PARADIGM SHIFT IN POULTRY FEEDING: THE DEVELOPMENT OF OMEGA 3 ENRICHED EGGS

BY

CHARLISA AFUA BROWN (B.Sc. AGRICULTURE)

A THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE
KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

OF

MASTER OF PHILOSOPHY
(ANIMAL NUTRITION)

FACULTY OF AGRICULTURE
COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

OCTOBER, 2016

DECLARATION

I**, Charlisa Afua Brown,** hereby declare that the work presented in this thesis is the result of my own effort and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

Charlisa Afua Brown (PG 1624914)	…………………..	…………………
(Candidate's Name and ID)	Signature	Date

Certified by:

Dr. Jacob A. Hamidu	…………………..	…………………
(Project supervisor)	Signature	Date

Mr. Worlah Y. Akwetey	………………..	…………………
(Head of Department)	Signature	Date

DEDICATION

This thesis is dedicated to my mentor Dr. Jacob Alhassan Hamidu and to my mum Mrs. Comfort Asibuo Brown.

ACKNOWLEDGEMENTS

Eternity will not be long enough to express my gratitude to you, my God Almighty for bringing me this far. If it had not been for the Lord on my side, where would I be? From the depth of my heart I say the glory is all yours and I am grateful.

I would like to express my profound gratitude to my academic supervisor, Dr. Jacob Alhassan Hamidu for his advice, encouragement, assistance and patience throughout this project. Thank you for trusting in me. I would also like to thank all the lecturers of the Department of Animal Science especially Dr. Oscar Simon Olympio and Dr. Kwaku Adomako who together with my supervisor assisted me greatly in my field work and also to Dr. Lawrence Borquaye, Prof. Ray Voegborlo, Joseph Nana Gyesi, Kingsley Adukpoh Enoch and Abigail H. Ntiamoah all of the Department of Chemistry for assisting me in my laboratory work.

My great appreciation goes to the Grand Challenge Canada under the Saving Brian Programme for funding my project. Special thanks to the proprietor of V.O.A.-LYS FARMS LIMITED, Mr. Victor Oppong Agyei for his immerse care and for building a poultry structure for my department purposely for my research.

I wish to express my appreciation to my father, Mr. John Charly Brown and my mum, Mrs. Comfort Asibuo Brown for their support, encouragement and prayers towards my education, God bless them. Many thanks to Dr. Kwabena Peprah, Samuel Akomeah Bright, Auntie Amina Ibrahim, Uncle Timothy Asibuo, Auntie Esther Opoku Kwarteng and all the members of my family for their love, care and prayers.

Finally, I will like to say a big thank you to Mr. Roland Adama, Victoria Kyeiwaa, Yaw Osei Bonsu, my able teaching assistants, Richard Koblah Agbehadzi, Charles Datsomor, Bernard Quaye and Michael Adu Amankrah for their immerse help through feed preparation to vaccination of my birds.

ABSTRACT

The objective of this study was to determine the enrichment levels of chicken eggs with the inclusion of varying levels of flaxseed oil in layer diet. As part this study, a preliminary trial was conducted to assess the potential of an indigenous seed in Ghana as a source of omega-3 polyunsaturated fatty acids. Oils from egusi (melon seeds) and werewere (neri seeds) seeds were extracted and omega-3 and omega-6 content evaluated using the Gas Chromatography technique. Following the results, 80 commercial Lohmann tradition layers (n=20 per treatment) were randomly allotted to 4 dietary treatments with varying levels of flaxseed oil (control (0%), 1.5%, 3% and 4.5%) from 39 weeks of age of layers until 70 weeks. Individual omega-3 and omega-6-fatty acids were extracted from the egg yolk beginning 41 to 70 weeks and analysed using Gas Chromatography technique. Egg quality was also determined as the level of flaxseed oil increased in the diet in addition to storage duration of eggs over a period of 14 days. At 70 weeks of production, 5 birds from each treatment were randomly selected, euthanized by cervical dislocation and dissected for evidence of fatty liver haemorrhage syndrome. The data was analysed using the SAS Proc. GLM procedure and ls means separated by the PDIFF procedure of SAS at $P<0.05$. The results showed that both egusi and werewere contained 61.18% and 54.03% linoleic acid (an omega-6 fatty acid) respectively but both had no trace of omega 3 fatty acids. By substituting feed ingredients such as maize, soyabean meal and fishmeal in layer diet with 3% flaxseed oil, there was about 0.2 mg/ml deposition of omega-3 and omega-6 fatty acids deposited chicken eggs. The level of deposition of omega 3 (n;3 PUFA) and omega 6 (n;6 PUFA) in the 3% inclusion of flaxseed oil was 2.58 fold higher than the control which had no flaxseed oil. For individual n-3 PUFA levels, the fold increases from control diet to the 3% oil inclusion were: 18:3 n-3 (α-linolenic acid, (ALA) = 1.55; 20:3 n-3 (Eicosatrienoic acid, (ETA) = 16.79; 20:5 n-3 (Eicosapentaenoic acid, (EPA) = 0.15; 22:3 n-3 (Docosatrienoic acid) = 27.81; 22:5 n-3 (Docosapentaenoic acid, (DPA) = 1.6; and 22:6 n-3

(Docosahexaenoic acid, (DHA) = 3.22. Haugh unit value, which is a measure of protein quality in the albumen of eggs was 81.2 in the 3% flaxseed oil group compared to the rest of the treatment (Control (81.0), 1.5 (79.9) and 4.5 (80.8). The treatment with 4.5% flaxseed oil had heavier ($P < 0.05$) egg weight of 63.81g compared to the rest (Control (61.3g), 1.5 (61.7g) and 3 (60.7g). When eggs were stored over a period of 14 days in ambient temperature there was strong reduction in albumen quality ($y = -2.3444x + 97.15$, $R^2 = 0.96$) with increasing days of egg storage, an indication of increasing loss of carbon dioxide and moisture from the eggs. There was little effect ($P>0.05$), of feeding treatments on egg quality. There was no difference ($P>0.05$) in live weight of birds, liver weight and abdominal fat weight between the treatments. The treatments with 3% and 4.5% flaxseed oil had higher ($P<0.05$) liver haemorrhage score compared to 1.5% flaxseed oil inclusion and control. This could mean continuously feeding of birds on high amount of the oil diets and PUFA impact the health of the birds negatively. However, there were no mortalities associated with the haemorrhage score. Therefore, further studies should examine the optimum interval to ensure proper egg enrichment without jeopardizing the health of the birds.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

AA = Arachidonic acid

ALA = Alpha- linolenic acid

AOAC = Association of Official and analytical chemist

ARMD = Age-related mascular degeneration

BHT = Butylated hydroxyl – toluene

CHO = Carbohydrate

CP = Crude protein

CVD = Cardio vascular disease

D6DE = Delta-6-desaturase enzyme

DGLA = Dihomo – gamma linolenic acid

DHA= Docosahexaenoic acid

DPA= Docosapentaenoic acid

DSA = Docosatetraenoic acid

EPA = Eicosapentaenoic acid

FAO = Food and Agricultural organisation

FLHS = Fatty liver haemorrhage syndrome

GLA= Gamma linolenic acid

GSS = Ghana statistical service

HDL = High density lipoprotein

HU = Haugh unit

ISSFAL = International society for the study of fatty acids and lipids

Kcal = kilo calorie

LA = linoleic acid

LCPUFA= Long chain polyunsaturated fatty acid

LDL = Low density lipoprotein

MoFA = Ministry of food and agriculture

mt = Metric tonne

MUFA= Monounsaturated fatty acid

NGO = Non - Governmental Organisation

NRC = National research council

SDA= Steridonic acid

Tbsp. = Tea spoon

VLDL = Very low density lipoprotein

CHAPTER ONE

1.0 INTRODUCTION

Egg is generally considered a complete food since it is an excellent source of easily digestible proteins, vitamins, minerals, carotenoids and fatty acids and form part of proper nutrition for all ages, especially, children (Song and Kerver, 2000; Ayim-Akonor and Akonor, 2014). Poultry eggs (i.e. table eggs) are a common food and one of the versatile ingredients used in cooking nutritious foods. Nonetheless, it is noticed that egg consumption is declining worldwide over the years (Sumner, 2008). While this puts most poultry industries at risk with most business avenues collapsing, it is a large source of protein that is ignored especially in developing countries. The industry suffers a lot due to the early controversial reports with respect to egg cholesterol in raising human blood or serum cholesterol levels that may put human health at risk (Weggemans *et al.,* 2001). This fear has as a result blurred this complete food from many people’s menu (Herron and Fernandez, 2004). However, many clinical researches more recently have demonstrated that serum cholesterol levels do not depend on consuming eggs (Song and Kerver, 2000; Chakrabarty *et al.,* 2004; Fernandez, 2006; Nakamura *et al.,* 2006; Djoussé and Gaziano, 2008; Chai *et al.,* 2009; Scrafford *et al.,* 2009).

Recently, some researchers Goldberg (2014) and Virtanen *et al.,* (2016) downplayed the argument on egg cholesterol and removed eggs from among the list of foods that increases the risk of cardiovascular diseases. However, such results and recent assurances need time to mature in consumer’s minds, before leading to increase in egg consumption.

The recent campaign to increase worldwide egg intake is forcing researchers and government to explore alternative procedures of checking the cholesterogenic properties of eggs. This led to the development of designer eggs or eggs fortified with other nutrients which have become popular in Europe and North America (Manohar *et al.,* 2007).

Polyunsaturated fatty acids (PUFA) are among the limited egg nutrients which are of significance to the human wellbeing, especially the omega 3 polyunsaturated unsaturated fatty acids (n:3-PUFA) and its interaction with omega 6 fatty acids for proper balance in the body (Simopoulos, 2000, Manohar *et al.,* 2007). Since the n:3-PUFA is not synthetized in human body (Meyer *et al.,* 2003) its fortification into eggs turned out to be an important technique in changing omega-6 (n:6) to omega-3 (n:3) proportion to the required ratio and therefore check the portion of cholesterol in eggs. The n:3 polyunsaturated unsaturated fatty acids (n:3 PUFA) have been perceived as good source of nutrient for human wellbeing (Palmquist *et al.,* 2005) for the past few decades.

It is essential to take n:3 fatty acids during pregnancy since it plays a part in normal foetal brain development which in this manner enhances neurodevelopment (Wahlqvist, 1998). Hence deprivation during pregnancy is associated with lower development and lower behavioural scores (Coletta *et al.,* 2010). The n:3 present in the diet of children conditions the visual and cerebral capacities, including intellectual capacity. Dietary n:3 PUFA may also play a key part to treat or eliminate various ailments such as coronary illness (Wahlqvist, 1998), mental problems (Ramakrishnan *et al.,* 2009), prevent some neuropsychiatric disorders, especially depression, and in addition dementia, strikingly in the elderly (Bourre, 2004). These and many confirm seventeen benefits derived from n:3 PUFA (Hjalmarsdotirr, 2016). These benefits include evidence that: omega-3s can fight depression and anxiety, omega-3s can improve eye health, promote brain health during pregnancy and early life, improve risk factors for heart disease, reduce symptoms of Attention deficit hyperactivity disorder (ADHD) in children, fight autoimmune diseases, decrease mental disorders, fight age-related mental decline and Alzheimer's disease, help prevent cancer, improve bone and joint health, alleviate menstrual pain, may improve sleep and fats are good for the skin.

Other past researches stressed the part this important nutrient plays in human health (MacLean *et al.,* 2006; Bernstein *et al.,* 2008; Brunner *et al.,* 2009; Yashodhara *et al.,* 2009). Also n:3 PUFA can play a vital role in lowering blood viscosity and pressure, plasma triglycerides, platelet aggregation and cardiac arrhythmia (Simopoulos, 2000).

Omega-3 fatty acid eggs are eggs fortified with flax products through flax fed to laying hens. These eggs contain the essential n:3 fatty acids, alpha-linolenic acid (ALA), plus two other n:3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic (DHA). The n:3 enriched eggs through this procedure provide about 12 times more n:3 fatty acids than regular eggs, based on an average n:3 content of 0.5 grams in n:3 enriched eggs versus 0.04 grams in regular eggs (Morris, 2003).

The feeding value of the egg can be influenced by the formulation and composition of layer diets (Van and Huyghebaert, 1995). And this has led several interested researchers in the production of designer eggs through this mechanism. Several experiments, for most part in western nations, have been directed on enrichment of n:3 unsaturated fats in eggs and the production parameters of layers (Cherian *et al.,* 2002; Bean and Leeson 2003; Mazalli *et al.,* 2004; Jia *et al.*, 2008), egg quality (Galobart *et al.,* 2001a; Ajuyah *et al.*, 2003; Cherian 2008), sensory evaluation (Ahn *et al.,* 1996; Gonzalez-Esquerra and Leeson 2000; Rymer and Givens, 2005) and health benefits (Lewis *et al.,* 2000; Narahari, 2003; Payet *et al.,* 2004). However, no study has been accounted to date on any part of designer eggs production and their effect on egg quality and consumer's acceptability in Ghana and for that matter Sub-Saharan Africa where this nutrition is crucially needed. Indigenous studies on this research is vital as accessibility of feed ingredients used to produce these designer eggs are different in various regions of the World and locally accessible ingredient might give a chance of more economical generation of these designer eggs. Numerous commercial organizations are creating a few assortments of designer eggs which are accessible at a premium cost in developed countries yet their strategy for production stays patent and sometimes as a business mystery which are not available for repetition. Hence this research seeks to add new information to the development of n:3 designer eggs in Ghana.

1.1 General Objective

The general objective of this study was to determine the effects of incorporating varying levels of flaxseed oil in layer diets on egg omega -3 fatty acid profile and potential consumption by human.

1.2 Specific Objectives

1. To study the egg consumption and production patterns in some municipalities in Ghana.
2. To ascertain the availability of indigenous n:3 enriched seeds in Ghana as potential substitute for flaxseed oil for eggs enrichments locally.
3. Evaluate n:3 enrichment procedures and enrichment potential through laying hens diet.
4. To investigate the effect of feeding n:3 enriched diets on important organ such as liver and abdominal fat deposition of the birds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE POULTRY INDUSTRY IN GHANA

Poultry farming is the raising of domestic birds for their eggs, meat and or feathers. These domestic birds include chicken, guinea fowl, turkey, ducks and geese (Ray and Roy 1991). Poultry farming involves several operations which comprises farming for basic needs at one end and highly commercial farming at the other end (Department of Animal Husbandry, 2005). The poultry industry provides employment, income and healthy protein. Despite all challenges the poultry industry in Ghana has a high potential to grow. Recently, the industry was identified as the primary sector with the potential to create job and help address the short fall in the supply of animal protein (Anang *et al.*, 2013).

Dating back to the 1960s, the Ghanaian government gave impetus to poultry production which resulted in a large number of poultry farms being established as a profit making enterprises. These farms were mainly suited at the southern parts of the country where there was ready market for fresh eggs and poultry meat (Buamah, 1992). However, the industry began to experience challenges later due to irregular supply of day old chicks and later the outbreak of New Castle Disease. As part of the government's effort to support the growth of the industry later, it subsidised custom duties on poultry inputs in 1970. Also both private and public Veterinary services opened their doors to Poultry farmers in the country (GSS and Macro, 2008).

According to the FAO (2014), poultry production in the southern regions of Ghana is growing steadily and has subsequently resulted in a rise in Ghana's livestock production. Between 2000 and 2007, poultry production in Ghana increased by 80% while that for total cattle production increased by a meagre 8% (FAOSTAT, 2013). National hen eggs increased from 23,320 tonnes in 2002 to 39,750 tonnes in 2011 (FAOSTAT, 2013).

The layer population keeps rising because there is a significant increase in the number of layer poultry farms in the Kumasi and Dormaa Ahenkro area between 2001 and 2011 (FAOSTAT, 2013). According to Gyening (2006), various commercial layer farms have enlarged their operations to house 50, 000 flock or more.

Both exotic and indigenous birds are raised in Ghana. Local Ghanaian Fowl (Village chicken) can mostly be found in more rural areas (Northern Upper East and Upper West) and others can be found in other regions. Exotic birds are usually kept for commercial purposes in Greater Accra, Brong-Ahafo and Ashanti regions which are urban areas with a ready market for their eggs. These Exotic Breeds are mostly imported or hatched from established parent stock in the country or imported eggs (FAO, 2013).

2.1.1 Challenges of the Poultry Industry

2.1.1.1 Poultry Feeds and Feed Ingredients

Insufficient feed in certain period of the year is a one of the major drawbacks of the poultry industry in Ghana. The quantity and quality of the feed at such scarce period is compromised. Fluctuating availability of feed ingredients for compound feed manufactures is also a setback. Some of the ingredients are not produced locally like fish meal while locally produced ingredients like maize is insufficient to meet the demands of the poultry industry (FAO, 2014). Ingredients like fishmeal, concentrate, premix and soybean which the industry cannot do without are imported. In 2010, 316 soybean meal, 9,058 metric tonnes of concentrate, 10,639 metric tonnes of fishmeal and 672 metric tonnes of premix was imported to the country. All these figures increased in 2011 except that for concentrate which declined to 516 metric tonnes. In 2011, 16,924 metric tonnes of soybean meal, 12,624 metric tonnes of fishmeal and

1,030 metric tonnes of premix was imported. In 2012, the value for soybean meal imported increased over 10 times to 51,817 metric tonnes respectively (Animal Production Directorate of MoFA, 2003).

Compounding the challenges of feeds, the increase in the cost of feed ingredients and a high interest rate on loans does not permit purchase of ingredients at appropriate time when market prices are lower. As a result, there is low output of the poultry industry as poultry farmers cut down on their flock size (FAO, 2014). Maize is one of the primary ingredient that is used in poultry production and is an example of an ingredient which has a price fluctuation since it is a seasonal crop. Its prices are low when it is in season and increases at a faster rate when it is out of season. Often prices are at peak between April and August but lower between September and December (Figure 1).

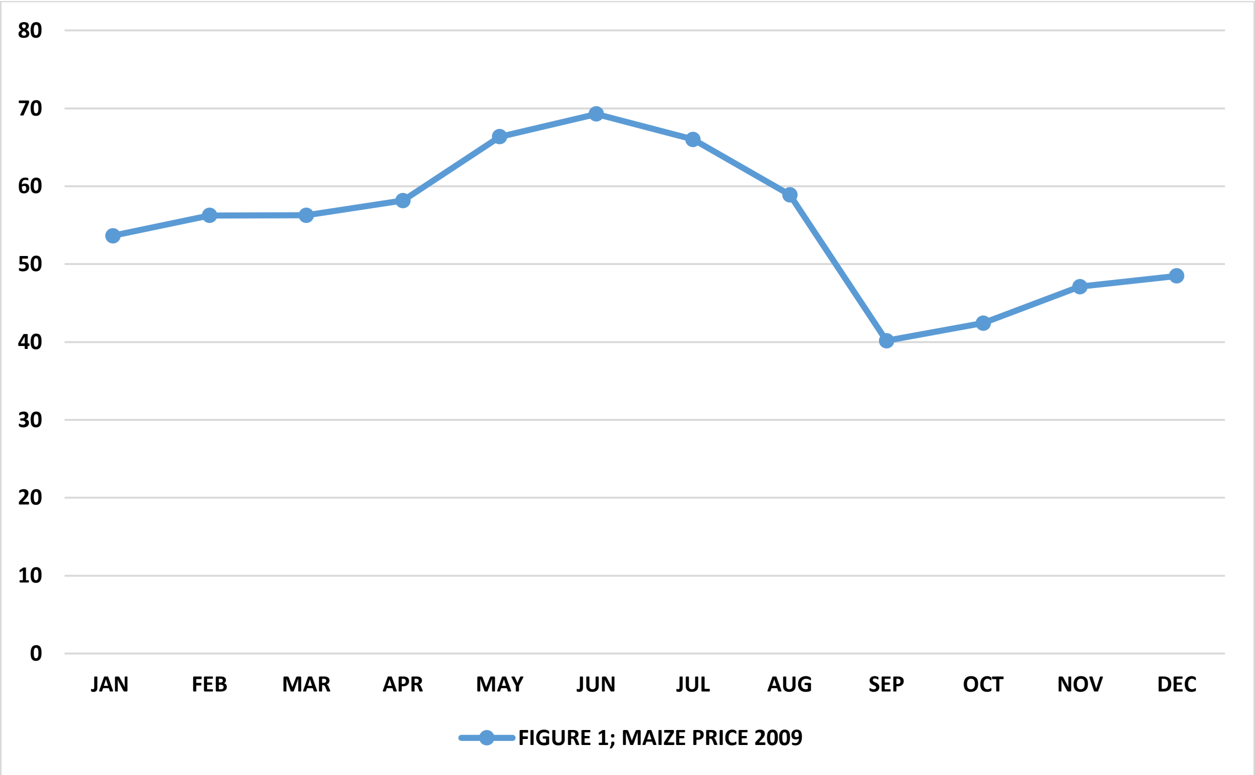


Figure 1: Trend in the price of maize in 2009; *Source:* SRID MoFA, 2011

Table 1: Poultry eggs production and supply in Ghana (tonnes)

Year	Production	Export	Import	Supply
2001	22 260	0	96	22 356
2003	24 380	196	62	24 246
2005	25 183	0	107	25 290
2007	31 270	16	51	31 305
2008	33 655	26	45	33 674
2009	36 700	4	20	36 716
2010	36 700	3	36	36 733
2011	39 750			

FAOSTAT, 2013

2.1.1.2 Price

The price of poultry products continuous to change rapidly at unpredictable amounts. According to some data compiled from ARI technical report by Anning (2006), the price of day-old chicks rose enormously between 2001 and 2005 from 75 percent to 122 percent. Also, the prices of poultry feed increased by 96.4 to 106.7 percent. He added that the rise in feed cost corresponds with the market price of maize which is produced locally but usually supplemented with imports. Subsequently, the cost of producing an egg increased from ¢367.3 to ¢762.0 from 2003 to 2004 and over a period of 5 years' price was 107.5 percent increase (Table 2). The price of poultry feed ingredients from the year 2012 to July 2013 also increased with the exception of soya meal. Both white maize and wheat bran increased by 7.10%. Oyster shell by 14.30% and day old chick by 33.30%. However, this did not affect the returns on investment (Table 3).

