0001
Error	10	36.92	3.69		
Corrected total	14	2713.74			

R-Square	CoeffVar	Root MSE	HHep Mean
0.986395	3.154412	1.921458	60.91333

Appendix Table 7:ANOVA result regarding average egg weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	226.196	56.549	34.50	<.0001
Error	10	16.393	1.639		
Corrected total	14	242.589			

R-Square	Coeff Var	Root MSE	Egwt Mean
0.932	2.559364	1.280365	50.02667

Appendix Table 8:ANOVA result regarding total egg mass production

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	366.529	91.63	120.46	<.0001
Error	10	7.61	0.761		
Corrected total	14	374.14			

R-Square	CoeffVar	Root MSE	TEM Mean
0.98	5.21	0.872	16.74

Appendix Table 9:ANOVA result regarding egg mass production per hen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.67	0.92	120.46	<.0001
Error	10	0.08	0.01		
Corrected total	14	3.74			

R-Square	CoeffVar	Root MSE	EMpHen Mean
0.98	5.21	0.0872	1.674

Appendix Table 10:ANOVA result regarding egg mass production per hen and day

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1255.217	313.803	121.41	<.0001
Error	10	25.85	2.585		
Corrected total	14	1281.057			

R-Square	CoeffVar	Root MSE	EMpHend Mean
0.98	5.184	1.6077	31.013

Appendix Table 11:ANOVA result regarding total feed intake

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	65.377	16.344	1.53	0.27
Error	10	106.70	10.670		
Corrected total	14	172.08			

R-Square	CoeffVar	Root MSE	TFI Mean
0.379	5.84	3.27	55.95

Appendix Table 12:ANOVA result regarding feed intake per hen and day

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	229.6	57.4	1.59	0.25
Error	10	362	36.2		
Corrected total	14	591.6			

R-Square	CoeffVar	Root MSE	FIpHend Mean
0.388100	5.807571	6.016644	103.6000

Appendix Table 13: ANOVAresult regarding Feed conversion ratio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.764	3.6911	21.09	<.0001
Error	10	1.7505	0.17505		
Corrected total	14	16.5145			

R-Square	CoeffVar	Root MSE	FCR Mean
0.89	11.52	0.4184	3.633

Appendix Table 14: ANOVA result regarding total Egg weight at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	25.183	6.296	0.61	0.6641
Error	10	103.007	10.301		
Corrected total	14	128.189			

R-Square	CoeffVar	Root MSE	eggwt Mean
0.196449	6.394	3.2095	50.193

Appendix Table 15: ANOVA result regarding Egg length at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	8.436	2.101	1.37	0.312
Error	10	15.393	1.539		
Corrected total	14	23.83			

R-Square	CoeffVar	Root MSE	egg length Mean
0.354017	2.333310	1.240699	53.17333

Appendix Table 16:ANOVA result regarding Egg width at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4.784	1.196	2.61	0.0995
Error	10	4.58	0.458		
Corrected total	14	9.364			

R-Square	CoeffVar	Root MSE	Eggwidth Mean
0.510893	1.647412	0.676757	41.08000

Appendix Table 17:ANOVA result regarding egg shape index at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.002	0.00042	2.84	0.0822
Error	10	0.0015	0.00015		
Corrected total	14	0.0031			

R-Square	Coeff Var	Root MSE	Shape index Mean
0.532	1.566	0.0121	0.773

Appendix Table 18:ANOVA result regarding Egg albumen hight at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10.695	2.674	6.21	0.0089
Error	10	4.3037	0.4304		
Corrected total	14	14.9989			

R-Square	Coeff Var	Root MSE	Albumen hight Mean
0.713064	10.39116	0.656028	6.313333

Appendix Table 19 :ANOVA result regarding Egg yolk height at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.287	0.323	0.76	0.5724
Error	10	4.213	0.421		
Corrected total	14	5.5			

R-Square	Coeff Var	Root MSE	Yolk hight Mean
0.233939	3.773849	0.649102	17.2

Appendix Table 20:ANOVA result regarding Egg yolk width at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4.916	1.229	0.30	0.8708
Error	10	40.84	4.084		
Corrected total	14	45.756			

R-Square	CoeffVar	Root MSE	yolkwd Mean
0.10744	5.6703	2.021	35.64

Appendix Table 21:ANOVA result regarding Egg yolk index at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.0032	0.0008	0.54	0.708
Error	10	0.015	0.00147		
Corrected total	14	0.018			

R-Square	Coeff Var	Root MSE	Yolk index Mean
0.178439	7.887128	0.038384	0.486667

Appendix Table 22:ANOVA result regarding Egg yolk color at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.53817333	3.63454333	4.22	0.0295
Error	10	8.6135	0.86134667		
Corrected total	14	23.152			