Table 2: Price changes of inputs of poultry production (Ghana Cedi, GH₵))

ITEM	2001	2002	2003	2004	2005	% Increase on 2001
Layer day old chicks (¢ × 1,000)	4.5	5	6.6	7	10	122.2
Average feed cost (¢×1000/45kg)						
Chick starter	75	75	93	112.8	155	106.7
Grower	65.5	65.5	75.7	89.8	130	98.5
Layer	72	72	87.3	107.5	147	104.2
Average cost of medication/bird						
Layer (up to 16 weeks)	2.3	2.5	2.6	2.3	2.6	
Cost of maize (¢× 1000)	1.2	1.3	1.8	3	4.4	266.7

Source: Compiled from data from ARI Technical Reports

Table 3: Prices of Inputs for Poultry Production (Ghana Cedi, GH₵)

Feed ingredient	Quantity (kg)	2012	July 2013	Change
Wheat Bran	25	7	7.5	7.10%
Maize – Yellow	50	37	40	8.10%
Soya Meal 49% - Local	50	70	70	0.00%
Oyster Shell	50	7	8	14.30%
Salt	1	0.4	0.5	25%
Layer Premix	1	2.9	2.96	2.10%
Poultry vaccines**				
HB1	1 dose	0.008	0.008	
Lasota	1 dose	0.008	0.008	
IBD 1	1 dose	0.01	0.01	
Newcavac	1 dose	0.085	0.085	
Fowl Pox	1 dose	0.015	0.015	
DOC***				
Local brown layer Single	Single	1.5	2	33.30%

Sources: * Asutsuare Poultry Farm Ltd ** VSD, 2013 ***Multivet, Gh.Ltd, 2013

2.1.1.3 Demand and Supply of Poultry Products

A survey of MOFA (2002) estimated the annual poultry production to be 14, 000 metric tonne and 200 million metric tonne (mt) for meat and eggs respectively. Only 34.28% of the meat demanded by Ghanaians was produced locally from 2007 to 2010 whilst 65. 72% of the demands were imported. According to MOFA (2002), the livestock and poultry subsector produced only 40% to the national animal protein supply. The remaining 60% is from mainly fish and few other sea foods.

Egg production for commercial purpose is next in line with the keeping of local Ghanaian chicken (village chicken) in the Ghanaian poultry industry (Okantah *et al.,* 2003). Eggs as compared to poultry meat face minor competition from importation. The table below shows the national egg production from 2001 and 2011. It was estimated that 257, 651 of eggs were produced locally in Ghana while only 601 was imported from other neighboring countries.

Table 4: Poultry egg demand and supply in Ghana (* 1000 mt)

Year	Eggs			
	Production	Export	Import	Demand
2001	22 260	0	96	22 356
2002	23 322	0	80	23 402
2003	24 380	196	62	24 246
2004	24 181	170	104	24 455
2005	25 183	0	107	25 290
2006	-	-	-	-
2007	31 270	16	51	31 305
2008	33 655	26	45	33 674
2009	36 700	4	20	36 716
2010	36 700	3	36	36 733
2011	36 750	-	-	-

Source: Ministry of trade and industry, 2011

2.1.1.4 Trends of Layer Production in Ghana

Egg production is a variable phenomenon and can be affected by unfavorable conditions, filthy environment, diseases and management conditions. A drop in egg production of 10- 15% has been reported by Mousa *et al.* (2002) in flocks having fatty liver syndrome. North (1984) reported house temperature of 65 to 75^{0}F for optimum egg production. However, an increase in house temperature up to 80^{0}F didn't cause a decline in egg production but egg size was reduced above 75^{0}F. Koelkebeck *et al.* (1999) reported that there is poor egg production performance when birds were supplied with water from farm well than birds receiving water from public health supply. Light exposure is reported to play a significant role in egg production as well (Alton *et al.*, 2000).

The output level of layer production in Ghana has been relatively constant over the last 9 years according to a survey from FAOSTAT (2013) which reported Ghana's output level of eggs (in shell) from 2002 to 2011 (Figure 2). From their report Ghana's layer output level rose slightly from 2001 to 2005 which was 22,260, 24,380 and 25, 183 tonnes for 2001, 2003 and 2005 respectively. There was a sharp increase in the output produced from 2005 to 2009; 25,183, 31,270, 33,655 and 36, 700 tonnes for 2005, 2007, 2008 and 2009 respectively which may be attributed to the effort of the NGOs. Most of these NGOs started operating by assisting the layer farmers at that time. Layer production from 2009 to 2010 was relatively constant and sharply increased from 36,700 tonnes in 2010 to 39,750 tonnes in 2011 (FAOSTAT, 2013).

Higher egg production has been reported to result in higher profit (Chung *et al.*, 1983). Some of the major layer production areas in Ghana are Brong Ahafo Region specifically Dormaa Municipality (Dormaa Ahenkro, Dormaa East and west and central), Sunyani; Greater Accra Region in the districts such as Ga-East, Ledzekuku-Krowor, Ashaiman and Ashanti Region (Kumasi) (Mensah-Bonsu *et al.*, 2010).

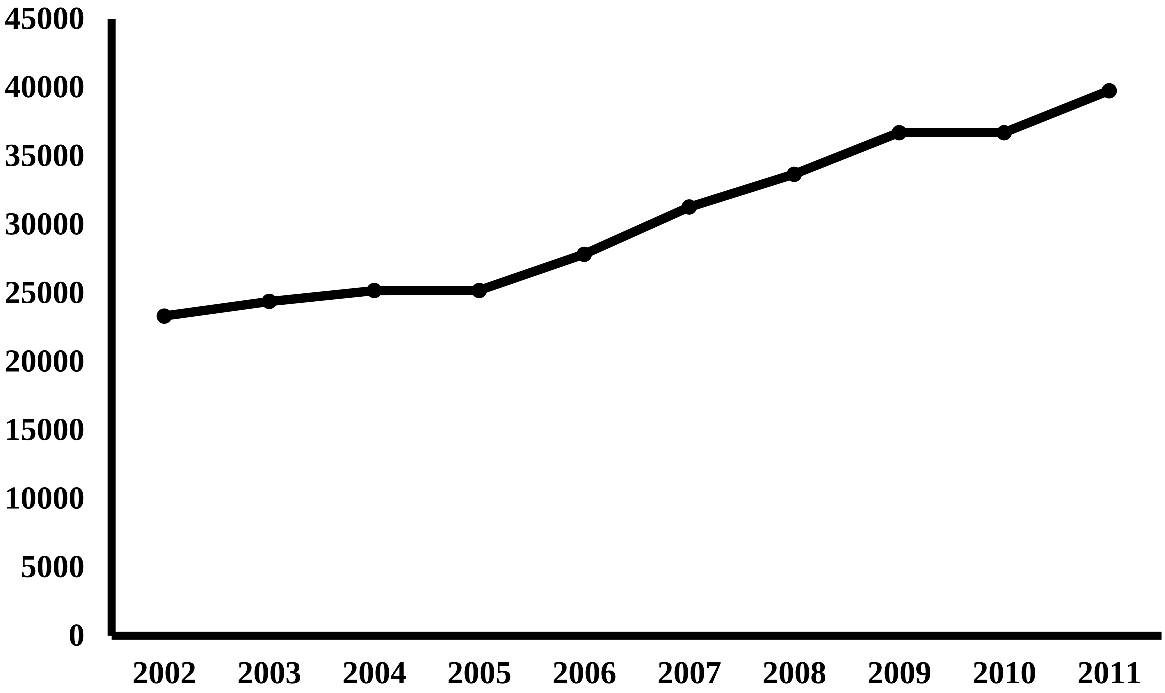


Figure 2: National hen egg (in shell) production (tonnes); *Source:* FAOSTAT, 2013

2.2 OIL SEEDS

Oilseeds are seeds that contains an appreciable amount of oil. The amount of oil in an oil seed ranges between 20% for soyabean and 40% for sunflower and canola (Sarwar *et al.,* 2013). Examples of seeds that contains oil include, flaxseed, coconut, cotton seed, olive seed, peanut, soyabean, canola etc.

2.2.1 Flaxseed

The seed from the flax plant is called flaxseed (*Linum usitatissimum* L.) and also called Linseed. It is a member of the Linaceae family. The Latin name of flaxseed means "very useful". It is an annual herb which has two varieties: yellow or golden (also known as golden linseeds) and the brown (Daun *et al.,* 2003). Flax was originally cultivated in Mesopotamia and its use has been documented as far back as 3000 BC (Cunnane *et al.,* 1995). The whole flaxseed is flat and it has an oval shape with pointed ends. Flaxseed contains a seed coat, two embryos, an embryo axis and a thin endosperm (Moris, 2007). The entire flaxseed plant can be used commercially either directly or after processing. Quality soluble fiber can be derived from the shell and the seed yields an oil rich in α-linolenic acid (often called n:3 fatty acid) and digestible proteins which makes it a functional food ingredient. (Oomah, 2001, Pengilly, 2003).

For human consumption, flax meal is made into the form of ground vacuum- packed grains which can be added to bread, pasta, egg products and in ready to eat foods (Mercier, 2014). Flaxseed has a spicy flavour. The flax as whole seed is stable and can last longer while in its grounded state it can go rancid at a higher temperature in about a week (Malcolmason, 2000).

Table 5: Proximate composition of flax based on common measures[a]

Form of Flax	Weight (g)	Common Measure	Energy (kcal)	Total Fat (g)	ALA (g)	Protein (g)	Total CHO[b] (g)	Total Dietary Fibre (g)
Proximate analysis	100	-	450	41	23	20	29	28
Whole seed	180	1 cup	810	74	41	36	52.0	50.0
	11	1 tbsp	50	4.5	2.5	2.2	3.0	3.0
	4	1 tsp	18	1.6	0.9	0.8	1.2	1.1
Milled seed	130	1 cup	585	53	30.0	26.0	38.0	36.0
	8	1 tbsp	36	3.3	1.8	1.6	2.3	2.2
	2.7	1 tsp	12	1.1	0.6	0.5	0.8	0.8
Flax oil	100	-	884	100.00				
	14	1 tbsp	124	14.0				
	5	1 tsp	44	5.0				

Source: Based on a proximate analysis conducted by the Canadian Grain Commission. The fat content was determined using the American Oil Chemists' Society (AOCS) Official Method Am 2-93. [a] The moisture content was 7.7%, [b]CHO= Carbohydrate. Total Carbohydrate includes carbohydrates like sugars and starches (1 g) and total dietary fibre (28 g) per 100 g flax seeds.

2.2.1.1 Chemical Composition of Flax Seed

Morris (2007) reported the proximate composition of flax. A proximate analysis of brown Canadian flax had 41% fat, 20% protein, 28% total dietary fibre, 7.7% moisture and 3.4% ash, which is the residue left after the samples were burnt in a muffle furnace (Table 5). Daun *et al.*, (2003) stated that the composition of flaxseed changes with some factors like genetics, seed varieties, growing environment and method of analysis.

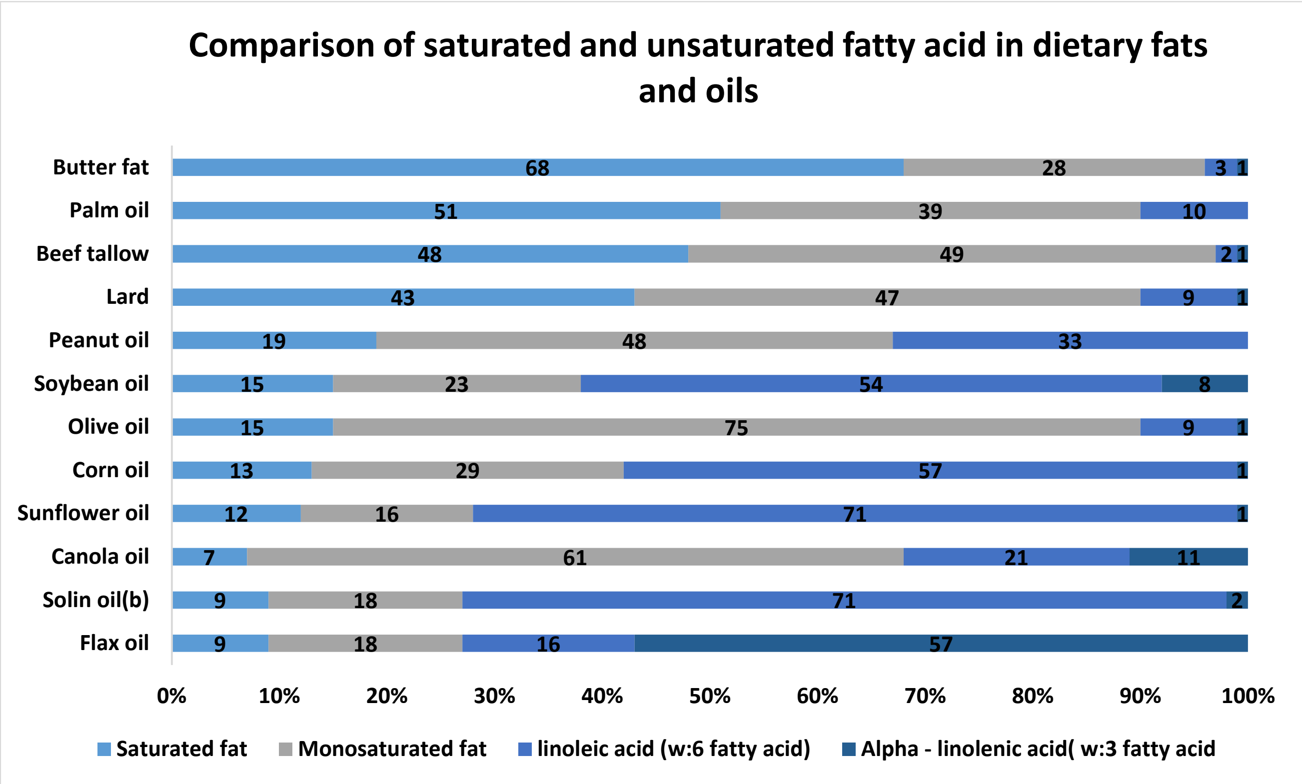


Figure 3: Comparison of saturated and unsaturated fatty acid in dietary fats and oils
[a]The solin oil values are those for Linola™.

Flaxseed oil contains 57 percent of alpha Linolenic acid (ALA) which makes it richest source of n:3. Flax oil also contain 16 percent of linoleic acid, 18 percent monosaturated fat and 9 percent saturated fats. From figure 3 it can be seen that flax, solin and canola oils have the lowest levels of saturated fatty acids which are nutritionally undesirable. Monounsaturated fatty acids is desirable and the level in flax is low.

From the report of the Canadian Grain Commission (2009) on fatty acid profile of flaxseed oil, it is quite evident that the level of α-linolenic acid is by far the largest component of the fatty acid profile of flaxseed. But it is when the profile of flaxseed is compared to other major oilseed crops that its value as a functional food crop becomes clear. Flaxseed may be considered a functional food because its high level of α-linolenic acid could be instrumental in promoting good health as well as preventing diseases (Simopoulos, 2000).

- **Local oil seeds**

2.2.2 Egusi (Melon) and Werewere (Neri) Seeds

Egusi and Werewere refers to the seeds of a highly nutritious types of water melon family called *Colocynthis citrullus* L and Citrullus lanatus subspecies mucosospermus respectively. Egusi melon and Neri are a native of West Africa and are largely cultivated in Nigeria, Ghana, Northern Namibia, Sierra Leone and Senegal. It is also called melon, *agushi or Egushi (Nigeria and Ghana respectively)* or *guna shanu* (Hausa), *ikpoghiri* (Itsekiri) and *ibara* (Congolese). Both are members of the *cucurbitaceae* family and they belong to the tribe Benicaseae. They are connected to cantaloupe, squash and pumpkin and other plants that grows on vines because such plants are also from the family *cucurbitaceae.*

According to Ogbonna (2013), there is a confusion in the nomenclature of the Egusi melon. Philip (1977) referred to the seed as *Citrullus vulgaris.* Other researchers called it *Citrullus lanatus* (Ogunremi, 1978; Okoli, 1984). This compelled Oyolu (1977) to recommend the name *Colocynthis citrullus.* He proposed that the vernacular name Egusi should be used with its Latin name to curb this confusion.

Cucurbit sp. can thrive both in the temperate and tropical countries. They belong to the economically most important vegetable crops worldwide (Paris, 2001). The primary part of the crops used in delicacies like soup and stew is the seeds. The Egusi seed is rich in oil and protein like soyabean (*Glycine max)* comprising of 50% oil and 35% protein (Sarwar et at., 2013), the seeds have both nutritional and cosmetic importance. The seeds contain vitamin C and B2, minerals, riboflavin, fat, carbohydrates and protein (Lazos, 1986). Being in the watermelon family it is a good source of carotenoid and lycopene. Lycopene has been found to be protective against a growing list of cancer (Cho *et al.*, 2004). Melon seed is among the most popularly consumed plant foods that are oilseeds (Oluba *et al.*, 2008). They can make a considerable contribution towards attaining a balanced diet (Fokou, *et al.*, 2004) especially because of its high levels crude protein and amino acids (Table 6 and Table 7).

2.2.2.1 Chemical composition of Egusi

Table 6: Chemical composition of Egusi

Composition	Ojieh et al., 2007	Umar et al., 2005
	Percentage	
Moisture	4.6	4.8
Ash	3.7	10
Ether extract	45.7	35.6
Crude protein	23.4	30.3
Crude fiber	12.0	3.3
NFE	10.6	24.4

Table 7: Amino acids composition (/100g) dry weight of watermelon seed

Amino acid	**Seed**
Essential Amino Acids	
Isoleucine	0.42
Leucine	1.20
Lysine	0.01
Phenylalanine	1.02
Threonine	0.45
Valine	0.7
Histidine	0.4
Methionine	0.3
Nonessential Amino Acids	
Alanine	0.9
Arginine	0.52
Aspartic Acid	1.34
Glutamic Acid	0.6
Glycine	1.2
Serine	0.6
Cysteine	1.1
Tyrosine	1.3
Proline	0.41
Ammonia	0.21
Total amino acids	**12.68**

Umar *et al.,* (2005)

2.3 FATTY ACID NUTRITION AND METABOLISM

Lipids are organic compounds which are the foundation for the structure and functions of living cells. They play a part in many biological functions which include increasing the fluidity of cell membrane (Fahy *et al.,* 2011). They also serve as an energy storage source and participate in key signalling pathways. They are considered extremely important for survival and overall human health and wellbeing. Essential nutrients cannot be produced by the body, and therefore must be derived from the diet. There are about 50 essential nutrients necessary for human health, including two fatty acids; linoleic acid (LA) and alpha-linolenic acid (ALA). Lipids are grouped into 8 categories; fatty acids, glycerophospholipids, glycerolipids, saccharolipids, sphingolipids, polyketides, sterols and prenols (Fahy *et al.*, 2009).

Fatty acids are made up of hydrocarbons linked up in chains with carboxylic acids as terminal functional groups. They are further categorised into saturated and unsaturated fatty acids according to their chemical structure. The unsaturated fatty acids can also be divided into two categorises depending on the number of double bonds they contain; monounsaturated fatty acid; MUFA (one double bond) and polyunsaturated fatty acids; PUFA (more than one double bond) (Bezard *et al.*, 1994). They can be classified as n: 6 or n: 3 depending on the location of the first double bond close to the methyl (Anderson and Ma, 2009). However, there are others, including those from the n:9, n:7 and n:4 families. However, the n:3 and n:6 are considered essential for the human body.

Table 8: Lipid names of major N: 6 and N: 3 fatty acids

N:6		**N:3**	
Common name	**Lipid name**	**Common name**	**Lipid name**
Linoleic acid	18:02	Alpha-linolenic acid	18:03
Arachidonic acid	20:04	Eicosapentaenoic acid	20:05
Alpha-linolenic acid	18:03	Docosapentaenoic acid	22:05
Nonadecadienoic acid	19:02	Docosahexanoic acid	22:06
Eicosadienoic acid	20:02		
Dihomogamma-linolenic acid	20:03		
Arachidonic acid	20:04		
Docosadienoic acid	01:42		
Docosatetraenoic acid	22:05		
Docosapentaenoic acid			

Source: AOCS Lipid Library 2014; De Oliveira *et al.*, 2013.

2.3.1 Essential Fatty Acids Metabolism

The main compound of the n:3 series is alpha-linolenic acid and it is most common n:3 fatty acid present in the diet (Brenna 2002). There are 3 metabolic fates of alpha-linolenic acid when it is consumed. The first is its desaturation (addition of a double bond) by delta-6-desaturase enzyme (D6DE) to yield stearidonic acid (SDA), and further elongation (addition of 2 atoms) and desaturation to be converted into long chain polyunsaturated fatty acid (LCPUFA) like Eicosapentaenoic acid (EPA), Docosahexanoic acid (DHA) and Docosapentaenoic acid (DPA). The other possibility is β-oxidation, with the haulting of the formation of acetate to complete oxidation to CO_2 or using acetate for biosynthesis (Burdge 2006).

LA is the parent compound of the n:6 PUFA series and most commonly found in vegetable oils. When LA is ingested, it becomes desaturated into gamma-linolenic acid (GLA) followed by elongation, to give dihomo-gamma-linolenic acid (DGLA) (Furuse *et al.,* 1991). Dihomo-gamma-linolenic acid forms arachidonic acid by a further hydrogenation, and can continue into docosatetraenoic acid, then the n:6 DPA through subsequent elongation-desaturation reactions.

Efficiency of ALA conversion to LCPUFA in humans is quite low, estimated to be 5-10% to EPA and 2-5% for full conversion to DHA (Arterburn *et al.,* 2006; Plourde and Cunnane, 2007). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) concluded that infants can convert approximately 1% of dietary ALA into DHA, while in adults the value is almost negligible (<0.1%) (Plourde and Cunnane 2007; Brenna *et al.,* 2009). If demands for n:3 PUFA are enough and serve to sustain membrane turnover and its renewal in adults, then it may be possible that this limited capacity to convert ALA into LCPUFA may be sufficient to maintain tissue function in healthy people who consume a balanced diet (Burdge 2004). However, maintaining adequate n:3 PUFA is necessary for people with diabetes and other metabolic disorders because their conversion enzymes may be compromised (Simopoulos *et al.*,1999).

ALA conversion to LCPUFA can be down regulated due to a high consumption of n:3 LCPUFA (Cunnane, 2003; Gibson *et al.*, 2013), LA (Emken, 1994; Brenna, 2002), saturated fat and trans fat in the diet. In addition, various lifestyle factors including smoking, alcohol consumption, stress, and magnesium deficiencies may also impact ALA conversion (Mahfouz and Kummerow, 1989; Marangoni, 2004; Ghezzi *et al.*, 2007). Stable isotope tracer studies have also indicated that ALA conversion occurs to a greater extent in women than in men, a 2.5-fold greater rate (Burdge and Calder, 2005). This could be advantageous for pregnant women whose children can derive the full benefit of ALA for brain development (Lewis *et al.*, 2000; Singh *et al.*, 2012).

2.3.2 Fatty Acid Composition in Chicken Eggs

The composition of an egg yolk depends on series of activities like synthesis of lipid in the liver, hepatic uptake and the amount of lipid in the diet (Sim and Qi, 1995). The content of fatty acid in an egg yolk is determined by the composition of fatty acids of the laying hens diets especially PUFAs. However, dietary mechanisms have no effect on the saturated fatty acids content of an egg yolk (Meluzzi *et al.*, 2000).

Ebeid *et al.*, (2008) reported from their study that, n:3 PUFA in an egg yolk increased when dietary n:3 increased. However, n:6 PUFA deceased linearly (P50:05) when compared with the control. Similarly, Schreiner *et al.*, (2004) reported a linear increase in n:3 PUFA in eggs yolks when they fed varying levels of n:3 PUFA to laying birds.

Linoleic acid (LA) is synthesized to arachidonic acid (AA) and Linolenic (LNA) is also converted to eicosapantanoic acid (EPA), docosapantanoic acid (DPA) and docosahexanoic acid (DHA) when consumed (Simopoulos, 2000). This means that n:3 and n:6 PUFAs are metabolised from LA and ALA. (Meluzzi *et al.,* 2000).