R-Square	CoeffVar	Root MSE	Yolk color Mean
0.627954	12.72748	0.928088	7.292000

Appendix Table 23: ANOVA result regarding Haugh unit at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	560.836	140.209	9.11	0.0023
Error	10	153.833	15.383		
Corrected total	14	714.669			

R-Square	Coeff Var	Root MSE	HU Mean
0.78475	4.837	3.9222	81.09

Appendix Table 24: ANOVA result regarding Egg shell thickness at 23 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00129	0.00032	1.21	0.365
Error	10	0.003	0.0003		
Corrected total	14	0.004			

R-Square	CoeffVar	Root MSE	shelthik Mean
0.326599	6.186	0.016	0.264

Appendix Table 25:ANOVA result regarding Egg weight at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	168.576	42.144	54.92	<.0001
Error	10	7.673	0.767		
Corrected total	14	176.249			

R-Square	CoeffVar	Root MSE	eggwt Mean
0.956463	1.607491	0.875976	54.49333

Appendix Table 26:ANOVA result regarding Egg length at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	27.991	6.998	18.48	0.0001
Error	10	3.787	0.379		
Corrected total	14	31.778			

R-Square	CoeffVar	Root MSE	egg length Mean
0.880837	1.129375	0.615359	54.48667

Appendix Table 27:ANOVA result regarding Egg width at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.620	1.905	9.10	0.0023
Error	10	2.09	0.209		
Corrected total	14	9.71			

R-Square	CoeffVar	Root MSE	Eggwidth Mean
0.784489	1.085050	0.457530	42.16667

Appendix Table 28:ANOVA result regarding shape index at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.002027	0.0005066	6.91	0.0062
Error	10	0.000733	0.000073		
Corrected total	14	0.00276			

R-Square	CoeffVar	Root MSE	Shape index Mean
0.734300	1.106394	0.008563	0.774000

Appendix Table 29:ANOVA result regarding Egg albumen height at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.227	0.3068	0.30	0.8700
Error	10	10.154	1.01533		
Corrected total	14	11.3804			

R-Square	CoeffVar	Root MSE	Albumen ht Mean
0.10783	19.284	1.0076	5.225

Appendix Table 30:ANOVA result regarding Egg yolk height at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.924	1.481	1.91	0.1853
Error	10	7.753	0.775		
Corrected total	14	13.677			

R-Square	CoeffVar	Root MSE	yolkht Mean
0.43313	5.10354	0.881	17.253

Appendix Table 31:ANOVA result regarding Egg yolk width at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.167	0.292	0.52	0.725
Error	10	5.63	0.563		
Corrected total	14	6.8			

R-Square	Coeff Var	Root MSE	Yolk width Mean
0.172	1.9962	0.751	37.6

Appendix Table 32:ANOVA result regarding Egg yolk index at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00351	0.000877	1.71	0.2243
Error	10	0.00513	0.00051		
Corrected total	14	0.0086			

R-Square	Coeff Var	Root MSE	Yolk index Mean
0.405864	4.946913	0.022657	0.458000

Appendix Table 33:ANOVA result regarding Egg yolk color at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.88260	1.4707	1.03	0.4395
Error	10	14.3217	1.4322		
Corrected total	14	20.2043			

R-Square	CoeffVar	Root MSE	yolkcol Mean
0.2912	15.617	1.1967	7.663

Appendix Table 34:ANOVA result regarding Haugh unit at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	42.669	10.667	0.18	0.9432
Error	10	590.487	59.049		
Corrected Total	14	633.156			

R-Square	CoeffVar	Root MSE	HU Mean
0.0674	10.89	7.684	70.56

Appendix Table 35:ANOVA result regarding Egg shell thickness at 28 weeks of age

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.01044	0.0026	3.21	0.0614
Error	10	0.008	0.00081		
Corrected total	14	0.01857			

R-Square	Coeff Var	Root MSE	Shell thikness Mean
0.562096	7.806295	0.028519	0.3653

BIOGRAPHICAL SKETCH

Galaye Ganebo the author was born in SNNPR wolaita zone Damot Gale District in 1977 G.C He started his primary Education at Grumo kosha elementary school in 1985G.C and completed Primary and secondary school at the same school in 1993G.C. He continued his secondary school at Soddo senior High school in 1994 G.C and completed in 1997 G.C.Then joined the former Debub university Awassa college of agriculture 1998G.Cand graduated with diploma in General Agriculture (Gage)2001G.C .After graduation ,he served at Damot Gale District in the office of Agricultural and rural Development .Then He joined Ambo university in advance standing program in 2006 G.C. and graduated with BSc degree in Animal production in 2008.G.C. Then after Graduation he served as an expert of live stock in Damot Gale District. He joined School of Graduate Studies at Hawassa University in 2015G.C to pursue his Degree of Master in the field of Animal Production.