Studies from Scheideler and Froning (1996) and Bean and Leeson (2003) showed that when flaxoil is fed to laying hens, the n:3 PUFA found in large amount in its egg yolk is ALA though significant quantities of DHA and DPA were also deposited. Sim and Qi (1995) reported that n:3 PUFA content in eggs produced by hens fed with flaxseed were in this order: ALA4DHA4DPA4EPA. This proved that laying birds can convert ALA in their diet to DPA, DHA and EPA.

Raeini-Sarjaz *et al.,* (2006) supplemented laying hens diet with 0, 5, 10 and 15% flaxseed and found that the total fat content of the egg yolk were not affected. Nevertheless, unsaturated fatty acids increased while saturated fatty acids decreased linearly with the increase in flaxseed content. Botsoglou *et al.,* (1998) stated that the addition of flaxseed to hen’s diet changes the fatty acid composition of the egg. Similar results from Raeini-Sarjaz *et al.,* (2006) showed that the total PUFA content of an egg yolk increased with increasing flaxseed in the hen’s diet and monosaturated fatty acid particularly oleic acid decreased significantly. This reduction of oleic acid was less distinct when treatments had a significantly higher n:6 to n:3 ratio. Grobas *et al.,* (2001) found in their study that 5 or 10% flaxseed oil decreased oleic acid while it increased PUFA content of the yolk; an increase in LA led to increased n:6 PUFA, whereas an increase in ALA led to an increase in n:3 PUFA. Goncuglu and Ergun (2004) fed laying hens with 0, 1, 2, 3 and 4% flaxoil and recorded a significant increase in n:3 and n:6 PUFA in the egg yolk. The n:3 PUFA mainly ALA and EPA in the egg yolk increases when 20% of flaxseed was incorporated into laying hens ration (Zellner *et al.,* 2006).

Ferrier *et al.,* (1995) reported that eating an egg from a hen fed with 10% flaxseed can provide 30% of the daily requirement for n:3 PUFA (1.1 to 1.5 PUFA per day). Such egg supplies about 264mg ALA, 10mg EPA and 82mg DHA. Beynen (2004) also found that feeding a diet rich in flaxseed resulted in eggs with higher levels of n:3; ALA, EPA and DHA and lower level of n:6; LA when compared with the control. Shapira *et al.,* (2008) concluded that the concentration of the total n:3 PUFA in n:3 fortified egg (5% flaxseed in feed) increased by 3.8 fold when compared with the control eggs, ALA to 6.8 fold and DHA to 2.4 fold. Similarly, there was a decrease of 3.6 fold when the n:6; n:3 PUFA ratio was compared with the control eggs. Souza *et al.,* (2008) recorded a linear increase in n:3 PUFA content of an egg yolk when flaxseed oil in the diet increased from 0 to 2%.

2.3.3 Health Benefits of Consuming n: 3 Fatty Acids

The n:3 fatty acids are very important nutrients for both the young and old (Holman, *et al.,* 1982; Bjerve, 1991). Dyerberg and Bang conducted a study on Greenland Eskimos and discovered the defensive function n: 3 PUFA present in fish plays against cardiovascular diseases (Dyerberg and Bang, 1979). Dyerberg and Bang's study provided the bedrock for most researches carried out on various aspects of n:3 PUFA in human nutrition.

Researchers and nutritionists in various parts of the world have put in serious efforts to investigate the advantages of consuming n:3 PUFA (Sim and Sunwoo, 2002) and discovered that n:3 PUFA have a positive effect on the heart (Wahlqvist, 1998), play a vital role in controlling and treatment of high blood pressure (Howe, 1997), diabetes (Mohan and Das, 2001), arthritis, and other inflammatory diseases (Babcock *et al.,* 2002) and auto-immune disorders (Kelley, 2001). This type of fatty acid performs a role in monitoring the growth of cancer (Rose and Connolly, 1999; Aronson *et al.,* 2001). Adequate consumption has also been found to delay the loss of immunological functions and promotes infant brain development, visual development, healthy skin, shiny hair and weight management (Neuringer *et al.,* 1988; Erasmus 1993; Temple 1996; Pandalai *et al.,* 1996; Rose 1997; Leizer *et al.,* 2000; Laca *et al.,* 2009). The n:3 PUFA have been found to normalize blood pressure, blood cholesterol levels and fat metabolism, decrease insulin dependence, and increase metabolic rate and membrane fluidity (Deckelbaum and Torrejon, 2012).

All the n:3 PUFA are important but, it is suggested that DHA has the most important biological effect as it contributes to the fluidity in cell membranes, necessary for optimal development of the nervous system and is especially abundant in neural and retinal tissues (Cherian and Sim 1992). For that reason, DHA is considered to be essential in visual and neurological development, particularly in premature infants (Neuringer *et al.*, 1988).

Regardless, there are conflicting reports in the literature on the advantages n:3 PUFA. Recent results from a systematic review showed that n:3 LCPUFA from marine sources, administered either through food or supplemented for a minimum of 6 months reduce cardiovascular diseases by 10%, cardiac arrest by 9% and coronary diseases by 18% (Delgado-Lista *et al.*, 2012). Another study showed that dietary and circulating levels of EPA and DHA, but not ALA, were inversely associated with CVD incidence (De Oliveira *et al.*, 2013). However, evidence supporting the health benefits of ALA appears to be scanty since it has not been studied as extensively as DHA. The only surety is that ALA can be converted to DHA (Simopoulos, 2000). For their health benefits, there are recommended levels of intake of these essential fatty acids around various regions in the world (Tables 9 and 10).

Table 9: Dietary recommendations for intake of polyunsaturated fatty acids

Country	EPA + DHA (g/day)	Source
Canada	1.2 – 1.6	Scientific review committee (1990)
NATO	0.8	Leskanish and Noble (1997)
ISSFAL	0.65	Simpoulos et al (1999)
United State of America	0.65	Kris-Etherton *et al.*, (2002)
WHO – NATO	0.3 – 0.5	Kris-Etherton *et al.*, (2002)
WHO - FAO	0.4 – 1.0	2003
American heart association	1.0	Kris-Etherton *et al.*, (2002)
UK SACN	0.45	Gebauer *et al.*, (2006)

Table 10: Recommendations for intake of the n:6:n:3 PUFA ratio

Country	N:3 : N:6	Reference
Australia	08:01	Ollis *et al.,* (1999)
Canada	04:01	Holub (2002)
UK	06:01	Widdowson (2005)
USA	9.8:1	Kris-Etherton *et al.*, (2002)
Japan	4-4.5:1	Okita *et al.,* (1995)
Japan	04:01	Sugano *et al.,* (2000)
FAO/WHO	5-10:1	Trautwein (2001)

2.4 NUTRIENT COMPOSITION OF EGG

Egg is one of the cherished sources of nutrients in human food and it plays a vital role to a healthy life. It has a low calorie content and supplies most nutrients including proteins when consumed (Hu *et al.*, 2001). A 60g egg contains 61.5% (36.9g) albumen, 29% (17.4g) yolk and 9.5% (5.6g) shell.

Ahn *et al.,* (1997) stated that the relative amount and composition of fats of an egg yolk are determined by several elements which includes the age of hens and size of the eggs. An increase in the size of eggs leads to a decrease in the proportion of egg yolk. The proteins in eggs are mostly taken as a standard reference (biological rating of 100) when it comes to examining the protein quality of other foods due to its high quality (Shrimpton, 1987). Eggs provides essential amino acids in high amount (Layman and Rodriguez, 2009). It is also worth noting that 2.3g of the total fat content (5.8g) of an egg is monounsaturated. That notwithstanding eggs also contain different types of vitamins, minerals and trace elements (Song *et al.,* 2000). The European Union classified egg as a high source of vitamin D. It was rated second in the ranking of the highest sources of vitamin D but it contains little vitamin C (Shrimpton, 1987). Bolton-Smith *et al.* (2000) stated that eggs contain little vitamin and a survey conducted by Benalam *et al.,* (2012) confirmed this by reporting that only 7mg/ 100g of vitamin K2 was found in raw whole eggs.

Almost all the minerals in an egg can be found in its shell and it is rich in calcium and phosphorus. It contains about 2.2g of calcium and 20mg of phosphorus. It can be used as a calcium supplement if is well grinded even though it is not edible (Sugura *et al.,* 1999). Calcium from an egg shell can be absorbed efficiently when compared with calcium from other foods (Stadelman and Schmieder, 2002).

Lipids have gained considerable attention among scientists, researchers and consumers amidst the many nutrients found in eggs. This is due to the bearing between dietary fat consumption and cardio vascular diseases. Cherian *et al.,* (2002) stated that a 60g chicken egg contains about 5.5 to 6g lipids, which is found in the egg yolk. The fatty acid profile of a hen's egg shows that 44% of the lipids in eggs are MUFA, followed by 29% saturated fatty acid and 11% PUFA. The lipids are normally present in the form free cholesterol, phospholipids and tricylglycerol (Sparks, 2006).

Conventional hen diets result in eggs with a n:6 : n:3 ratio of about 13:1, much higher than recommended for optimal health (Scheideler and Froning 1996). However, an enriched egg can provide upwards of 500 mg total n:3 PUFA, which may contain up to 290 mg of combined EPA and DHA. This could provide adults with half of the necessary recommendation (500 mg EPA and DHA daily) to reduce the risk for developing CVD (Harris *et al.,* 2009). N:3 and conventional eggs contains the same amount of energy, protein, saturated fat and monosaturated fats but n:3 eggs contains more LA, AA, ALA, DHA when compared with conventional eggs (Table 11).

Table 11: Nutrient content of enriched and conventional eggs[1]

Nutrient	N:3 Egg	Conventional Egg2
Energy (calories)	70	70
Protein (g)	6	6
Total fat (g)	5.0	5.0
Saturated fat (g)	1.5	1.5
Monosaturated fat (g)	2.0	2.0
Total n:6 fatty acids(g)	0.8	0.6
Linoleic acid(mg)	6405	540
Arachidonic acid(mg)	305	80
Total n:3 fatty acid (g)	0.43	0.1
Alpha-linoleic acid (mg)	1745	31
Eicosapentanoic acid (mg)	175	1
Docosapentanoic acid (mg)	105	4
Docosahexanoic acid (mg)	1085	344
Cholesterol (mg)	195	195

Source: Gillingham *et al.*, 2005

1Abbreviations: g = grams, mg = milligrams.

2Nutrient content for 1 large hard-boiled chicken egg, (Canadian Nutrient File, 2014)

On average, a commercially available n:3-enriched egg contains about 75 mg DHA for an egg from a flax-fed hen, and 125 mg DHA from a hen fed both flaxseed and fish oil (Johnson, 2014)

2.5 EGG QUALITY

Egg quality involves both the internal egg quality, focusing on the content of the egg and external egg quality, focusing on the egg shell. Egg size cannot be left out since small eggs cannot be sold as table eggs.

2.5.1 Egg Weight

Dietary lipids have been acknowledged to enhance the egg weight (Jensen *et al.,* 1958) in laying hens although the mechanism remains unclear. March and McMillan (1990) reported that linoleic acid (n:6 PUFA) can increase the synthesis of lipoproteins which are deposited in the developing yolk. Current research conducted on feeding n:3 PUFA to laying hens showed contrary results which shows that the effects on egg weight and egg mass is compromised. Various studies demonstrated that egg weight was not influenced by feeding varying sources and levels of n:3 PUFA in the diet of laying hens (Baucells *et al.,* 2001; Schreiner *et al.,* 2004; Carrillo-Dominguez *et al.,* 2005). Feeding of 4% flaxseed oil did not affect egg weight of the hens (Celebi and Utlu, 2006). Grobas *et al.,* (2001) reported that hens fed 5 or 10% flaxseed oil in the feed produced eggs without any change in egg weight or egg mass during a period of 12 weeks. They also reported similar egg weight in the hens fed on diets with 5% flaxseed oil when compared to the controls. Novak and Scheideler (2001) noted that the weight of an egg and its mass was not affected by feeding 10% flaxseed to the hens. Schumann *et al.,* (2000) also observed no change in egg weight in laying hens at 4% flaxseed oil in the diet. Rowghani *et al.,* (2007) reported similarly that egg weight in laying hens did not differ when they fed diets having 0, 3 or 5% canola oil. In a different study by Farrel (2002), there was no significant effect on egg weight when canola oil was added to laying hens diet. Shafey *et al.,* (2003) recorded that adding sunflower and olive oil had no effect on the weight of egg and its mass when fed to laying hens. However, many researchers have reported a negative effect of n:3 PUFA on egg weight and egg mass in laying hens. Eggs laid by hens on diets with flaxseed oil were 4% smaller than the control eggs (Augustyn *et al.,* 2006). Pappas *et al.,* (2005) reported a similar result that the addition of n:3 PUFA to laying hens diet significantly decreased the egg weight and its components.

2.5.2 Egg Yolk Weight

The diet of the bird plays a significant role in determining the colour of the egg yolk though some diseases can affect it too. Diets containing alfafa meal and yellow maize causes the yolk of an egg to be yellow when fed to hens while diets with white maize, wheat and grain sorghum produces a light coloured yolk. Marigold petals an example of natural yellow – orange material can be combined to light coloured feeds to intensify the colour of the egg yolk (Jacqueline *et al.,* 2011).

Egg yolk weight is an important factor for interior egg quality. Any deviation in egg yolk weight can significantly alter the whole-egg weight. Most studies by various researchers indicated that the egg yolk weight was not much influenced by feeding n:3 PUFA to laying hens. Grobas *et al.,* (2001) revealed that the hens fed n:3 PUFA rich diets (with 5 or 10% flaxseed oil) produced similar egg yolk weights when compared to the hens without supplementation during a period of 12 weeks. The increase in dietary n:3 PUFA content (by feeding flaxseed and flaxseed oil) in experimental groups did not change egg yolk weight (Augustyn *et al.,* 2006). Beynen (2004) and Sosin *et al.,* (2006) stated that feeding n:3 PUFA to the hens kept the yolk weight constant. Similarly, Horniakova (1997) found no change in yolk weight of hens with 2 or 6% canola oil supplementation in the rations. Shafey *et al.,* (2003) observed no change in the yolk weight of laying hens fed olive and sunflower oil in the diets. Scheideler *et al.,* (1998) found no significant effect of flaxseed on wet yolk weight percentage. Najib and Al-Khateeb (2004) reported close values for yolk index when they fed different levels of full fat canola seeds to the laying hen. On the other hand, Van Elswyk (1997) suggested that the changes in egg quality by feeding flaxseed to the hens also included a decrease in yolk weight. He proposed that the yolk weight reduction was related to changes in circulating estradiol in blood brought about by either n:3 PUFA or the antinutritional characteristic of linseed. Hens fed flaxseed produced eggs with a smaller (P50:05) percentage of wet yolk weight compared to the control birds (Bean and Leeson, 2003). Novak and Scheideler (2001) found a reduction in wet yolk percentage when laying hens were fed with flaxseed. In contrast, few studies have reported an increase in yolk weight in the hens fed diets rich in PUFA (Rowghani *et al.,* 2007) but the possible cause of this increase was not indicated.

2.5.3 Egg White Quality

The albumen height is an important criterion for analysis of internal quality of egg and it represents protein quality (Silversides *et al.,* 1993). Extended storage time and higher storage temperature decrease the albumen height, and thus degrade the internal quality of the egg (Scott and Silversides, 2000; Raji *et al.,* 2009). A fresh egg normally has its yolk in the middle immersed in a thick jelly-like albumen when it is gently broken onto a flat surface. When eggs kept for some weeks are broken onto a flat surface, one can observe a flattened and displaced yolk at one side and a thin (watery) albumen at the other side of the plate. Thin albumen with a large surface area gives an indication of an egg with poor quality (Jacqueline *et al.,* 2011).

DEFRA (2010) reported that, the sign of an egg with good quality is a jelly-like albumen with a cloudy colour. They reported that carbon dioxide is lost from an egg through its shell pores when the egg stay long thereby reducing its acidity. This results in an alkaline egg content with a transparent and watery albumen. The rate of deterioration increases with a higher temperature as more carbon dioxide is lost with high temperatures.

Egg white quality in laying hens seemed to be unaffected by n:3 PUFA supplementation. Galobart *et al.,* (2001b) reported no change in Haugh Unit value in the hens fed 5% flaxseed oil. Similarly, Jiang *et al.,* (1991) found no change in Haugh Unit value in the hens fed flaxseed enriched diets. Albumen quality in terms of weight percentage of egg was not affected by dietary flaxseed oil fed to the laying hens (Raes *et al.,* 2002). Similarly, the egg white weight was not altered by 5% canola oil supplementation to laying hens (Rowghani *et* *al.,* 2007). Scheideler *et al.,* (1998) found no effect of feeding flaxseed on the egg white percentage in the eggs. Horniakova (1997) observed no change in albumen weight when laying hen's diet was supplemented with 2 and 6% canola oil. On the other hand, Wang (1996) found an increase in albumen weight when 8% safflower oil was added to the bird's diet. This is associated with the higher content of oleic acid found in safflower oil. Grobas *et al.,* (2001) revealed that the hens fed 5 or 10% flaxseed oil produced eggs with no change in Haugh unit value but with an increase in albumen weight when compared to the hens without supplementation. Novak and Scheideler (2001) found a significant increase in the percentage of the albumen in eggs produced from hens fed on rations with flaxseed. Whereas, Augustyn *et al.,* (2006) reported that the eggs from hens fed flaxseed or flaxseed oil had less albumen than control eggs.

The Haugh Unit is used to measure the quality of the proteins in eggs and it relies in the albumen height. Raymond Haugh in 1937 introduced this test, (Kluger, 2010). Measuring Haugh unit is a very necessary production count of egg quality following other estimates like shell thickness. The albumen height is measured with a micrometer after an already weighed egg is carefully broken onto a flat surface. The albumen height and the egg weight is used in the computation of Haugh unit. The higher the albumen height, the higher the quality of the proteins in an egg. A limitation of the Haugh unit is that, it does not determine the content of other principal nutrients like vitamins or micronutrients (Haugh, 1937).

The formula for calculating the Haugh Unit is

$$HU - 100 \times \log (h - 1.7w^{0.37} + 7.6)$$

Where:

- HU= Haugh Unit
- h = albumen height (mm)
- w = egg weight (g)

The value of Haugh unit should be between 20 and 110 (Haugh, 1937).

2.5.4 Albumen Weight

The albumen weight is the weight of the albumen, which can be measured with a compass. It is usually calculated with the formula below;

ALBUMEN WEIGHT (g) =Weight of egg – (Weight of Shell + Weight of yolk)

(Reddy *et al.*, 1991; Monira *et al.*, 2003; Fayeye *et al.*, 2005).

2.5.5 Eggshell Quality

Shell quality, with a particular reference to shell thickness and shell strength, is an important determinate in egg appearance and is very important in egg handling. Patterson *et al.*, (2001) observed that eggs fortified with n:3 PUFA recorded a larger number of egg breaking and leaking when compared with the control eggs. The quality of an egg shell can be estimated as egg specific gravity, shell breaking strength, shell weight or shell thickness. The age of strain and hen, environmental and nutritional factors may influence the shell strength (Roberts, 2004).

Inclusion of flaxseed, as n:3 PUFA source, in the diet of laying hens can decrease eggshell quality with reference to shell weight (Van Elswyk, 1997). Novak and Scheideler (2001) observed that laying hens fed with flaxseed laid eggs which recorded a decline in wet shell percentage. However, most of the researchers found no effect of feeding diets fortified with n:3 PUFA on eggshell quality to the laying hens. Galobart *et al.*, (2001b) stated that egg shell thickness remained similar in the hens fed 5% flaxseed oil as compared to the controls. Grobas *et al.*, (2001) demonstrated that the laying hens fed 5 or 10% flaxseed oil produced eggs with similar shell weight but the thickness of the shell reduced. Raes *et al.*, (2002) found no visible change in egg shell quality nor egg shell weight when flaxseed oil were incorporated in the diet of layers. Filardi *et al.,* (2005) also found no change in eggshell weight and eggshell thickness when canola oil was supplemented at 3.12% in the ration of laying hens.

2.6 EGG CHOLESTROL

The egg marketing board in 1957 sold eggs with ease with the conception that eggs contains proteins so the best way to start the day was adding an egg to the breakfast menu (Guter and Low, 2008). By 1960, egg consumption increased and an individual consumed about 5 eggs every week (National Food Survey, 2001). Later, studies suggested that egg contains cholesterol which raised serious debate since these studies by researchers suggested that foods rich in cholesterol may increase blood cholesterol which might subsequently increase the chances of acquiring cardio vascular diseases (CVD) (Kannel *et al.*, 1969).

As a result, egg became considered as a contributing factor to coronary diseases due to its cholesterol content. Other studies were conducted and in spite of the findings by researcher's that there was no link between egg consumption and blood cholesterol concentrations (Song and Kerver, 2000; Fernandez, 2006; Nakamura *et al.,* 2006; Djoussé and Gaziano, 2008; Chai *et al.,* 2009; Scrafford *et al.,* 2009), the consumption of eggs is still on the decrease (Herron and Fernandez, 2004). In fact, there are two types of cholesterol, the low density lipoprotein (LDL); bad cholesterol and the high density lipoprotein (HDL); good cholesterol. Cholesterol in egg is the high density lipoprotein. Blesso *et al.,* (2013) investigated if feeding people with metabolic syndrome with eggs along with carbohydrate restriction would change lipoprotein metabolism and influence atherogenic lipoprotein profiles. They found that there were greater increases in HDL-cholesterol and large HDL particles, and reductions in total very low density lipoprotein protein (VLDL) and medium VLDL particles for those who consumed 3 whole eggs per day compared to people who consumed equivalent amount of yolk-free egg substitute ($P<0.05$). This clearly proves that egg contains HDL. Schmier *et al.,* (2009) conducted a cost-benefit analysis and found that removing eggs from the diet makes an individual prone to aggerelated mascular degeneration (ARMD), which leads to a higher healthcare cost. This is because the cholesterol in eggs is the good cholesterol that is the high density lipoprotein.

2.7 FATTY LIVER HAEMORRHAGE SYNDROME

Fatty liver haemorrhage syndrome (FLHS) is a metabolic disease of laying hens which is a non-infectious disease meaning that it cannot be transmitted from one bird to the other. It normally occurs in caged layers which are fed with a diet high in energy. The features of FLHS are fat build up in the liver and abdominal cavity, ruptured liver and haemorrhage and death (Crespo and Shivaprasad, 2003). Fatty liver haemorrhage syndrome can result in a drop in egg production and a drop in flock numbers as a result of the high mortality rate associated with this disease. This negatively affects the poultry industry by imposing a great economic loss on farmers (Squires and Leeson, 1988). It is normally difficult to distinguish between birds with the syndrome and healthy birds, however increased body weight, decreased egg production and a rise in mortality can be used as a sign of this metabolic disease (Leeson, 2007).

The atielogy of FLHS is not clear but many researchers associate it with nutrition (Akiba *et al.,* 1983). These authors stated that feeding laying hens with a high energy diet will result in FLH. In addition, energy metabolism and hormonal levels during egg production can cause this disease (Scheele, 1997). This author explained that high oestrogen levels leads to high fed intake and hence high energy accumulation. Other attributable factors include genetic factors (Squires and Leeson, 1988), housing and environmental factors (Shini *et al.,* 2006), stress (Akiba *et al.,* 1983) and bacterial endotoxins (Alisi *et al.,* 2012). Trott *et al.,* (2013) reported that there is equal occurrence of FLHS in both floor and caged birds.

Many researchers have studied the role dietary fatty acids play in FLHS. Caston *et al.,* (1995), Schumann *et al.,* (2000) and Cherian and Hayat (2009), fed laying hens with varying levels of flaxseed and recorded no significant change in hen's liver when compared with the control. Bean and Leeson (2003) found a contrary results and stated that feeding 10% flax resulted in significantly higher levels of n:3 fatty acids in both brown and white egg layers but long-term use of flax seed increases the incidence of liver haemorrhages in supplemented laying hens. The cause of the increased liver haemorrhages in flax fed birds is unclear. Some researchers have attributed the increased haemorrhage to the high content of long chain unsaturated fatty acids in flax. They hypothesized that these unsaturated fatty acids are prone to oxidative rancidity and further recommended that antioxidants like vitamin E and butylated hydroxyl-toulene (BHT) be supplemented with the diets to reduce lipid oxidation in the liver of flax fed birds (Cherian and Hayat, 2009). Prolonged lipid peroxidation and oxidation destroys the hepatic cellular membrane and this has been stated to increase the susceptibility of bird's liver to haemorrhage (Spurlock and Savage, 1993). It should however be noted that, dietary antioxidants might not change liver oxidation products or haemorrhage score when birds are already suffering from FLHS so it should be included in diets early to avoid its initiation (Schumann *et al.,* 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

The study was carried out at the Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi. The study area is located in the semi-deciduous forest zone of Ghana. The area is located between latitude $06^{0}43^{1}$N and longitude $01^{0}36$. The study area lies in the forest zone with an average rainfall of 30mm and 200mm in January and June respectively with an average temperature between 23^{0}C minimum and 35^{0}C maximum in February and August respectively (Abubakari, 2013). The study was carried out in 4 parts including egg production and consumption survey, potential of two local seeds (Egushi and Werewere) as sources of PUFA for egg enrichment, dietary enrichment of hen feed in the development of n:3 fatty acid eggs and egg quality analysis enriched eggs and over a period of time.

3.1 Experiment One: Egg production and consumption patterns in some selected Municipalities in Ghana.

3.1.1 Data Collection

A cross sectional study of egg production and consumption pattern was conducted in 7 municipalities/districts in Ghana. These included Ejisu-Juaben Municipality, Ewutu-Efutu Senya District, Sehwi Wiaso Municipality, Teshie Municiaplity, Shai Osudoku Municiaplity, Yegi Municipality and Asuogyamang Municipality. These study areas were chosen because of their cosmopolitan and both urbanized and rural natures.

Approximately, 150 respondents were randomly sampled from each municipality/ district with different demographic characteristics participated in the research. Data were collected using a self-administered questionnaire consisting of three main sections:

demographic characteristics, egg producers and consumption pattern and consumer preference and perceptions. The questionnaire contained both opened and close ended questions. In order to capture people with varying educational background, questions were sometimes read out to respondents who could not read and write and their responses summarised on the questionnaire. The study was segmented into three areas; consumers, egg sellers and egg producers with 50 questionnaires being administered to each group or the number respondents available for interview in each category in each municipality.

3.1.2 Data Processing

Responses from the survey were tallied and entered into Microsoft Excel version 2010 and frequency chart developed as representation of response obtained.

3.2 **Experiment Two:** The potential of two local seeds (Egushi and Werewere) as sources of PUFA for egg enrichment

3.2.1 Proximate Composition and Amino Acid Determination

Seeds of Egushi (Melon seeds) and Werewere (Neri seeds) were obtained from the open market and transported to the Department of Animal Science, KNUST for proximate analysis following the procedures of the Association of Official and Analytical Chemist (AOAC, 1995). In this method, 2 g of grinded seeds from each of the two seeds were weighed and oven dried at 135^oC till a constant weight was attained to determine the moisture content. The Soxhlet extraction method (AOAC,1995) was used to determine the fat content. Kheldjal method (AOAC, 1995) was used to determine the crude protein fraction. Ash, the inorganic residue was obtained by burning a sample at 600^oC in a furnace. Crude fiber was determined by digestion method (AOAC, 1995). The individual amino acid composition of each seed was determined through a collaborative and consultative work at the Evonik Africa (Pty) Limited laboratory in South Africa.

3.2.2 Fatty Acids Determination

To further elucidate the presence of n:3 polyunsaturated fatty acids in these seed, they were subjected to fatty acids extraction and identification at the lipid laboratory at the Department of Agriculture, Food and Nutritional Science; University of Alberta, Edmonton-Canada. In this analysis, 2 g of each seed was placed in 50 ml falcon tube and digested using electronic ultrasonic homogenizer in the presence of 20 ml of hexane. The seed and its contents were completely shredded and broken to release oil content. The content was allowed to stand overnight at room temperature to allow the particles to settle. The oil content which was part of the hexane was gently taken out with a Pasteur pippitte into a 1.5 ml microcentrifuge tube leaving the slurry behind. The hexane was allowed to evaporate and the oil collected for analysis using Gas Chromatography technique (Aglient Thechologies, 6890N, Network GC Sys Jms-Q1000GC(A) Ultra Quad GC/MS). The total fatty acids profile including saturated, monounsaturated and all polyunsaturated were analysed. The n:3 fatty acids and n:6 fatty acids that were targeted included, α-linolenic acid (C18:3 n-3), docosatrienoic acid (C22:3 n-3), eicosapentaenoic acid (C20:5 n-3), docosapentaenoic acid (C22:5 n-3), docosahexaenoic acid (C22:6 n-3), linoleic acid (C18:2 n:6), γ-linolenic acid (C18:3 n:6), nonadecadienoic acid (C19:2 n:6), eicosadienoic acid (C20:2 n:6), dihomogamma-linolenic acid (C20:3 n:6), arachidonic acid (C20:4 n:6), docosadienoic acid (C22:2 n:6), docosatetraenoic acid (C22:4 n:6), docosapentaenoic acid (C22:5 n:6) and 11,14,17-eicosatrienoic acid (C20:3 n-3) (De Oliveira *et al.*, 2013).

3.3 Experiment Three: Comparative analysis of essential fatty acids deposited in eggs through layer hen diet

3.3.1 Experimental Diet and Experimental Design

One thousand Lohmann Tradition layer day old chicks were obtained from a commercial hatchery, Akate Farms and Trading Company Limited in Kumasi. The chicks were brooded for six weeks and fed a commercial starter mash with 20% crude protein and a metabolisable energy content of 2780 kcal/kg for six weeks. The birds were transferred to a grower diet from seven weeks until eighteen weeks of age. The ration had a crude protein of 17% and 2750kcal/kg metabolisable energy (calculated). The birds were again placed on a layer diet from week 20 until experimental logistics were ready for the study. At 39 weeks of age 20 birds each was allocated to four experimental diets containing varying levels of flaxseed oil T1 (0%), T2 (1.5%), T3 (3%) and T4 (4.5%) in a Completely Randomised Design (CRD) (Table 12) and each bird represented an experimental unit.

The birds were given a period of two weeks after placement on the experimental diets to adjust to the treatments. After two weeks egg samples were collected and taken to the Physical Laboratory at the Department of Chemistry, KNUST for analysis. Feed and water were given ad-libitum throughout the experiment. Regular vaccination and medication schedules were strictly adhered to until completion of experiment. Birds were vaccinated against Gumboro, Fowl pox and New Castle disease at scheduled times while dewormer and sulphur powder (lice treatment) were given on schedule or when needed. Strict biosecurity measures were practiced during the research period to maintain birds in healthy state.

Table 12: Percent Composition of experimental diet

INGREDIENTS	TREATMENT WITH INCLUSION LEVELS (%)			
	T1	T2	T3	T4
Maize	61.80	57.30	52.36	47.65
Fishmeal	9.60	8.00	7.58	7.40
Soyabean meal	11.60	15.00	15.00	15.00
Wheatbran	9.00	9.00	13.36	16.73
Oyster shell	7.50	8.70	8.21	8.22
Flaxoil	0.00	1.50	3.00	4.50
Vitamin Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Calculated Nutrient Composition (%)				
Crude Protein	17	17	17	17
Metabolisable Energy (Kcal/kg)	2750	2750	2750	2750
Calcium	3.29	3.50	3.50	3.50
Phosphorus	0.25	0.25	0.24	0.23
Crude fiber	2.93	3.28	3.36	3.60
Lysine	1.00	1.00	1.00	1.00
Methionine	0.37	0.35	0.35	0.34
Cysteine + methionine	0.69	0.64	0.64	0.63

3.3.2 The N:3 and N:6 Fatty Acid Determination

The n:3 and 6 determinations was carried out at the Physical laboratory of the Department of Chemistry KNUST. Three eggs were randomly collected weekly from each treatment after 2 weeks of feeding the birds with the experimental diet. This was repeated every week and a total of twenty-eight eggs was used for each treatment. Egg yolk from eggs collected were analyzed by gas chromatography to assess the fatty acid composition. The raw egg yolk samples (0.5 g) were placed in 25 × 150 mm Teflon-lined screw-capped test tubes and the modified Folch method (Folch et al. 1957) was used to extract the total fat. The egg yolk samples were mixed with 12 mL of Folch solution (chloroform : methanol, 2:1, vol/vol) and kept overnight. Then, 2 mL of 0.88% NaCl (wt/vol) was added, mixed gently, and centrifuged (21°C) at 1,500 × *g* for 3 min. After phase separation, the top layer was carefully siphoned off. A total of 5 mL of the bottom layer was removed and dried in a block heater under a stream of nitrogen in 20-mL glass vials. The dried fat was resolubilized in 0.5 mL of chloroform and 25 µL of the reconstituted mixture (extracted fat and chloroform) and was then derivatized using 2 mL of methylating reagent (1 *N* methanolic HCl; Sigma) in a water bath at 60°C for 60 min. Then, 50 µL of distilled water, a known amount (500 µL) of internal standard (1 mg/mL of chloroform; heptadecanoic acid, 17:0; Sigma), and 2 mL of hexane were added, mixed thoroughly, and centrifuged (21°C) at 1,500 × *g* for 3 min. The top hexane layer was separated and transferred to another test tube containing about 10 to 20mg of anhydrous sodium sulfate to absorb any moisture from hexane. After centrifugation (3 min at 1,500 × *g* at 21°C), 1 mL of hexane solution (after adjusted to limit 0.2 to 0.3 mg lipids in 1 mL of hexane) was transferred to a gas chromatograph vial. Fatty acid composition of the 1 µL of solution injected into the gas chromatograph (model 3400, Varian, Palo Alto, CA) equipped with a flame ionization detector and an SGE BP20 capillary column (30 m × 0.25 mm ID × 0.25-µm film thickness; Scientific Instrument Services Inc., Ringoes, NJ) was determined. The initial column temperature was set at 50°C for 0.2 min, increased to 120°C at a rate of 20°C/min, and held for 5 min. Column temperature was then elevated to 230°C at rate of 10°C/min and held at the final temperature for the total running time (30 min). Helium was used as the carrier gas at a flow rate of 3.0 mL/min. A cool-on column injection method was used, with an initial and final injector temperature of 60°C (0.2 min) and 230°C (28 min) respectively, increasing at a rate of 150°C/min. The detector was set at 240°C and the column head pressure of the carrier gas (helium) was 175 kPa. The cool-on-column injection method used in our study is appropriate because it eliminates sample discrimination, does not alter the sample because of its inert nature, and provides high analytical precision and accuracy that is needed for detection of LC PUFA (Yuwono and Indrayanto, 2009). A fatty acid standard (15A, Nu- Chek Prep Inc., Elysian, MN) was injected after every 10 samples to monitor the chromatographic conditions during the duration of sample analysis. The fatty acid peak integration was performed using the Galaxie chromatography data system (Varian, Walnut Creek, CA). Fatty acids were quantified using heptadecanoic acid (17:0) as an internal standard (Varian) and were identified by comparison of authentic standards (GLC-463, Nu-Chek Prep Inc.).

Total n:3 PUFA levels were calculated as 18:3 n:3 (linolenic acid; LNA) + 20:5 n:3 (eicosapentaenoic acid; EPA) + 22:5 n:3 (docosapentaenoic acid; DPA) + 22:6 n:3 (docosahexaenoic acid; DHA). Total n:6 PUFA levels were calculated as 18:2 n:6 (linoleic acid; LA) + 18:3 n:6 (γ-linolenic acid) + 20:2 n:6 (eicosadienoic acid)+ 20:3 n:6 (dihomogamma-linolenic acid) + 20:4 n:6 (arachidonic acid) + 22:4 n:6 (docosatetraenoic acid) Total PUFA levels were calculated as the sum of total n:3 acids + total n:6 fatty acids. Long-chain n:3 PUFA levels were calculated as the sum of EPA + DPA + DHA.

3.4 **Experiment Four:** Effect on egg storage on egg quality characteristics of n:3 fortified egg

3.4.1 Experimental Site and Design

Egg quality analysis was carried out at the egg embryology laboratory Department of the Animal Science, KNUST. A total of 900 eggs were collected between week 45 and 52 for the egg quality analysis. Fifteen eggs from each treatment were collected 15 consecutive times. The eggs were stored at room temperature for zero to fourteen days. The eggs were carefully broken by cracking the narrow anterior part of the oval shape to allow the easy flow out of the albumen after it was weighed with an electronic scale. The albumen height was measured using the tripod micrometer. The highest portion of the albumen close to the chalaza was measured. The yolk was carefully separated from the albumen and it was weighed on an electronic scale. The egg shell was gently washed to remove the egg membrane. It was then weighed after it was air dried at room temperature for two days. The shell weight was taken using an electronic scale. The albumen weight was calculated as the difference between the weight of the egg and the weight of the yolk plus shell. Haugh unit was calculated using the formular of Haugh (1937); $HU= 100 * \log (h - 1.7w^{0.37}+ 7.6)$ where HU is the Haugh unit, h the albumen height in millimetres and w the egg weight in grams. The yolk colour was measured using the Roche colour fan (RCF 193). The completely randomised design was used. The completely randomised design was used.

3.5 Experiment Five: Assessing the effects of n:3 fortified diets on fatty liver haemorrhage syndrome as stress of treatments on bird.

3.5.1 Sampling

Five birds were randomly selected from each treatment at the end of the experiment. They were individually weighed before euthanizing each of them by cervical dislocation. Each bird was dissected and strict concentration was paid to the presence of blood clot and fat in the coelomic cavity. Each liver was gently removed, weighed on a digital scale and examined for haemorrhage and haematomas. Haemorrhage was examined on both the dorsal and ventral sections of the liver. It was graded on a scale of 0 -5, with score 0 indicating no haemorrhages; score 1, up to 10 subcapsular or ecchymotic haemorrhages; score 2, more than 10 subcapsular or ecchymotic haemorrhages; and scores 3-5, large haematomas and massive liver haemorrhage accompanied with rupture liver capsule.

3.6 Statistical Analysis

Data was analyzed with the Generalized Linear Model Procedure of SAS (SAS Proc GLM) at $P < 0.05$. The LS means was separated by the Students Newman Keuls test (SNK) (SAS Institute, 2012).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 **Experiment One:** Egg production and consumption patterns in some selected Municipalities in Ghana

4.1.1 Demographic Characteristics of Producers

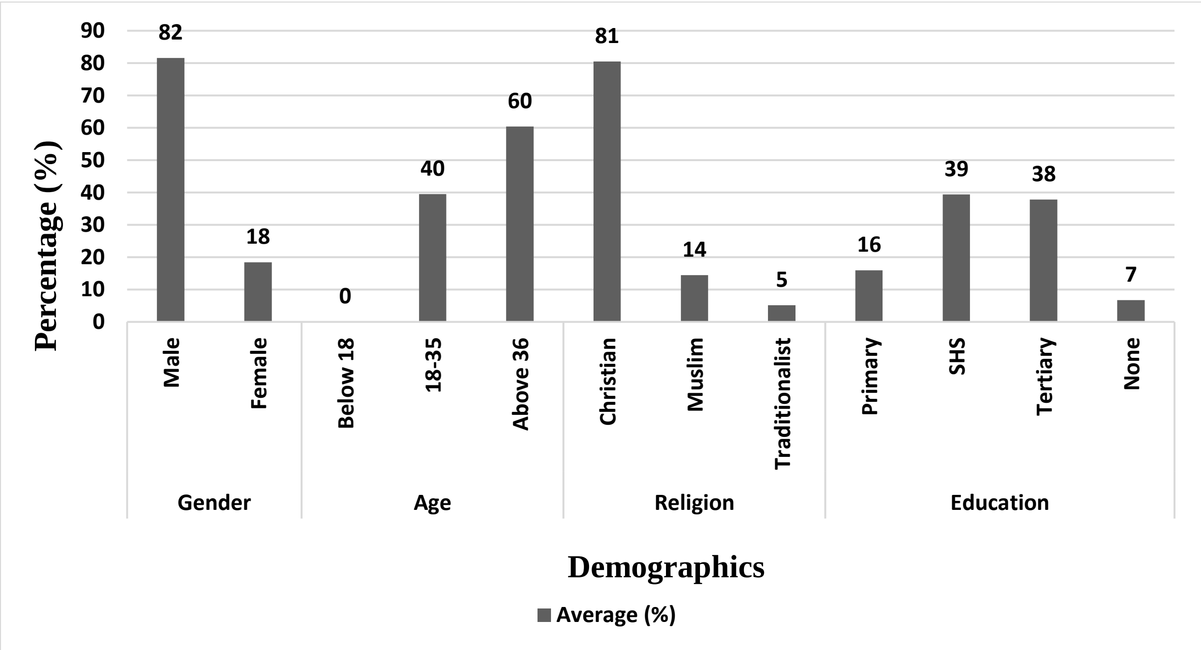
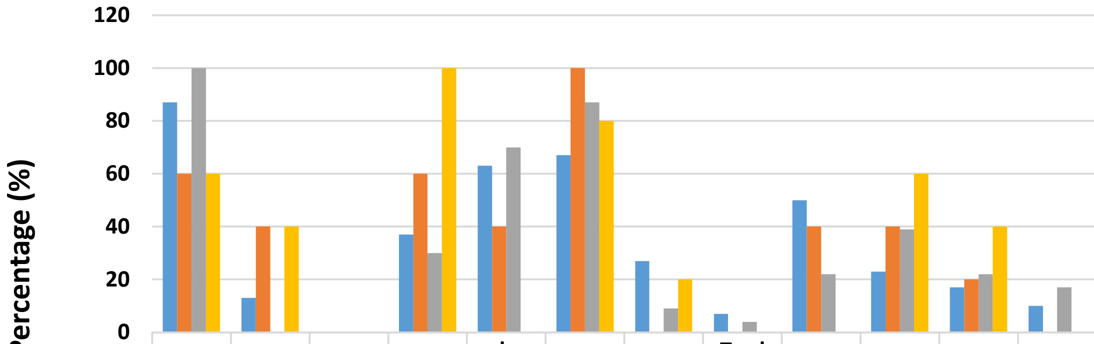
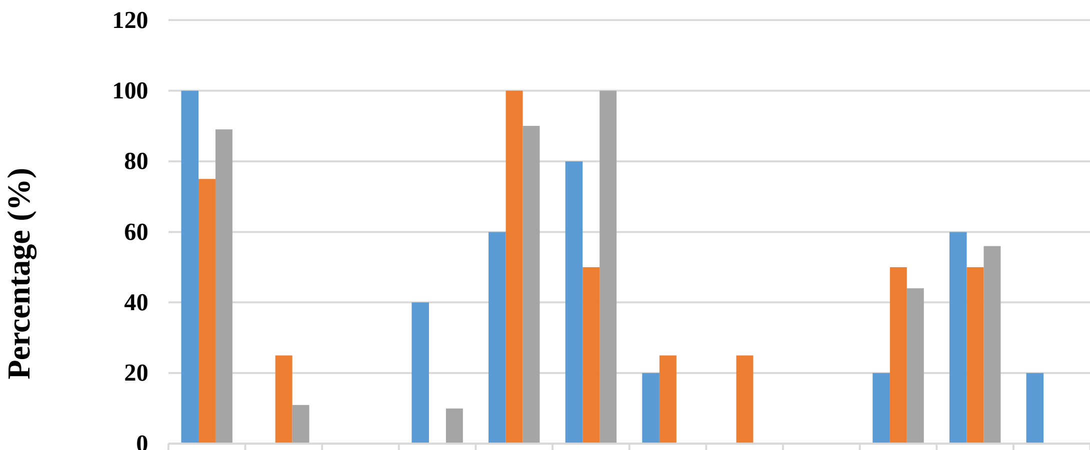


Figure 4a: Average demographic characteristics of producers from seven municipalities



	Mal e	Fem ale	Belo w 18	18-35	abo ve 36	Chri stian	Mus lim	Trad ition alist	Prim ary	SHS	Terti ary	Non e
Perecntage (%) Ewutu-Senya	87	13	0	37	63	67	27	7	50	23	17	10
Perecntage (%) Sehwi	60	40	0	60	40	100	0	0	40	40	20	0
Perecntage (%) Ejisu-Juabeng	100	0	0	30	70	87	9	4	22	39	22	17
Perecntage (%) Shai Osudoku	60	40	0	100	0	80	20	0	0	60	40	0

Figure 4b: Demographic characteristics of producers from Ewutu- Senya, Sehwi, Ejisu-Juabeng and Shai Osudoku municipalities.



	Male	Femal e	Below 18	18-35	above 36	Christ ian	Musli m	Tradit ionalis t	Prima ry	SHS	Tertia ry	None
Percentage Yeyi	100	0	0	40	60	80	20	0	0	20	60	20
Percentage Teshie	75	25	0	0	100	50	25	25	0	50	50	0
Percentage Asuagyamang	89	11	0	10	90	100	0	0	0	44	56	0

Figure 4c: Demographic characteristics of producers from Yeyi, Teshie and Asuogyamang municipalities.

From Figure 4a, it can be seen that more males are into egg production as 82% of the respondents were males with only 12% female respondents producing eggs. This result can be attributed to drudgery nature of poultry keeping and the high energy demands of poultry egg production particularly if the farm has a low level of mechanisation. Mukhtar (2012) had a similar result of more males engaging in egg production than females in Bauchi state, Nigeria. Many of the respondents were above 36 years with the remaining falling within the ages of 18 and 35 (Figure 4a). These findings show that poultry egg production is done by middle aged men and adults and might be due to the huge capital investment it requires and the perception of the youth that farming is an occupation of the elderly. Eighty one percent of these egg producers were Christians, 14% were Muslims and 5% were traditionalist. This could be as a result of the communities the questionnaires were administered being densely dominated by Christians. The educational background from Figure 4a shows that most of the producers had secondary education and had tertiary education with few having primary education and no formal education. This means that majority of the producers were literate which is very necessary because efficient poultry production requires good record keeping which requires reading and writing. Also administration of medication also requires some level of reading.

4.1.2 Demographic Characteristics of Egg Sellers

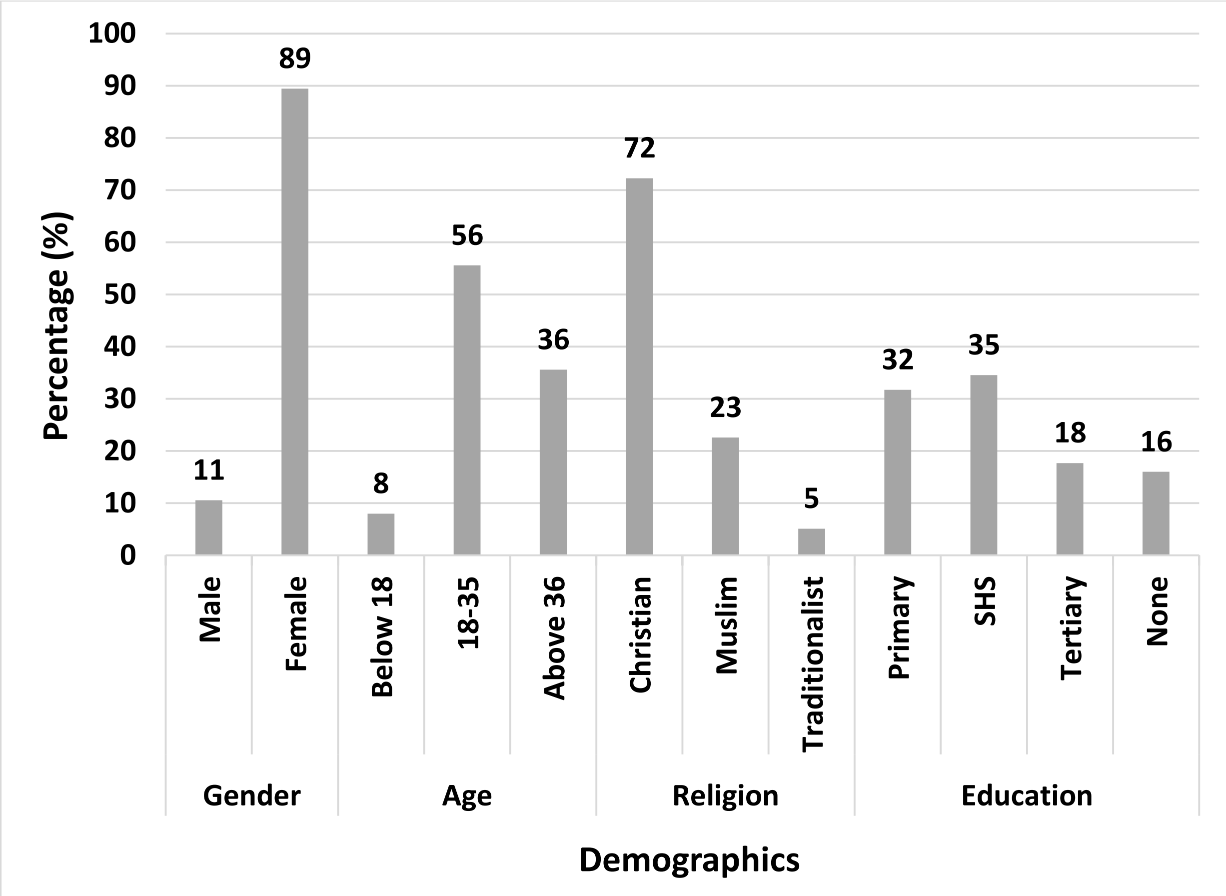
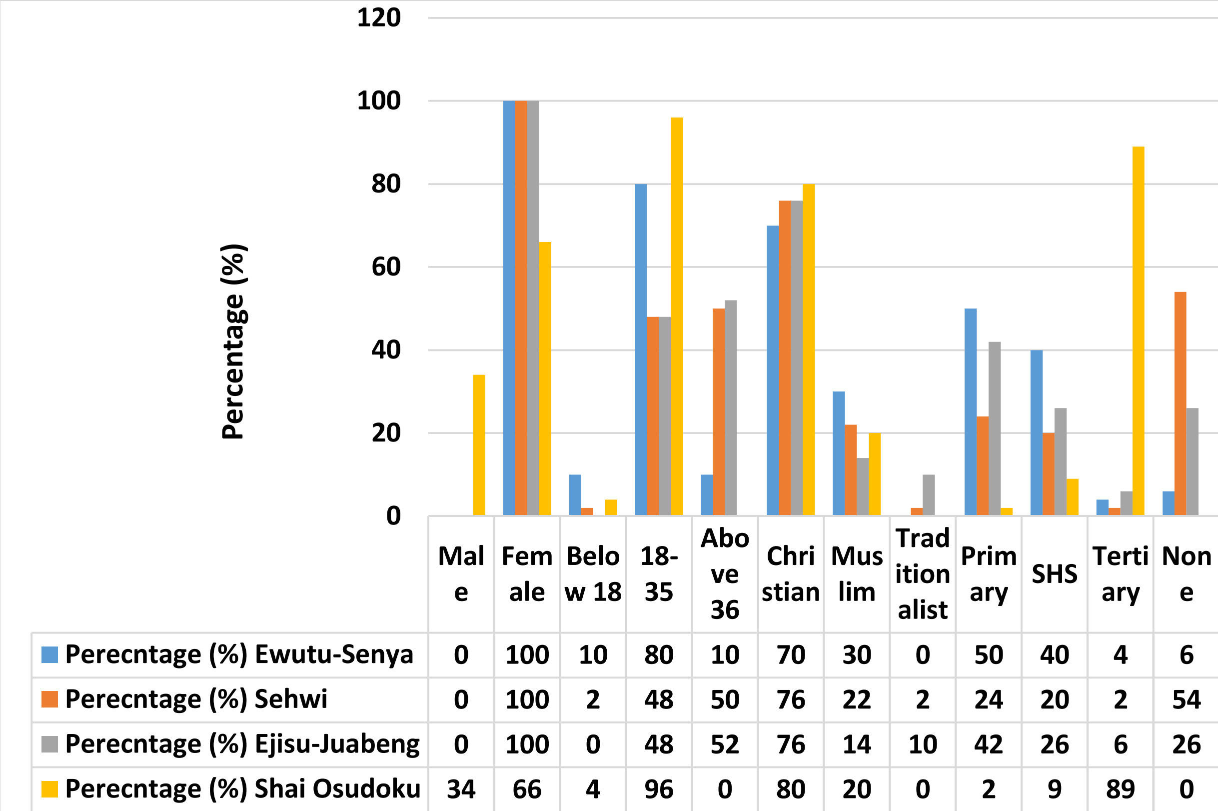
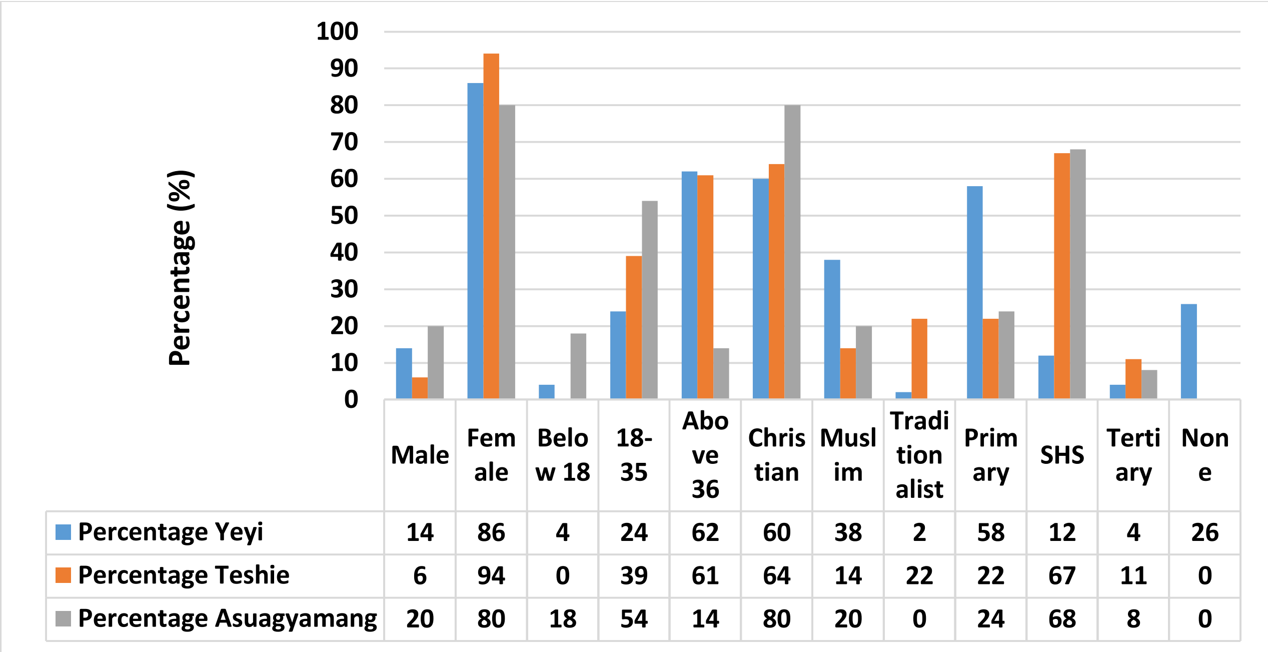


Figure 5a: Average demographic characteristics of egg sellers in the seven municipalities



	Male	Female	Below 18	18-35	Above 36	Christian	Muslim	Traditionalist	Primary	SHS	Tertiary	None
Perecntage (%) Ewutu-Senya	0	100	10	80	10	70	30	0	50	40	4	6
Perecntage (%) Sehwi	0	100	2	48	50	76	22	2	24	20	2	54
Perecntage (%) Ejisu-Juabeng	0	100	0	48	52	76	14	10	42	26	6	26
Perecntage (%) Shai Osudoku	34	66	4	96	0	80	20	0	2	9	89	0

Figure 5b: Demographic characteristics of egg sellers in Ewutu- Senya, Sehwi, Ejisu-Juabeng and Shai Osudoku municipalities.



	Male	Female	Below 18	18-35	Above 36	Christian	Muslim	Traditionalist	Primary	SHS	Tertiary	None
Percentage Yeyi	14	86	4	24	62	60	38	2	58	12	4	26
Percentage Teshie	6	94	0	39	61	64	14	22	22	67	11	0
Percentage Asuagyamang	20	80	18	54	14	80	20	0	24	68	8	0

Figure 5c. Demographic characterictics of egg sellers in Yeyi, Teshie and Asuagyaman

Figure 5a shows that majority of the egg sellers in these municipalities were females with few males. This could possibly be due to the fact that majority of the producers (farmers) were males and their female counterparts did the marketing and also attributed to the fact that women have the good strategies of selling. Also fifty-six percent of these egg sellers fell within the ages of 18 to 35 years, 36 % were above 36 years and the remaining 8% were below 18 years. This indicates that most of the egg sellers in these municipalities were middle aged women. Majority of the respondents were Christians followed by Muslims with few traditionalist (Figure 5a). This could be due to the location of the communities the questionnaires were administered being densely populated by Christians. The low number of traditionalist could also be attributed to the religious beliefs of many traditionalists who see eggs as sacred and to be meant for mostly rituals. Thirty-five percent of the respondents had secondary education, 32% primary education, 18% tertiary education with the remaining 16% having no formal education. It can be said that most of these egg sellers had formal education which is a good indication. As with demographics, the educational levels of the respondents from the individual districts followed same trend with a larger proportion between 80 and 94% being females.

4.1.3 Demographic Characteristics of Consumers.

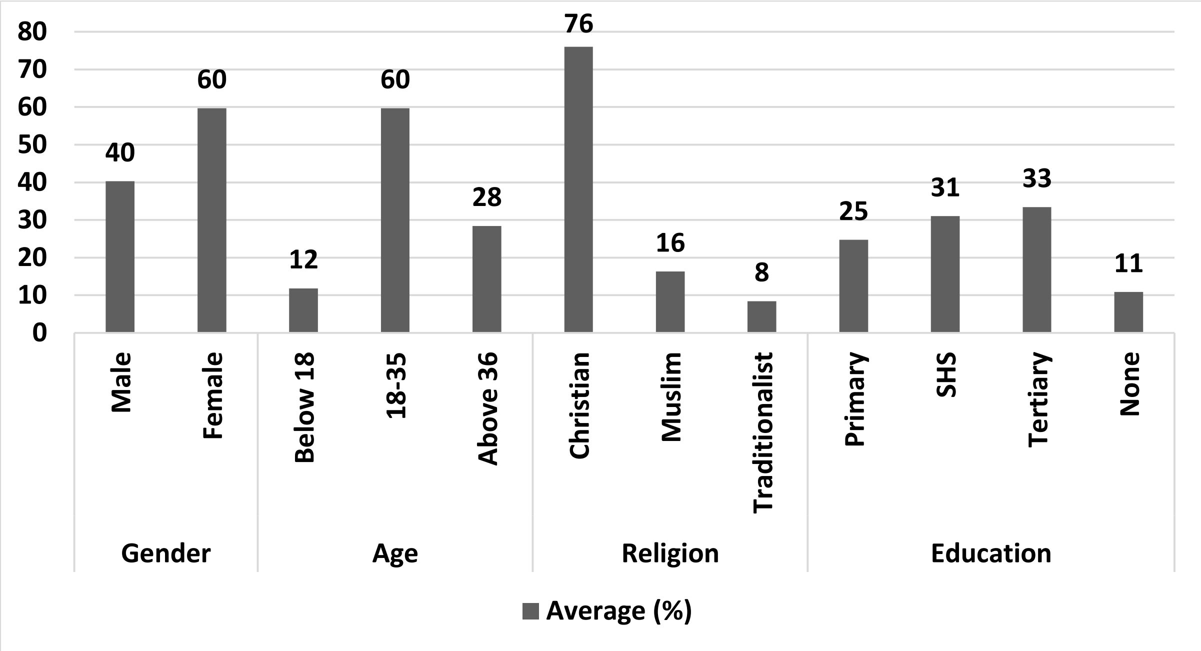
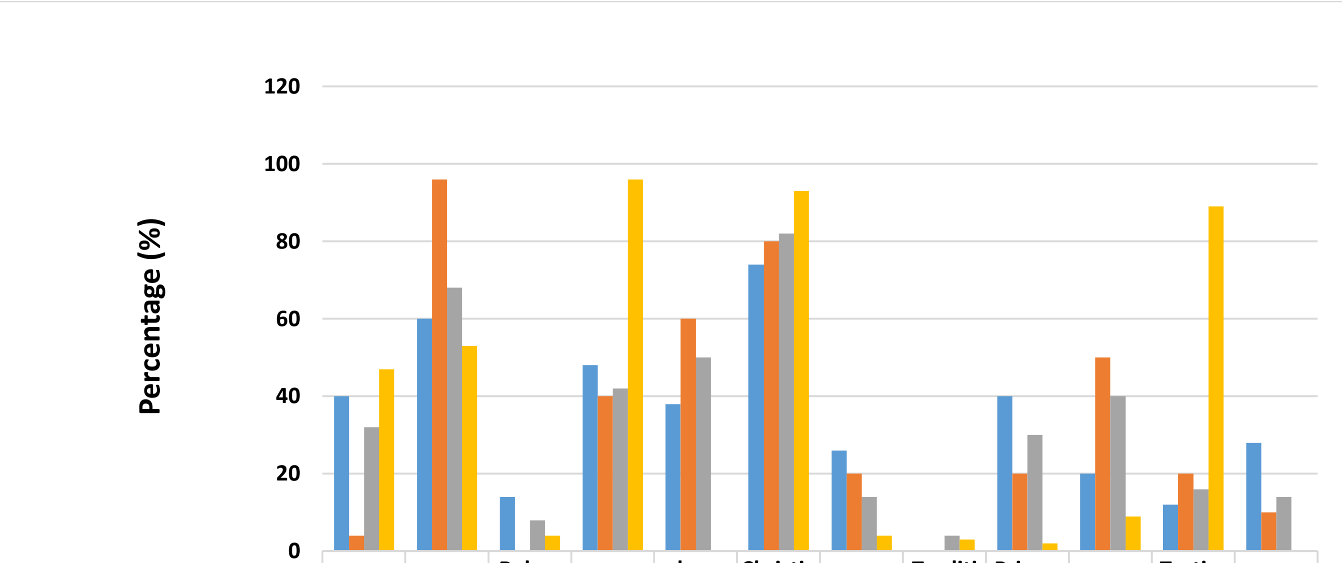
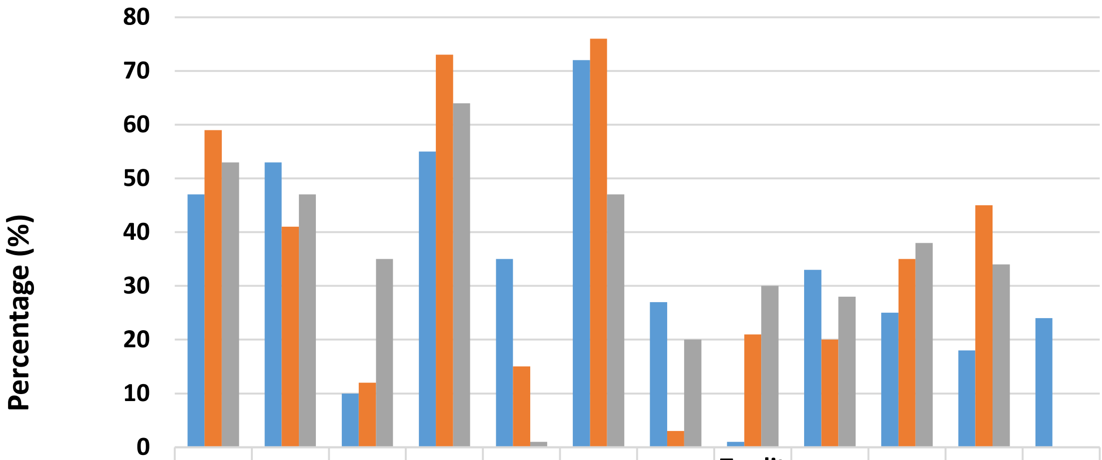


Figure 6a: Average demographic characteristics of consumers in the seven municipalities



	Male	Female	Below 18	18-35	above 36	Christian	Muslim	Traditionalist	Primary	SHS	Tertiary	None
Perecntage Ewutu-Senya	40	60	14	48	38	74	26	0	40	20	12	28
Perecntage Sehwi	4	96	0	40	60	80	20	0	20	50	20	10
Perecntage Ejisu-Juabeng	32	68	8	42	50	82	14	4	30	40	16	14
Perecntage Shai Osudoku	47	53	4	96		93	4	3	2	9	89	0

Figure 6b: Demographic characteristics of consumers in Ewutu-Senya, Sehwi, Ejisu- Juabeng and Shai Osudohu municipalities



	Male	Female	Below 18	18-35	above 36	Christian	Muslim	Traditionalist	Primary	SHS	Tertiary	None
Percentage Yeyi	47	53	10	55	35	72	27	1	33	25	18	24
Percentage Teshie	59	41	12	73	15	76	3	21	20	35	45	
Percentage Asuagyamang	53	47	35	64	1	47	20	30	28	38	34	0

Figure 6c: Demographic characteristics of consumers in Yeyi, Teshie and Asuagyamang municipalities.

A large number of the respondents interviewed from all seven districts were females with few males. Sixty percent of these egg consumers fell within the ages of 18 to 35 years, 28% were above 35 years and 12% were below 18 years. Most of the egg consumers interviewed were within their productive years and this is encouraging because most people in their production years in Africa and Ghana to be precise normally have a perception that consuming eggs raise their blood cholesterol level and hence shun from egg consumption. Majority of the respondents were Christians followed by Muslims with few traditionalist (Figure 6a). These findings could also be due to the location of their areas the questionnaire were administered. Also 33% had tertiary education, 31% had senior high school education, 25% of the respondents had basic education and 11% of the respondents interviewed had no formal education. This high level of formal education among the respondents could be linked with the high egg consumption rate among the middle aged respondents.

4.1.4 Quality Characteristics of Eggs as Food among Consumers in the Municipalities

From Table 13 it can be seen that, in the Ewutu Senya municipality, majority (half) of the respondents had fish as their best source of animal protein in their diets while the remaining respondents said the best protein source in their diets were eggs and meat. Ewutu-Efutu Senya is located in a coastal area where fish is in abundance and sold at a lesser cost and since fish is also a high protein source just like egg, they would opt for fish first. This may be the reason for eating more fish in the District as compared to the other source of animal protein (Egg and Meat). Questions relating to the rating of eggs as source of protein were asked and it was realised that majority of the respondents rated eggs as a high source of protein and few respondents rated the protein in eggs as low. This shows that majority of the egg consumers knew the health benefits and the nutrients provided by eggs, but due to the easy access to fish which is also a good source of protein and also its low cost, they tend to take in more fish than eggs. In fact, they recommended that such educational programs should continue since it will help better the consumption of more eggs than other source of animal protein like meat. The study showed that 58% of the respondents consumed egg twice per week, 28% consumed egg once a week and only 10% of the respondents consumed egg three times per week. This shows that majority of the respondents recorded a low egg consumption rate as compared to the required egg consumption being one per day. The low consumption rate might be due to the cost and also the spiritual misconceptions of the people about eggs. A particular interest is the 52% of respondents in Shai-Osudoku that consumed eggs 4 times in a week. This district is located in the Greater Accra Region, the capital of Ghana where household income levels are often higher and people understand the importance of eggs than most forest zones.

Table 13: Quality characteristics of eggs as food among consumers in the Municipalities

MUNUCIPALITY		Ewutu-Senya	Ejisu-Juabeng	Sehwi-Wiawso	Asuogyamang	Teshie	Shai-Osudoku	Yegi
EGG CHARACTERISTICS	PARAMETERS				PERCENTAGE (%)			
Best protein in diet	Meat	20	36	36	64	31	0	31
	Fish	50	30	0	16	24	53	49
	Egg	30	34	60	20	16	47	17
	Beans	0	0	4	0	9	0	3
						12		
Rating of eggs as a source of protein	Very high	24	60	60	38	42	47	19
	High	56	30	36	62	30	53	39
	Low	20	10	4	0	8	0	39
	No idea							3
Eggs eaten per week	Once a week	28	40	20	45	18	7	21
	Twice a week	58	36	80	30	21	8	20
	Thrice a week	10	6	0	20	18	18	34
	Four times a week	0	18	0	5	43	15	22
	More than 4 times						52	3

The survey shows that majority of the respondents in the Sehwi Wiaso municipality said eggs were the best source of protein in their diet. 36% said the best source of protein in their diet was meat while 4% said beans was the best source of protein in their diet (Table, 13). Egg provides a good source of high quality protein and contains many vitamins and minerals. Egg in diets can also improve health by preventing heart disease, improving skin and hair and boosting brain development (Wahlqvist, 1998). This may be some of the reasons for eating more eggs in the Sefwi Wiawso Municipality as compared to the other source of animal protein (Beans and Meat). Questions relating to respondent's knowledge about rating of eggs as source of protein showed that majority of these respondents in the Sehwi municipality acknowledged that eggs are very high source of protein and a high source of protein with few respondents rating the protein in eggs as low (Table 13). This shows that majority of the egg consumers knew the health benefits and the nutrients provided by eggs. The study showed that majority of the respondents consumed egg twice per week whilst few of the respondents consumed egg three times per week. This shows that majority of the respondents recorded a low egg consumption rate as compared to the required egg consumption being one per day. The low consumption rate might be due to the spiritual misconceptions of the people about eggs.

In the Ejisu – Juabeng municipality, questions relating to respondent's knowledge about rating of eggs as a source of protein showed that majority of the respondents rated the protein in eggs as high and very high while few rated the protein in eggs as low (Table 13). This shows that majority of the egg consumers knew the health benefits and the nutrients provided by eggs, probably due to the extension education provided by the (FAO) nutritionist, as acknowledge by the respondents.

The study showed that 60% of the respondents consumed egg twice per week, 30% consumed egg once a week and only 10% of the respondents consumed egg three times per week. This shows that majority of the respondents recorded a low egg consumption rate as compared to the required egg consumption being one per day which is in agreement with Ayim-Akonor and Akonor, (2014) whose work revealed that consumption of eggs among participants in the Accra Metropolis where 94.2% of the populace were found to consume eggs at least once in a month. The low consumption rate might be due to fear of eggs in raising their serum cholesterol and also the spiritual misconceptions of the people about eggs that egg consumption in pregnant women causes bald head in their unborn babies.

In Asuogyamang municipality, majority of the respondents rated meat as the best source of protein in their diet with few of the respondents rating egg and fish as their best source of protein in their diet. All the respondents rated egg as a good source of protein with 32% rating the protein in eggs as very high and 68% rating eggs as a high source of protein. Though all of the respondents regarded eggs to be a good source of proteins, however the consumption rate was lower than that of chicken. This could be due to the perception of consumers that eggs increase the risk of heart related problems due to its high cholesterol content (Houston *et al.*, 2011). Egg consumption of consumers were assessed and it was recorded that majority of the consumers ate eggs once a week and twice a week while few ate eggs three times a week and four times in a week. This means that the rate of egg consumption was very low, though eggs are inexpensive source of high quality proteins, essential vitamins and minerals for a healthy diet and healthy life (Zaheer, 2015).

In the Teshie municipality, 31% of the respondents said meat was the best source of protein in their diet, 24% said fish, 16% said eggs and 9% said beans was the best source of protein in their diet. Most of the respondents rated the proteins in eggs as very high and high while few respondents rated it as low. Egg consumption in this municipality was high as majority of the consumers consumed eggs four times in a week. Few of the respondents consumed eggs once in a week (Table 13). The respondents knowledge about the protein content relatively reflected in their rate of egg consumption as majority of them consumed eggs four times in a week.

In the Shai Osudoku municipality, 53% of the respondents rated fish as the best protein in their diet and 47% said eggs were the best source of protein in their diet. Forty-seven percent of the respondents rated the protein content of eggs as very high and the remaining 53% rated the protein content in eggs as high. Also 7% of the respondents said they consume eggs once a week, 8% of the respondents said they ate eggs twice a week, 18% of the respondents ate eggs three times in a week, 15% of the respondents consumed eggs four times in a week whilst 52% of the respondents consumed more than four eggs in a week. Egg consumption was high in this municipality as compared to the other municipalities in this study. This could be attributed to the high educational background of the respondents hence their knowledge about the nutritional value of eggs.

In the Yegi municipality, majority of the respondents 49% ranked fish as their best protein source, 31% of the consumers rated meat as the best source of protein in their diet and 17% of the consumers chose egg as their best protein source in their diets whiles only 3% of the total consumers interviewed had option for beans (cowpea). Majority of the respondents chose fish because, the study area is located near the Volta Lake, hence easy access to fish at affordable prices. Nineteen percent of the respondents rated the protein in eggs as very high, 39% rated eggs protein as high, 39% also rated the protein in eggs as low and 3% of the respondents did not have any idea about the protein content of eggs. This results can be attributed to the low educational background of the consumers. Twenty-one percent of the respondents in Yegi municipality ate eggs once a week, 20% ate eggs twice a week, 34% also ate eggs three times in a week and 22% ate eggs four times in a week and 3% ate more than four eggs in a week. Egg consumption in this municipality was relatively high even though the most of the consumers did not have a fair idea about the nutritional content of eggs.

4.1.4 Flock Size, Egg Production and Market of Eggs of the Various Municipalities.

4.1.4.1 Ewutu-Senya Municipality

- **Flock size**

Number of layers kept by farmers

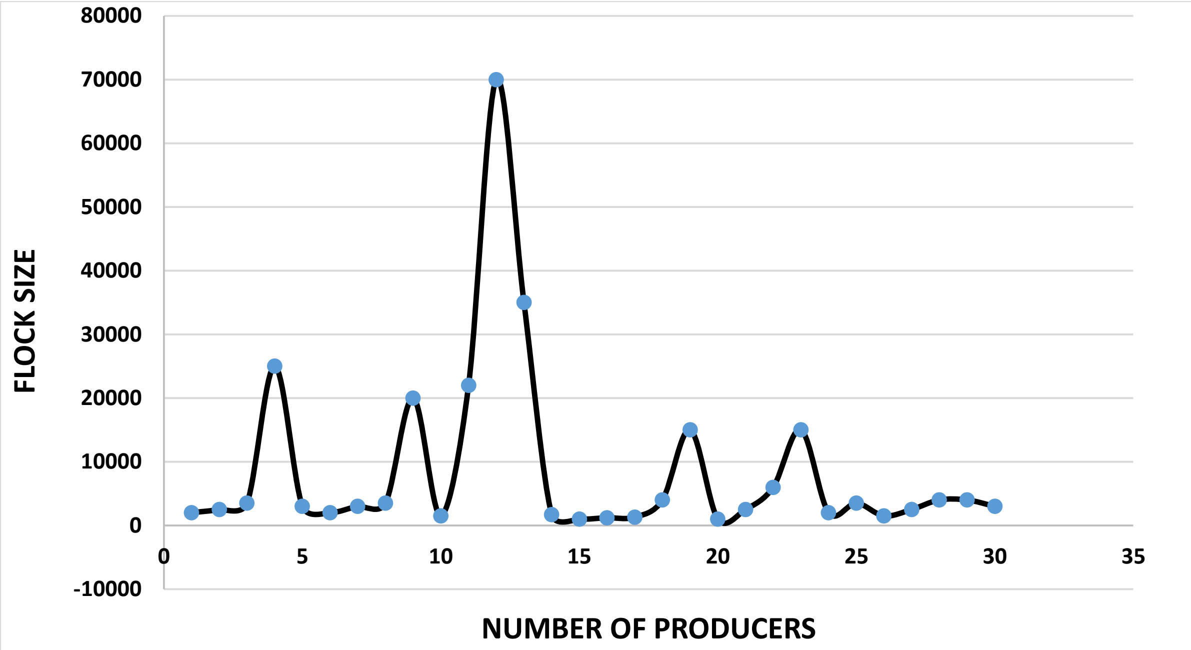


Figure 7: Number of layers kept among farmers in the Ewutu-Senya municipality.

Figure 7 shows that 23 out of the 30 farmers interviewed in this municipality operated on a small- scale commercial farming since their flock size were less than 5000 birds while the remaining 7 operated on a large scale. FAO (2014) reported that farms with 50 – 5000 birds can be qualified as a small scale, 5000 – 10000 flock size falls within the medium scale and over 10, 000 flock size fall within the large scale system of farming. The reason why most of the farmers operated on a small scale can be attributed to the location of this municipality being in the fishing community.

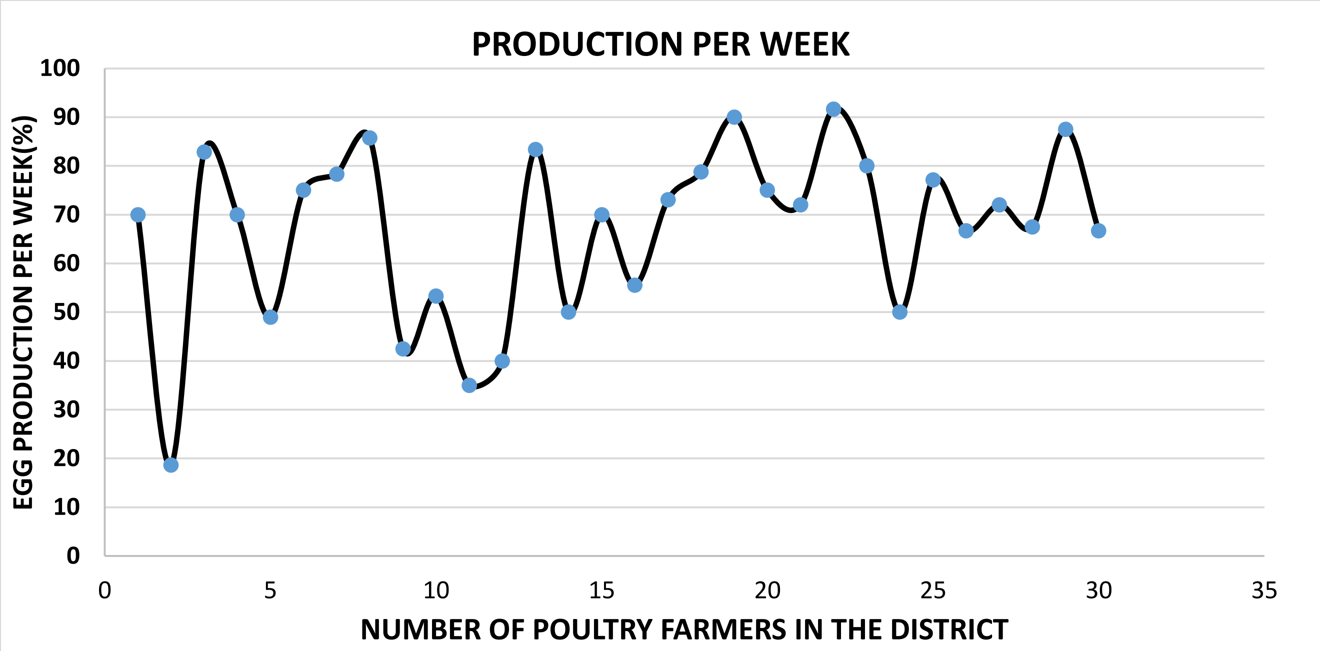


Figure 8: Egg production per week (%) of farmers interviewed in the Ewutu-Senya Municipality

Seven farmers had their egg production between 20 – 50% with majority of them recording a production of between about 58 – 92%. It can be said that, farmers within this municipality practised most of the cultural practices of poultry keeping hence their higher egg production. The higher egg production could also be attributed to the age of their birds meaning that most of the farmers birds were not spent and were in their productive ages.

4.1.4.2 Sehwi Wiaso Municipality

- **Flock size**

Table 14: Number of layers kept among farmers in the Sehwi Wiaso Municipality

Producers	Flock Size
1	1000
2	2000
3	1500
4	1000
5	900

The results show that all the farmers interviewed operated on a small-scale based on the categorisation of scale by FAO (2014). The number of layers kept by most of the farmers in the municipality were below 2000 and only one farmer kept exactly 2000 layers (Table 6). This could be attributed to the high capital investment required for poultry production and the unwilling of banks to give loans with low interest rates to farmers.

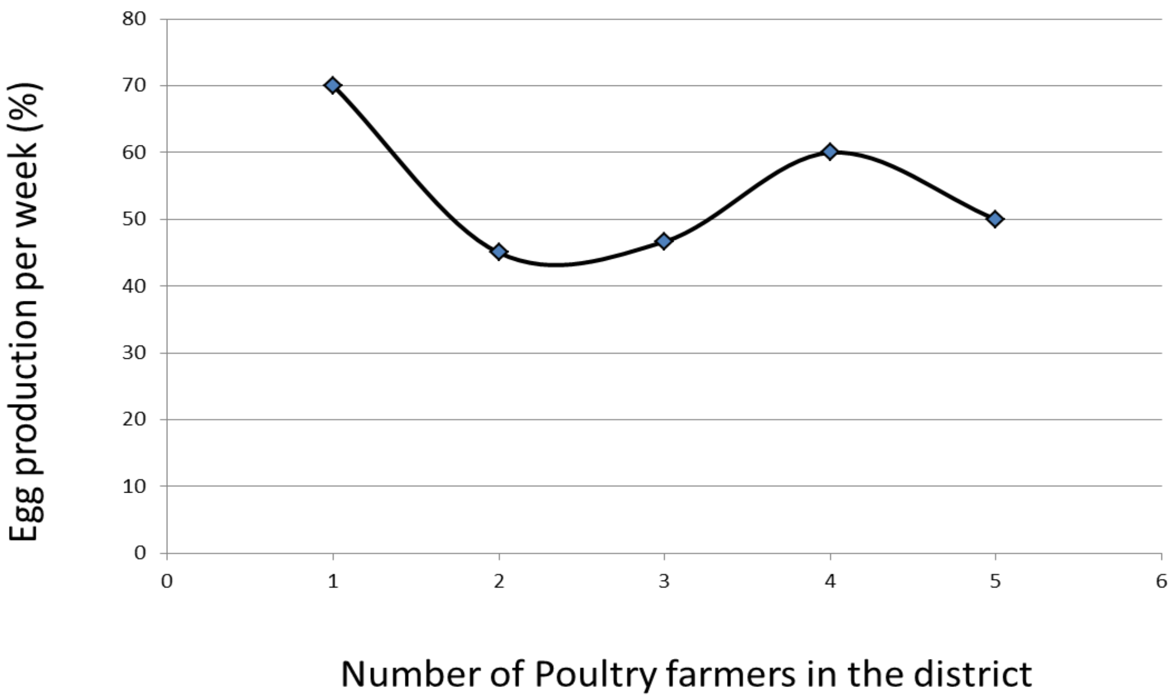


Figure 9: Shows weekly egg productions of farms in Sehwi Wiaso

Figure 9 shows that egg production in the municipality was low. Four of the farmers had a production percentage of below 70% with only farmer having a percentage of 70%. This low production value could be due to the age of the birds, poor cultural practices and high incidence of diseases.

- **Egg Sold per Week**

Table 15: Eggs sold per week by egg sellers in Sehwi Wiawso municipality

Sellers (%)	Number of crates sold per eeek
20	10 - 50
80	100 - 200

The report shows that 20% of the egg sellers sold 10 - 50 crates of eggs per week whiles 80% of them sold 100 – 200 crates of eggs per week in the Sefwi Wiawso Municipality (Table 7). This table shows that egg has a ready market at Sefwi and it has a high demand. Comparing the flock size of the farmers interviewed to the number of eggs sold per week, one can say that majority of the eggs sold in this Municipality were brought in from outside the Municipality. However, this indicates that egg production is generally low in the study area.

4.1.4.3 Ejisu Juabeng Municipality

- **Flock size and egg production per week**

Table 16: Flock size and egg production per week by farmers in Ejisu Juabeng Municipality

Producers	Layers Kept	Production Per Week
1	7000	34300
2	8000	39200
3	4000	19600
4	11000	53900
5	10000	49000
6	1000	4900
7	15000	73500
8	2000	9800
9	1000	4900
10	4500	20475
11	10000	45500
12	1200	5460
13	1200	5460
14	11000	50050
15	4000	18200
16	2000	9100
17	20000	98000
18	4000	16800
19	1500	7350
20	2000	9800
21	4500	18900
22	1000	4900
23	2000	8400
24	2000	8400
25	4500	18900
26	1500	6300
27	5000	21000
28	2000	8400
29	1500	6300
30	3500	14700

Table 16 shows that majority of the farmers (19) kept birds on a medium scale with less than 5000 birds. Only 11 farmers kept birds from 5000 to 20000. Production of eggs in the Ejisu-Juabeng Municipality was high as compared to the population and this might be due to the higher number of layers kept by individual farmers and also the ready market for the farmers products. The egg production per week of farmers in the Ejisu-Juaben Municipality is relatively high. Most of the farmers (27) had their egg production per week ranging from 6300-126000 eggs per week, with few of them (3) having a very low egg production per week. The high production could be due to the training farmers in Ejisu received from their farmer association in this Municipality.

- **Eggs Sold Per Week**

Table 17: Number of eggs sold per week in Ejisu Juabeng municipality

Eggs Taken per Week	Percentage of Sellers
10-50 crates	92
51-60 crates	4
61-70 crates	4
TOTAL	**100**

Majority of the egg sellers interviewed in the municipality sold between 10 to 50 crates of egg in a week and a few sold between 51 and 70 crates (Table 17). Egg sold by egg sellers in this municipality was low as compared to the eggs produced by farmers. It can therefore be stated that majority of the eggs produced in this municipality were sent to other areas outside the municipality for sales.

4.1.4.4 Asuogyaman Municipality

- **Flock Size**

Table 18: Flock size of farmers interviewed at Asuogyamang Municipality

Numbers of Farmers	Flock Size
1	150
1	600
3	1000
2	2000
1	6000
1	35000

Table 18 shows that, only two of the farmers interviewed fall within the small scale commercial egg production while six fall within the medium and just one been into the large scale commercial production. All the farmers kept their birds under intensive system of housing; the essence of this system was for the convenience of egg collection and controlling of diseases (UFAW, 1994). Most farmers were willing to expand their firm if only they got access to the necessary resources.

- **Egg Production Per Week**

In the assessment of egg production per week it was recorded that on the average about 190,000 to 201,000 eggs were produced by the farmers in the district. Farooq *et al.* (2001) found positive association of egg production and reported major contribution of eggs in total returns. Egg production is a dependent variable and is influenced by several factors like strain of chicken, feeding, mortality, culling, health and management practices, age at point-of-lay, peak lay and persistency of lay Tolimir and Masic (2000). Some the farmers use the manual technique for feed production such as using the spade in mixing already crushed poultry ingredients; this might affect the birds if the mixing is not properly done and in turn affecting the number of eggs produced. The table below shows the egg production per week among poultry farmers in the district of Asuogyaman .

Table 19: Egg production per week.

Number of Layers	Egg Production Per Week
150	840
600	3360
1000	5600
1000	5600
2000	8400
2000	11200
2000	10080
6000	33600
35,000	196000

From the table above, the rate of egg production per week in the district is relatively high as compared to the number of poultry farms available. According to the farmers they rated eggs to be one of the highest sources of animal proteins available in the district. Under normal circumstance, egg production increases as the number of the layers increases under enabling environments such as provision of potable water, good ventilation and clean dry place (Mburu and Ondwasi, 2005), which is in accordance with the present study as seen from the table above.

4.1.4.5 Teshie Municipality

- **Flock Size and Egg Production Per Week**

All the producers interviewed in the Teshie municipality operated on a small scale. This could be attributed to the location of the area which is a city so most of the farmers kept them on your backyards. Egg production in these farms were high with all the farmers having a production percentage of above 75%. This can be attributed to the small scale hence their ability to manage the birds properly.

Table 20: Number of producers, flock size and egg production of farmers in the Teshie Municipality

Producers	Flock Size	Average Egg/Day	Production Percentage
Farmer 1	600	465	78
Farmer 2	450	350	78
Farmer 3	500	410	82
Farmer 4	900	850	94

- **Egg Sold Per Week**

Table 21: Number of eggs sold by egg sellers in Teshie Municipality

Crates Taken Per Week	Percentage of Sellers
0-100	44
101-200	25
201-300	17
201-400	14

Majority of the egg sellers said they usually bought between 0 to 100 crates of eggs to sell. Twenty-five percent bought between 101 and 200 eggs, 17% bought between 201 and 300 crates of egg and 14% bought between 301 and 400 crates of egg. This shows that large quantity of eggs was sold in this municipality. The reason may be due to the high demand of egg in this urban area. It can be deduced that most of the eggs sold in this municipality are brought from other areas since egg production is low in the municipality.

4.1.4.6 Yegi Municipality

- **Flock Size and Egg Production Per Week**

Table 22: Flock size and egg production in Yegi Municipality

Producers	Flock Size	Average Egg Production/Week	Average Production % Per Week
Farmer 1	800	3990	71.25
Farmer 2	1000	5880	84
Farmer 3	2000	11760	84
Farmer 4	2500	14700	84
Farmer 5	3000	17850	85

From the table 22 above, it can be seen that one of the farmers interviewed in the municipality operated on a small scale while the remaining farmers operated on a medium scale. This could be attributed to the location of the municipality. This municipality is located in a fishing community where cat fish are mostly produced. Egg production in these farms were high as most of the farms had a production percentage of 84% with one farm recording 71% and the other recording 85%.

- **Egg Sold Per Week**

Table 23: Number of eggs sold by egg sellers in Yegi Municipality

Percentage of Farmers (%)	Number of Crates Taken
26	10-25
36	26-45
38	46 and above

Twenty-six percent of egg sellers in the Yegi municipality bought 10 to 25 crates of eggs to sell every week. Also 36% bought between 26 and 45 crates and 38% bought more than 46 crates of egg to sell every week. There is a probability that most of the eggs sold in this municipality were brought in from different areas to be sold there since egg production in Yegi was low due to the location of the area.

4.1.4.7 Shai Osudoku Municipality

- **Flock Size**

Table 24: Number of producers and flock size in the Shai- Osudoku Municipality

Number of Farmers	Flock Size
1 farmer	10,000
1 farmer	2,000
2 farmers	4, 000
1 farmer	500
2 farmers	6000
1 farmer	7000
1 farmer	8000
1 farmer	500

Two farmers operated on a small scale in this municipality, one farmer operated on a large scale and seven farmers operated on a medium scale with a flock size of between 2000 to 8000 birds (FAO, 2014). This finding can be due to the location of this municipality being in the urban area and unavailability of loans with flexible interest rates to farmers.

- **Eggs Sold per Week in the Shai – Osudoku Municipality**

Table 25: Number of egg sellers and eggs sold per week

Percentage of Egg Sellers (%)	Number of Crates Taken
58	10-30
42	31-60

Majority of the farmers sold between 10 to 30 crates of eggs a week and the remaining respondents interviewed sold between 31 and 60 crates. This means that most of the eggs produced in this Municipality was sold in the Municipality.

4.2. Experiment Two: The potential of two local seeds (Egushi and Werewere) as sources of PUFA for egg enrichment

4.2.1 Proximate Composition of Egusi and Neri Seeds

Table 26: Proximate Analysis of Egusi and Neri Seeds

Seeds	Moisture (%)	Ash (%)	Crude Protein (%)	Fat (%)	Crude Fibre (%)	NFE (%)
Egusi	9.50	3.50	35.00	44.00	7.55	0.45
Neri	9.00	2.50	25.9	34.00	28.33	0.27

Table 26 shows the proximate composition on dry matter basis for egusi and neri. Both egusi and neri seeds are rich in oil and protein. Neri seeds recorded a high crude fibre content when compared with egusi and this was because the seeds used were the readily available seeds on the market where egusi seeds are dehulled and neri seeds are undehulled. The moisture content of the seeds were close with egusi recording 9.5% and neri recording 9%. The moisture content of the seeds falls within the range Arkroyed and Doughty (1964) reported for legumes; 7 – 10%. However, the moisture content was higher than the study of that Shaibu (2013) reported. The ash content of egusi in this study (3.5%) is close to that recorded by Ojieh *et al.,* (2008). Pomeranz and Clifton (1981) recommended that the ash content of seeds and tubers for animal feed formulation should be in the range of 1.5 -2.5% which the ash content of neri (2.5%) falls within. This shows that the mineral content of the seeds are good. The crude protein value of both seed compares favourably with those of protein rich foods such as soybean, cowpeas, pigeon peas and pumpkin with protein contents ranging between 23.1 and 33.0% (Olaofe *et al.,* 1994). This protein value also falls within the recommended daily allowance for children (23.0 – 36.0 g) (NRC, 1989). The Egusi seed is rich in oil and protein like soyabean (*Glycine max)* comprising of 50% oil and 35% protein (Sarwar *et al.,* 2013). The ether extract (crude fat) content of 44% obtained for egusi melon in this study agrees closely with that reported by Oluba *et al.,* (2008). It is however too high compared to that obtained for soybean (23.5%) (Paul and Southgate, 1980). With the high amount of crude fat obtained for egusi melon in this study, egusi melon could be regarded as an oil seed (Sarwar *et al.*, 2013).

4.2.2. Amino Acid Profile of Egusi and Werewere

Table 27a: Total contents of Amino acids after hydrolysis of protein Egusi (Melon seed)

Amino Acid	Content (%)[a]	AA (%) in CP	Content (As is)
Methionine*	0.823	2.201	0.849
Cysteine	0.551	1.475	0.569
Methionine + Cysteine	1.374	3.675	1.418
Lysine*	1.128	3.017	1.164
Threonine*	1.245	3.331	1.285
Arginine*	5.387	14.406	5.558
Isoleucine*	1.334	3.567	1.376
Leucine*	2.550	6.820	2.631
Valine*	1.537	4.111	1.586
Histidine*	0.867	2.320	0.895
Phenylalanine*	1.795	4.800	1.852
Glycine	1.760	4.707	1.816
Serine	1.523	4.072	1.571
Proline	1.267	3.388	1.307
Alanine	1.563	4.181	1.613
Aspartic acid	3.011	8.053	3.107
Glutamic acid	6.448	17.245	6.653
Total (without NH_3)	32.789	87.693	33.832
Ammonia	0.559	1.496	0.577
Total	**33.348**	**89.189**	**34.409**

*Essential amino acid

a = Figures standardized to a dry matter content of 88%; AA = Amino acid; Dry matter (%): 90.80; CP = Crude protein; CP (%)[a]: 37.39; CP (% as is): 38.58

Table 27b: Total contents of Amino acids after hydrolysis of protein Werewere (Neri)

Amino Acid	Content (%)[a]	AA (%) in CP	Content (As is)
Methionine*	0.650	2.426	0.677
Cysteine	0.350	1.308	0.365
Methionine+ Cysteine	1.000	3.733	1.042
Lysine*	0.744	2.777	0.775
Threonine*	0.770	2.874	0.802
Arginine*	3.582	13.368	3.731
Isoleucine*	0.931	3.475	0.970
Leucine *	1.591	5.937	1.657
Valine *	1.078	4.024	1.123
Histidine*	0.591	2.207	0.616
Phenylalanine*	1.260	4.704	1.313
Glycine	1.428	5.331	1.488
Serine	1.101	4.110	1.147
Proline	0.823	3.071	0.857
Alanine	1.096	4.092	1.142
Aspartic acid	2.028	7.571	2.113
Glutamic acid	4.314	16.102	4.494
Total (without NH_3)	22.338	83.375	23.270
Ammonia	0.389	1.451	0.405
Total	**22.727**	**84.826**	**23.675**

*Essential amino acids

a = Figures standardized to a dry matter content of 88%; AA = Amino acid; Dry matter (%): 91.67; CP = Crude protein; CP (%)*: 26.79; CP (% as is): 27.91.

From tables 27a and 27b it can be seen that when both seed samples were standardized to a dry matter content of 88% they contained nine (9) of the essential amino acids with arginine (5.387%, 3.582%) recording the highest value, methionine (0.823% 0.650%), lysine (1.128%, 0.744%), threonine (1.245%, 0.770%), isoleucine (1.334%, 0.931%), leucine (2.550%,1.591%), valine (1.537%,1.078%), histidine (0.867%,0.591%), and phenylalanine

(1.795%,1.260%) for egusi and neri respectively. It can be seen that, egusi recorded higher values for essential amino acids than neri. Both seeds also contained considerable quantities of other nonessential amino acids like alanine, aspartic acid and glutamic acid together with other conditional amino acids cysteine, serine, glycine, and proline which egusi recorded higher values than neri. Conditional amino acids are usually not essential except in times of stress and illness. The results show that arginine, glutamic acid and aspartic acid were the three most abundant amino acids in egusi melon and neri. This observation is in partial agreement with the report of Aremu *et al.,* (2006) who worked on egusi. The total amino acid content of egusi is 33.348% and that of neri is 22.727%. This results shows that egusi and neri are rich in amino acids especially arginine, isoleucine and leucine.

4.2.3 Fatty Acid Profile of Egusi and Neri Seeds

Table 28: Fatty acid Composition of Egusi and Neri seeds

SEEDS	% Methyl Palmitate C16:0	% Methyl Heptadecanoate C17:0	% Methyl Stearate C18:0	% Methyl Oleate C18:1	% Methyl Linoleate C18:2	% Methyl Arachidate C20:0
Egushi	10.65	6.18	10.10	11.77	61.18	0.11
Neri	16.03	6.56	10.85	12.42	54.03	0.11

Table 28 shows the fatty acid composition of egusi and neri. It can be seen that palmitic acid (10.65%, 16.03%) heptadecanoic acid (6.18%, 6.565), steric acid (10.10%, 10.855), oleic acid (11.77%, 12.42%) and linoleic acid (61.18%, 54.03%) are the principal fatty acids in egusi and neri respectively with arachidonic acid (0.11%) for both egusi and neri. The linoleic acid content of egusi and neri in this study is close to that reported by Oluba *et al.,* (2008). Findings from these results show that both egusi and neri are richer in linoleic acid than animal fat which is richer in oleic acid (29% – 48%) (NRC, 1994). Younis *et al.,* (2000) worked on *Cucurba pepo* seed which is in the same family as egusi and neri and reported a similar results. They found that the seed contains mostly palmitic, oleic, stearic and linoleic acids with linoleic acids being the most. The results also show that egusi and neri contain no n:3 PUFAs but rich in n:6 PUFA. Egusi seed contains 61.29% of PUFA and neri contains 54.14% of PUFA which is mostly linoleic acid which is an essential fat**.**

4.3 Experiment Three: Comparative analysis of essential fatty acids deposited in eggs through layer hen diet

4.3.1 N:3 Polyunsaturated Fatty Acid Content of Eggs

Table 29: Effects of flaxseed oil inclusion levels on n:3 PUFA content in egg yolk (mg/ml)

Flaxseed oil inclusion	Total omega 3	C18:3	C20:3	C20:5	C22:3	C22:5	C22:6
T1 (Control)	0.076b	0.193a	0.014a	0.147a	0.012a	0.003a	0.004b
T2 (1.5%)	0.074b	0.193a	0.012a	0.139a	0.013a	0.002a	0.005b
T3 (3%)	0.191a	0.298a	0.227a	0.118a	0.303a	0.004a	0.015a
T4 (4.5%)	0.064b	0.173a	0.011a	0.116a	0.010a	0.002a	0.005b
SEM	0.034	0.078	0.108	0.028	0.126	0.001	0.002
P-Value	0.033	0.668	0.400	0.820	0.301	0.293	0.002

[abc] Different superscripts within the same column indicate significant differences among means (P ≤É 0.05)

There were no significant difference (P>0.05) in the individual n:3 PUFA except in C22;6 where treatment with three percent flaxseed oil recorded a significantly (P<0.05) higher levels when compared with the other treatments. Even though there were no significant difference in the amount of C18:3 (ALA) in the egg yolk, it can be found from the Table 29 that the egg yolk contained more ALA when compared with the other n:3 PUFAs. This is because the n:3 PUFA in flaxseed seed oil is ALA which is 54.8% (Mazalli *et al.,* 2004) hence the egg yolk recording high levels ALA. This is in agreement with Van Elswyk (1997); Bean and Leeson (2003) and Fraeye *et al.* (2012) study which proved that feeding flaxseed enriched feed mainly resulted in ALA enriched egg yolk, up to 200 mg per egg. Although flaxseed oil have no EPA, DPA and DHA in it, the presence of these fatty acid in their egg yolk confirms the ability of laying hens to convert ALA into its long chain metabolites (Schumann *et al.*,2000). C22:6 (DHA) recorded significantly higher value than the other treatments. Mazalli *et al.,* (2004) found that including flaxseed oil at 3% or ground flaxseed at 9% compared with 3% fish oil or other vegetable oil sources at 3% led to the highest concentration of DHA in egg yolk. Four percent flaxseed oil and fish oil were tested by Baucells *et al.,* (2000) and it was found that flaxseed oil recorded 1.56% DHA and fish oil recorded 3.18%. The drop in the DHA in egg yolk as flaxseed oil inclusion exceeded 3% is in agreement with this study. Treatment with 3% flaxseed oil also recorded a significantly higher total n:3 PUFA in the egg yolk when compared with the other treatments. A lot of earlier researchers have proved that the amount of n:3 in the egg yolk increased linearly as flaxseed and flaxseed oil levels increased in the diet (Scheideler and Froning 1996, Van Elswyk, 1997). However, the result in this study showed that total n:3 significantly increased only when birds were f

ed with 3% flaxseed oil when compared with the other treatments. There was no significant difference between the control diet and diets with 1.5% and 4.5% flaxseed oil. The significantly higher total n:3 value for treatment with 3% flaxseed oil than 4.5% might be attributed the high fiber content of the 4.5% flaxseed oil diet and also the oil content. High fiber in a diet of poultry has a laxative effect on the birds as Scheideler and Froning (1996) hypothesized. It was observed that birds on the 4.5% flaxseed oil diet excreted loose manure. Even though the diet was isocaloric, it was observed that birds on the 4.5% flaxseed oil consumed less feed hence eating insufficient amount of n:3. The low feed consumption noticed could be due to the oily nature of the feed. The control diet was however comparable to diets with 1.5% and 4.5% flaxseed oil.

4.3.2 N:6 Polyunsaturated Fatty Acid Content of Eggs

Table 30: Effect of flaxseed oil inclusion levels on the n:6 Pufa content of egg yolk (mg/ml)

Flaxseed Oil Inclusion	C18:2	C18:3	C19:2	C20:2	C20:3	C20:4	C22:2	C22:4	C22:5	Total n:6
T1 (Control)	0.025	0.271	0.009	0.204	0.096	0.006	0.003	0.002	0.006	0.623^{b}
T2 (1.5%)	0.026	0.236	0.005	0.195	0.089	0.017	0.003	0.002	0.006	0.579^{b}
T3 (3%)	0.296	0.491	0.023	0.161	0.347	0.016	0.004	0.003	0.014	1.355^{a}
T4 (4.5%)	0.029	0.211	0.012	0.151	0.076	0.010	0.002	0.003	0.006	0.501^{b}
SEM	0.134	0.142	0.006	0.038	0.133	0.005	0.001	0.001	0.003	0.037
P-Value	0.384	0.490	0.164	0.717	0.417	0.385	0.536	0.893	0.196	0.041

^{ab} Different superscripts within the same column indicate significant differences among means ($P \leq 0.05$)

There were no significant (P>0.05) difference in all the individual n:6 PUFA. The C22:4 (arachidonic acid) increased slightly in this study even though there were no significant difference in the increase. This findings are not in agreement with a previous studies by Van Elswyk *et al.,* (1992) who found a 70% decrease in the level of arachidonic acid in n:3 fortified eggs when compared to the control eggs. This is because ALA and LA compete for the enzyme D-6 saturase which catalyses the first step in the elongation process. Alpha linolenic acid has more affinity for the enzyme so feeding ALA rich diets like flaxseed oil should decrease the population of AA (Van Elswyk, 1997). However, Grobas *et al.,* (2001) also recorded an increase in arachidonic acid level when they fed 5% and 10% flaxseed oil to laying birds. They expressed finding this result as unexpected and postulated the reason for the findings to be associated with the low levels of ALA (0.8%) they used in their study which decreased the reduction of D-5 desaturase activity in laying hens.

There was a significant (P<0.05) difference in the total n:6 PUFA values with treatment of 3% flaxseed oil recording a significantly higher value when compared with the other treatments. This result is not in agreement with previous studies where researcher fed flaxseed and flaxseed oil to laying hens. In their study n:6 concentrations decreased as inclusion of flaxseed increased Scheideler and Froning (1996), Cachaldora *et al.,* (2008), Rodica *et al.,* (2009), Amaar *et al.,* (2014). Grobas *et al.,* (2001) also found a relatively high numerical increase in the total n:6 PUFA when they fed 5% and 10% flaxseed oil to laying hens when compared to the eggs from the birds on the control diet even though the increase were not statistically significant.

4.4. Experiment Four: Effect of egg storage on egg quality characteristics of n:3 fortified egg

4.4.1 Effects of Incorporating Flaxseed Oil in Layer Diet and Egg Storage Duration in Ambient Temperature on Egg Quality Assessment

The effects of the different flaxseed oil treatments were statistically significant (P<0.05) for initial egg weight, final egg weight, wet shell weight and dry shell weight when compared with the control. Treatment with 4.5% flaxseed oil recorded a significantly higher fresh egg weight when compared with the other treatments. This increase in egg weight could be explained by the report by Jensen *et al.,* (1958), who stated that dietary lipids enhances the weight of eggs. This results does not conform to the findings of other researches where researchers fed flaxseed oil to birds. These researchers proved that, there was no significant difference in the egg weight of flax fed birds as compared to egg from birds which were not fed with flaxseed (Baucells *et* *al.*, 2000, Schumann *et al*., (2000), Grobas *et al.*, 2001, Celebi and Utlu, 2006). However, other reseachers also found significant differences in egg weight when they fed flaxseed to layer hens (Dunn- Horrocks *et al.*, 2011, Cherian and Quezada, 2016). In their study flax fed hens recorded a higher egg weight when compared with other diets which is in agreement with this study. Nonetheless, (Pappas *et al.*, 2005, Augustyn *et al.*, 2006) found a negative effect of n:3 PUFA on egg weight. Mode of storage and duration of storage can affect the weight of an egg. This is because carbon dioxide (CO_2) and moisture are lost from egg as it ages. This loss of CO_2 is rapid when eggs are stored in an ambient temperature hence causing a decline in egg weight (Mountney, 1976). From table 32 it can be noted that egg weight declined when eggs were stored. Treatment with 4.5% flaxseed oil recorded a significantly higher stored egg weight than the other treatments followed by treatment with 1.5% flaxseed oil. There was no significant ($P>0.05$) difference between treatments with no flaxseed oil and treatments with 1.5% and 3% flaxseed oil but treatments with 1.5% flaxseed oil recorded a significantly ($P<0.05$) higher stored egg weight than treatment with 3% flaxseed oil. It was noted from this study that; final egg weight was lower than their respective initial egg weights when compared. There was a drop of 0.1g when eggs were stored for one day, the drop in egg weight increased to 0.73g when storage period increased to 8 days and 1.86g lost in weight when eggs were stored for 14 days.

Table 31: Effects of incorporating flaxseed oil in layer diet and egg storage duration in ambient temperature on external egg quality

Flaxseed Oil Inclusion	Initial Egg Weight (g) 1	Final Egg Weight (g) 2	Wet Shell Weight (%) 2	Dry Shell Weight (%) 5
T1 (Control)	61.390^{b}	60.930^{bc}	10.628^{a}	10.482^{a}
T2 (1.5%)	61.786^{b}	61.127^{b}	9.795^{b}	9.634^{b}
T3 (3%)	60.713^{b}	60.058^{c}	10.070^{b}	9.924^{ab}
T4 (4.5%)	63.810^{a}	62.958^{a}	9.868^{b}	9.937^{ab}
SEM	0.348	0.321	0.198	0.226
Egg Storage Duration in Ambient Temperature				
0	61.775^{abc}	61.775^{ab}	9.858^{b}	9.774^{ab}
1	62.267^{ab}	62.167^{ab}	10.423^{ab}	10.136^{ab}
2	59.283^{c}	59.000^{c}	9.621^{b}	9.481^{b}
3	62.560^{ab}	62.349^{a}	10.120^{ab}	9.979^{ab}
4	62.884^{ab}	62.606^{a}	10.0912^{ab}	9.997^{ab}
5	59.883^{bc}	59.1^{c}	9.361^{b}	9.226^{b}
6	62.624^{ab}	62.159^{ab}	10.193^{ab}	10.000^{ab}
7	62.5325^{ab}	61.894^{ab}	10.415^{ab}	10.2375^{ab}
8	63.289^{a}	62.556^{a}	10.202^{ab}	9.998^{ab}
9	62.614^{ab}	61.381^{ab}	9.999^{ab}	10.628^{ab}
10	62.568^{ab}	61.704^{ab}	10.261^{ab}	10.120^{ab}
11	60.553^{abc}	59.523^{bc}	9.317^{b}	9.218^{b}
12	62.885^{ab}	61.724^{ab}	10.045^{ab}	9.873^{ab}
13	62.381^{ab}	62.178^{ab}	11.630^{a}	11.552^{a}
14	60.772^{abc}	58.909^{c}	9.822^{b}	9.696^{ab}
SEM	0.675	0.622	0.383	0.438
Source of Variation				
Flaxseed oil inclusion	<.0001	<.0001	0.0133	0.0636
Egg storage	<.0001	<.0001	0.0148	0.0527
Interactions	0.6609	0.6685	0.4071	0.362

^{abc} Different superscripts within the same column indicate significant differences among means (*P* ≤ 0.05); initial egg weight is the weight of the egg on the day of lay; final egg weight is the stored egg weight, wet shell weight is the weight of the shell when it was air dried for three days and dried shell weight is the weight of the shell when it was oven dried at 90^{o}C for three days.

Table 32: Effects of incorporating flaxseed oil in layer diet and egg storage duration in ambient temperature on internal egg quality

Flaxseed Oil Inclusion	**Wet Yolk Weight (%) 1**	**Albumen Weight (%) 3**	**Albumen Height (Mm) 3**	**Haugh Unit 4**
T1 (Control)	27.813	64.485	6.890	81.002
T2 (1.5%)	26.882	62.253	6.791	79.964
T3 (3%)	26.991	61.858	6.893	81.187
T4 (4.5%)	26.271	62.573	6.937	80.813
SEM	0.540	1.355	0.071	0.511
Egg Storage Duration in Ambient Temperature				
0	24.685^{b}	65.458	9.280^{a}	95.478^{a}
1	25.162^{b}	64.257	9.260^{a}	95.346^{a}
2	26.381^{b}	63.515	8.692^{b}	93.314^{ab}
3	26.495^{b}	63.047	8.200^{c}	89.814^{cd}
4	26.285^{b}	63.185	8.433^{bc}	90.637^{bc}
5	25.857^{b}	63.483	7.600^{d}	87.341^{d}
6	27.445^{ab}	61.618	6.906^{e}	81.852^{e}
7	26.980^{b}	61.580	6.808^{e}	81.140^{e}
8	26.727^{b}	61.914	6.086^{f}	75.694^{f}
9	26.567^{b}	61.469	5.649^{gf}	72.407^{gf}
10	28.094^{ab}	60.259	5.633^{gf}	72.596^{gf}
11	27.492^{ab}	61.483	5.267^{gh}	70.869^{g}
12	27.442^{ab}	60.658	5.783^{f}	73.928^{gf}
13	31.477^{a}	70.390	5.089^{h}	67.624^{h}
14	27.748^{ab}	59.566	4.475^{i}	63.085^{i}
SEM	1.046	2.624	0.138	0.989
Source of Variation				
Flaxseed oil inclusion	0.2513	0.5333	0.5159	0.3376
Egg storage	0.0067	0.4524	<.0001	<.0001
Interactions	0.756	0.4136	0.275	0.2728

[abcdefgh] Different superscripts within the same column indicate significant differences among means ($P \leq 0.05$); wet yolk weight is the weight of the fresh egg yolk

There was a significant (P<0.05) difference between shell weight relative to egg weight (% wet shell weight) between the control diet and diet with flaxseed oil. % wet shell weight was lowest in eggs from hens fed with flaxseed oil. These findings are in agreement with

Cherian and Quezada (2016) who reported low egg shell weight of flaxseed fed birds when compared with the control diet with no flaxseed. Egg storage did not affect egg shell weight. Even though there was a significant difference in shell weight as egg storage duration increased, there was no significant difference as the storage days progressed.

Flaxseed oil did not have any significant effect on yolk weight relative to egg weight (% wet yolk weight), albumen weight relative to egg weight (% albumen weight), albumen height and Haugh unit when compared with the control. A significant increase was recorded for % wet yolk weight as storage duration increased. As storage duration increases, water is lost from the albumen to the egg yolk making it increase in size and weight. This loss of water from the albumen into the yolk decreases the albumen weight with long storage. Even though there was no significant increase in % albumen weight, it can be seen from table 32 that, % albumen weight kept declining as storage duration increased. There was a significant reduction in albumen height as storage duration increases. This is because, as egg ages, carbon dioxide (CO_2) is lost from the egg through the shell. This increases the pH of the albumin making it alkaline, (Scott and Silversides, 2000). This causes the gelly-like albumen to become watery and transparent because Mucin fiber a substance which gives eggs its gel-like texture is lost as CO_2 is lost (Mountney, 1976). The decrease in albumen height with storage agrees with the findings of Falvey *et al.,* (1998) and Samli *et al.* (2005). A reduction in albumen height with storage will directly affect Haugh unit as egg ages. There was a significant decrease in Haugh unit from 95.35 in one day storage through to 75.69 on the eighth day of storage to 63.09 when eggs were stored in ambient temperature for 14 days. It can be seen from the table that egg from this study exceeded the recommended HU for freshness 70HU at day 13 of storage (Van Niekerk, 2014). Eggs from treatments with 0%, 1.5% and 3% flaxseed oil had a significantly higher yellowish yolk as compared to eggs from treatment with 4% flaxseed oil. The yellow colour of egg yolk is normally derived from xanthophyll pigment in the hen’s diet. The lighter egg yolk recorded in this study can be due to less of these xanthophylls in the hen's diet. Yellow maize was used throughout this research and the inclusion level of maize reduced as the level of flaxseed oil increased. This could be the reason for the lighter egg yolk recorded in treatment with 4.5% flaxseed oil. The result of this study agrees with a previous study by Cherian and Quezada (2016) who recorded a paler yolk in eggs from treatment with flaxseed with a 9% drop in maize when compared with their control.

4.5 Experiment Five: Assessing the effects of n:3 fortified diets on fatty liver haemorrhage syndrome as stress of treatments on bird.

4.5.1 Effects of Flaxseed Oil Diet on the Health of Laying Birds

Table 33: Effects of flaxseed diet on live weight, liver and abdominal fat content of birds

Flaxseed Inclusion level	Live Weight (g)	Liver Weight (g)	Fat Weight (g)	Heamorrhage Score
T1 (Control)	1.97^{a}	37.814^{a}	58.73^{a}	1.2^{b}
T2 (1.5%)	1.87^{a}	31.508^{a}	73.82^{a}	1.4^{b}
T3 (3%)	1.84^{a}	31.564^{a}	49.84^{a}	3^{a}
T4 (4.5%)	1.92^{a}	31.802^{a}	94.73^{a}	2^{a}
SEM	0.075	2.573	12.785	0.447
P-value	0.633	0.266	0.110	0.049

^{ab} Different superscripts within the same column indicate significant differences among means ($P \leq 0.05$)

Birds fed with diets with 3% and 4.5% flaxseed oil inclusion levels had their livers infected with more haemorrhages and haematomas as compared with their counterparts who were fed with 1.5% and no flaxseed oil even though all the treatments had the same metabolizable energy content. The difference in haemorrhage score is unclear and might be attributed to the long chain unsaturated fatty acid flaxseed oil contain. This fatty acid undergoes oxidative rancidity at a faster rate (Cherian and Hayat, 2009). This oxidative rancidity of the fat in the liver destroys the hepatic cellular membrane of the birds making them more prone to haemorrhages. Bean and Leeson, (2003) reported a similar finding with this study when they fed laying hens with 10% flaxseed and found an increase incidence of fatty liver haemorrhage when compared with the control. Other studies, by Caston *et al.*, (1994), Schumann *et al.*, (2000) and Cherian and Hayat (2009) showed no significant difference in liver haemorrhage score when they fed laying birds with flaxseed.

CHAPTER FIVE

5.0 CONCLUSION AND RECCOMENDATION

5.1 Conclusion

Based on the results of this study, the following conclusions were made. Most egg producers are males and they are above 36 years. Most of them are formally educated. Egg sellers are mostly females within the ages of 18 and 35 years and most of them have received formal education. Most egg consumers interviewed were females and they were within the ages of 18 and 35 years. Most egg consumers eat two eggs in a week. Egusi and neri seeds do not contain omega 3 PUFA but omega 6 PUFA particularly linoleic acid. It is rich in other saturated fatty acids, rich in protein and in all the essential amino acids in addition to other non-essential and conditional amino acids. Feeding 3 percent flaxseed oil to laying increased the omega 3 and 6 PUFA content of eggs by 2.54 folds. Flaxseed oil did not have any significant effect on both the internal and external quality of eggs. Prolong storage of egg in ambient temperature reduces the protein quality. Flaxseed oil had no effect on live weight, liver weight and abdominal fat weight of birds. Feeding flaxseed oil at 3percent increases the fatty liver haemorrhage score of birds.

5.2 Recommendation

Further studies should be carried on the effects of flaxseed oil on production parameters like hen day production and feed conversion efficiency. Also studies on the histopathology of the liver and abdominal fat content of birds consuming flaxseed oil to further ascertain the health impact of flaxseed oil on laying hens. A potential study that space the addition of oil at 3% is also recommended.

REFERENCES

1. Abubakari, S. (2013). Development of Intensity-Duration-Freqency-Curves for Kumasi (Doctoral dissertation).
2. Ahn, D. U., Lutz, S., and Sim, J. S. (1996). Effects of dietary α-linolenic acid on the fatty acid composition, storage stability and sensory characteristics of pork loin. *Meat Science*, *43*(3), 291-299.
3. Ahn, D. U., Kim, S. M., & Shu, H. (1997). Effect of egg size and strain and age of hens on the solids content of chicken eggs. *Poultry Science*, *76*(6), 914-919.
4. Ajuyah, A. O., Wang, Y., Sunwoo, H., Cherian, G., and Sim, J. S. (2003). Maternal Diet with Diverse Omega–6/Omega–3 Ratio Affects the Brain Docosahexaenoic Acid Content of Growing Chickens. *Neonatology*, *84*(1), 45-52.
5. Akiba, Y., Takahashi, K., Kimura, M., Hirama, S., and Matsumoto, T., (1983). The influence of environmental temperature, thyroid status and a synthetic oestrogen on the induction of fatty livers in chicks. *British Poultry Science* 24, 71-80
6. Alisi, A., Ceccarelli, S., Panera, N., and Nobili, V. (2012). Causative role of gut microbiota in non-alcoholic fatty liver disease pathogenesis. Fro*nt Cell Infect Microbiology*, 2, 132.
7. Alton, K. B., Van Heek, M., Farley, C., Compton, D. S., Hoos, L., Sybertz, E. J., and Davis, H. R. (2000). Comparison of the activity and disposition of the novel cholesterol absorption inhibitor, SCH58235, and its glucuronide, SCH60663. *British Journal of Pharmacology*, *129*(8), 1748-1754.
8. Amaar, Y. G., Reeves, M. E., and Firek, M. (2014). Ampk and hdac pathways and regulation rassf1c gene expression. *Cancer Research*, *74*(19 Supplement), 2454-2454.

9. Anang, B. T., Yeboah, C., and Agbolosu, A. A. (2013). Profitability of Broiler and Layer Production in the Brong Ahafo Region of Ghana. *ARPN Journal of Agricultural and Biological Science,* 8(5), 423-430.

10. Anderson, B. M., and Ma, D. W. (2009). Are all n:3 polyunsaturated fatty acids created equal? *Lipids in Health and Disease*, *8*(1), 1.

11. Animal Production Directorate (2003). State of Ghana's Animal Genetic Resources Accra, 66

12. Aning K. G. (2006). The structure and importance of the commercial and village based poultry in Ghana. [http://www.fao.org/docs/eims/upload/213723/agal_poultryreview_ghana_aug06.pdf]. Retrieved December 09, 2015, from

13. AOAC International. (1995). Official Methods of Analysis of AOAC International. 16th Edition Arlington: AOAC International.

14. AOCS Lipid Library. (2014). Fatty acids: Methylene-interrupted double bonds [Internet]. Available from [http://lipidlibrary.aocs.org/Lipids/fa_poly/index.htm] (*Accessed 2015 August 1).*

15. Aremu, M. O., Olaofe, O., and Akintayo, T. E. (2006). A comparative study on the chemical and amino acid composition of some Nigerian under-utilized legume flours. *Pakistan Journal of Nutrition*, *5*(1), 34-38.

16. Arkroyed, W. R., & Doughty, J. (1964). Legumes in human nutrition. *Food and Agricultural Organization Nutrition Studies Publication,* 19.

17. Aronson, W. J., Glaspy, J. A., Reddy, S. T., Reese, D., Heber, D., and Bagga, D. (2001). Modulation of omega-3/omega-6 polyunsaturated ratios with dietary fish oils in men with prostate cancer. *Urology, 58*(2), 283-288.

18. Arterburn, L. M., Hall, E. B., & Oken, H. (2006). Distribution, interconversion, and dose response of n− 3 fatty acids in humans. *The American journal of clinical nutrition*, *83*(6), S1467-1476S.

19. Augustyn R., Barteczko J., and Smulikowska, S. (2006)– The effect of feeding regular or low α-linolenic acid linseed on laying performance and total cholesterol content in eggs. *Journal of Animal and Feed Sciences*,15, (Suppl. 1), 103-106.

20. Ayim-Akonor, M., and Akonor, P. T. (2014). Egg consumption: patterns, preferences and perceptions among consumers in Accra metropolitan area. *International Food Research Journal*, *21*(4).

21. Babcock, T. A., Helton, W. S., Hong, D. and Espat, N. J. (2002): Omega-3 fatty acid lipid emulsion reduces LPS-stimulated macrophage TNF-alpha production. Surg Infect (Larchmt) 3, 145–149.

22. Baucells, M. D., Crespo, N., Barroeta, A. C., Lopez-Ferrer, S. and Grashorn, M. A. (2000). Incorporation of different polyunsaturated fatty acids into eggs. *Poultry Science* ,79(1),51-59.

23. Bean, L. D., and Leeson, S. (2003). Long-term effects of feeding flaxseed on performance and egg fatty acid composition of brown and white hens. *Poultry Science*, *82*(3), 388-394.

24. Bernstein, R. K., Accurso, A., Dahlqvist, A., Draznin, B., Feinman, R. D., Fine, E. J., and Manninen, A. H. (2008). Dietary carbohydrate restriction in type 2 diabetes mellitus and metabolic syndrome: time for a critical appraisal. *Nutrition and Metabolism*, *5*(1), 1.

25. Beynen, A. C. (2004). Fatty acid composition of eggs produced by hens fed diets containing groundnut, soya bean or linseed. *NJAS-Wageningen Journal of Life Sciences*, *52*(1), 3-10.

26. Bezard, J., Blond, J. P., Bernard, A., and Clouet, P. (1994). The metabolism and availability of essential fatty acids in animal and human tissues. *Reproduction Nutrition Development*, *34*(6), 539-568.

27. Bjerve, K. S. (1991). Omega 3 fatty acid deficiency in man: implications for the requirement of alpha-linolenic acid and long-chain omega 3 fatty acids. *World review of nutrition and dietetics*.

28. Blesso, C. N., Andersen, C. J., Barona, J., Volek, J. S., and Fernandez, M. L. (2013). Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in individuals with metabolic syndrome. *Metabolism*, *62*(3), 400-410.

29. Bolton-Smith, C., Price, R. J., Fenton, S. T., Harrington, D. J., and Shearer, M. J. (2000). Compilation of a provisional UK database for the phylloquinone (vitamin K 1) content of foods. *British Journal of Nutrition*, *83*(04), 389-399.

30. Botsoglou, N. A., Yannakopoulos, A. L., Fletouris, D. J., Tserveni-Goussi, A. S., and Psomas, I. E. (1998). Yolk fatty acid composition and cholesterol content in response to level and form of dietary flaxseed. *Journal of Agricultural and Food Chemistry*, *46*(11), 4652-4656.

31. Bourre, J. M. (2004). Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing. *Journal of Nutrition*, *8*, 163-174.

32. Brenna, J. T. (2002). Efficiency of conversion of α-linolenic acid to long chain n:3fatty acids in man. *Current Opinion in Clinical Nutrition and Metabolic Care*, *5*(2), 127-132.

33. Brenna, J. T., and Lapillonne, A. (2009). Background paper on fat and fatty acid requirements during pregnancy and lactation. *Annals of Nutrition and Metabolism*, *55*(1-3), 97-122.

34. Brunner, E.J., Mosdøl, A., Witte, D.R., Martikainen, P., Stafford, M., Shipley, M.J., and Marmot M.G., (2008). Dietary patterns and 15-y risks of major coronary events, diabetes, and mortality. *American Journal of Clinical Nutrition* 87(5): 1414-1421.

35. Buamah F. T. (1992). Feeding Chicken for Egg Production. Ghana Publishing Corporation. Accra. 120-122.

36. Burdge, G. C. (2006). Metabolism of α-linolenic acid in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *75*(3), 161-168.

37. Burdge, G. C., and Calder, P. C. (2005). Conversion of $\ alpha $-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reproduction Nutrition Development*, *45*(5), 581-597.

38. Cachaldora, P., Garcia-Rebollar, P., Alvarez, C., De Blas, J. C., and Mendez, J. (2008). Effect of type and level of basal fat and level of fish oil supplementation on yolk fat composition and n-3 fatty acids deposition efficiency in laying hens. *Animal Feed Science and Technology*, *141*(1), 104-114.

39. Canadian Grain Commission. (2009). Quality of Western Canadian flaxseed 2009, export quality data, July, 2009. [http://www.grainscanada.gc.ca/flaxlin/harvest-recolte/2009/hqf09-qrl09-eng.htm.] (Accessed April 21, 2016).

40. Canadian Nutrient File. (2014). [Internet]. Available from [http://webprod3.hcsc.gc.ca/cnf-fce/index-eng.jsp.] (Accessed 2015 July 4).

41. Carrillo-Dominguez, S., Carranco-Jauregui, M. E., Castillo-Dominguez, R. M., Castro-Gonzalez, M. I., Avila-Gonzalez, E., and Perez-Gil, F. (2005). Cholesterol

and n:3and n:6 fatty acid content in eggs from laying hens fed with red crab meal (Pleuroncodes planipes). *Poultry science*, *84*(1), 167-172.

42. Caston, L. J., Squires, E. J., and Leeson, S. (1994). Hen performance, egg quality, and the sensory evaluation of eggs from SCWL hens fed dietary flax. *Canadian Journal of Animal Science, 74*(2), 347-353.

43. Celebi, S. and Utlu, N. (2006) Influence of animal and vegetable oil in layer diets on performance and serum lipid profile. *International Journal of Poultry Science*, 5, 370– 373.

44. Chai, S.C; S. Hooshmand; R.L. Saadat; and B.H. Arjmandi (2009). No relationship between egg consumption and cardiovascular risk factors in postmenopausal women. *FASEB Journal*, 23,550.11.

45. Chakrabarty, G. A. Y. A. T. R. I., Manjunatha, S., Bijlani, R. L., Ray, R. B., Mahapatra, S. C., Mehta, N. A. L. I. N., and Manchanda, S. C. (2004). The effect of ingestion of egg on the serum lipid profile of healthy young Indians. *Indian Journal of Physiology and Pharmacology*, *48*(3), 286-292.

46. Cherian G. and. Hayat Z. (2009). Long-term effects of feeding flaxseeds on hepatic lipid characteristics and histopathology of laying hens. Department of Animal Sciences, Oregon State University, Corvallis 97331 poultry science.

47. Cherian, G. (2008). Omega-3 fatty acids. In *Wild-Type Food in Health Promotion and Disease Prevention* , 169-177. Humana Press.

48. Cherian, G., and Quezada, N. (2016). Egg quality, fatty acid composition and immunoglobulin Y content in eggs from laying hens fed full fat camelina or flax seed. *Journal of Animal Science and Biotechnology*, *7*(1), 1.

49. Cherian, G., and Sim, J. (1992). Preferential accumulation of n:3 fatty acids in the brain of chicks from eggs enriched with n:3 fatty acids. *Poultry Science*, *71*(10), 1658-1668.

50. Cherian, G., Holsonbake, T. B., and Goeger, M. P. (2002). Fatty acid composition and egg components of specialty eggs. *Poultry Science*, *81*(1), 30-33.

51. Cho, E., Smith-Warner, S. A., Spiegelman, D., Beeson, W. L., van den Brandt, P. A., Colditz, G. A., and Goldbohm, R. A. (2004). Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. *Journal of the National Cancer Institute*, *96*(13), 1015-1022.

52. Chung, S. B., Cheong, I.C., and Ohh, B.K., (1983). The relative economic importance of the major traits in laying hens. Research Reports of the Officer of Rural Developments, S. Korea. Livestock and Veterinary, 25,13-20.

53. Coletta, J. M., Bell, S. J., and Roman, A. S. (2010). Omega-3 Fatty acids and pregnancy. *Reviews in Obstetrics and Gynecology*, *3*(4), 163.

54. Crespo, R., Shivaprasad, H.L., (2003). Developmental, metabolic, and other noninfectious disorders, In: Diseases of Poultry, 11 ed. Iowa State Press, Ames, pp. 1055-1102.

55. Cunnane, S. C. (2003). Problems with essential fatty acids: time for a new paradigm. *Progress in Lipid Research*, *42*(6), 544-568.

56. Daun, J., Barthet, V., Chornick, T., Duguid, S. (2003). Structure, composition, and variety development of flaxseed. In: Thompson, L., Cunanne, S. edition. Flaxseed in Human Nutrition. 2nd edition Champaign, Illinois, pp. 1-40.

57. De Oliveira Otto, M. C., Mozaffarian, D., Lemaitre, R. N., Fretts, A. M., Hotamisligil, G., Tsai, M. Y., and Nettleton, J. A. (2013). Trans-Palmitoleic acid, other dairy fat biomarkers, and incident diabetes: The Multi-Ethnic Study of

Atherosclerosis (MESA). *The American Journal of Clinical Nutrition*, *97*(4), 854-861.

58. Deckelbaum, R. J., and Torrejon, C. (2012). The omega-3 fatty acid nutritional landscape: health benefits and sources. *The Journal of nutrition*, 142(3), 587S-591S.

59. Defra, (2010): Preliminary outbreak assessment. *Available at: [http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/ai/latest*situation/index.html] (assessed December 5, 2015).

60. Delgado-Lista, J., Perez-Martinez, P., Lopez-Miranda, J., and Perez-Jimenez, F. (2012). Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. *British Journal of Nutrition*, *107*(S2), S201-S213.

61. Djousse, L. and Gaziano, J.M. (2008), "Egg consumption in relation to cardiovascular disease and mortality: the physicians' health study", *American Journal of Clinical Nutrition*, 87(4), 964-969.

62. Dunn-Horrocks, S., Pichardo-Fuchs, M., Lee, J., Ruiz-Feria, C., Creger, C., Hyatt, D., and Farnell, M. (2011). Effect of omega-3 enriched layer rations on egg quality. *International Journal Poultry Science*, *10*, 8-11.

63. Dyerberg, J., and Bang, H. O. (1979). Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *The Lancet*, *314*(8140), 433-435.

64. Ebeid, T., Eid, Y., Saleh, A., and Abd El-Hamid, H. (2008). Ovarian follicular development, lipid peroxidation, antioxidative status and immune response in laying hens fed fish oil-supplemented diets to produce n-3-enriched eggs. Animal-Cambridge University Press-, *2*(1), 84.

65. Emken, E. A. (1994). Metabolism of dietary stearic acid relative to other fatty acids in human subjects. *The American journal of clinical nutrition*, *60*(6), 1023S-1028S.

66. Erasmus, U. (1993). Fats that heal, fats that kill: the complete guide to fats, oils, cholesterol, and human health. Book Publishing Company.

67. Fahy, E., Cotter, D., Sud, M., and Subramaniam, S. (2011). Lipid classification, structures and tools. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1811*(11), 637-647.

68. Fahy, E., Subramaniam, S., Murphy, R. C., Nishijima, M., Raetz, C. R., Shimizu, T., and Dennis, E. A. (2009). Update of the LIPID MAPS comprehensive classification system for lipids. *Journal of lipid research*, *50*(Supplement), S9-S14.

69. Falvey, L., Hills, R. And Sdoodee, R. (1998).Review of the Australian Centre for International Agricultural Research Training Program. *Accessed 2016 May 26*

70. FAO, I. (2013). WFP. 2013. *The state of food insecurity in the world*.

71. FAO.(2014). Poultry Sector Ghana. FAO Animal Production and Health Livestock Country Reviews. No. 6. Rome.

72. Faostat, F. (2013). Statistical Yearbook 2013: World Food and Agriculture. *FAO Food and Agricultural Organziation UN Rome Italy*.

73. Farooq, M., Durrani, F. R., Aleem, M., Chand, N., and Muqarrab, A. K. (2001). Egg traits and hatching performance of Desi, Fayumi and Rhode Island Red chicken. *Pakistan Journal of Biological Science*, *4*(7), 909-911.

74. Farrel, D.J. (2002) Adding value to the hen's egg. Nutr. Rep. Int., 45, 1052– 1057. 8

75. Fayeye, T. R., Adeshiyan, A. B., and Olugbami, A. A. (2005). Egg traits, hatchability and early growth performance of the Fulani-ecotype chicken. *Growth*, *74*, 48.

76. Fernandez, M. L. (2006). Dietary cholesterol provided by eggs and plasma lipoproteins in healthy populations. *Current Opinion in Clinical Nutrition and Metabolic Care*, *9*(1), 8-12.

77. Ferrier, L. K., L. J. Caston, S. Leeson, J. Squires, B. J. Weaver, and B. J. Holub, (1995). α-Linolenic acid-and docosahexaenoic acid-enriched eggs from hens fed flaxseed: Influence on bloodlipidsandplateletphospholipidfattyacidsinhumans. *American Journal of Clininical Nutrition* 62,81–86

78. Filardi, R.D.S., Junqueira, O.M., de Laurentiz, A.C., Casartelli, E.M., Aparecida, R.E. and Francelino, A.L. (2005) Influence of different fat sources on the performance, egg quality, and lipid profile of egg yolks of commercial layers in the second laying cycle. *Journal of Applied Poultry Research*, 14, 258– 264.

79. Fokou, B., Achu, M. B. And Chaunges, T.M. (2004). Preliminary nutritional evaluation of five species of egusi seeds in Cameroon. *African Journal of Nutrition and Agricultural Development*, 4 : 8

80. Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497-509.

81. Fraeye, I., Bruneel, C., Lemahieu, C., Buyse, J., Muylaert, K., and Foubert, I. (2012). Dietary enrichment of eggs with omega-3 fatty acids: A review. *Food Research International*, *48*(2), 961-969.

82. Furuse, K., Fukuoka, M., Masuda, N., Negoro, S., Takada, M., Matsui, K., and Ogawara, M. (1991). A randomized trial in inoperable non-small-cell lung cancer:

vindesine and cisplatin versus mitomycin, vindesine, and cisplatin versus etoposide and cisplatin alternating with vindesine and mitomycin. *Journal of Clinical Oncology*, *9*(4), 606-613.

83. Galobart, J., Barroeta, A. C., Baucells, M. D., Codony, R., and Ternes, W. (2001a). Effect of dietary supplementation with rosemary extract and α-tocopheryl acetate on lipid oxidation in eggs enriched with ω3-fatty acids. *Poultry Science*, *80*(4), 460-467.
84. Galobart, J., Barroeta, A.C., Baucells, M.D., Cortinas, L. and Guardiola, F. (2001b). a-Tocopherol transfer efficiency and lipid oxidation in fresh and spray-dried eggs enriched with o3-polyunsaturated fatty acids. *Poultry Science,*80, 1496– 1505.
85. Gebauer, S. K., Psota, T. L., Harris, W. S., and Kris-Etherton, P. M. (2006). n− 3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *The American journal of clinical nutrition*, *83*(6), S1526-1535S.
86. Ghana Statistical Service. (2002). Population and Housing Census. Summary Report of Final Results. 62.
87. Ghezzi, S., Rise, P., Eligini, S., Colli, S., and Galli, C. (2007). Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *76*(6), 363-369.
88. Gibson, G. R., Fava, F., Gitau, R., Griffin, B. A., Tuohy, K. M., and Lovegrove, J. A. (2013). The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk'population. *International Journal of Obesity*, *37*(2), 216-223.

89. Gillingham, L. G., Caston, L., Leeson, S., Hourtovenko, K., and Holub, B. J. (2005). The effects of consuming docosahexaenoic acid (DHA)-enriched eggs on serum lipids and fatty acid compositions in statin-treated hypercholesterolemic male patients. *Food research international*, *38*(10), 1117-1123.

90. Goldberg, E. M, Gakhar N., Ryland, D., Aliani, M., Gibson, R. A. and House J. D. (2012). Fatty acid profile and sensory characteristics of table eggs from laying hens fed hempseed and hempseed oil. *Journal of Food Science*, 77(4):S153-60.

91. Goncuglu, E., and Ergun, A. (2004). The effect of flaxseed oil to egg quality, fatty acids and cholesterol content of egg yolk in laying hens. In *22nd World Poultry Congress, Istanbul, Turkey*.

92. Gonzalez-Esquerra, R., and Leeson, S. (2000). Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs. *Poultry Science*, *79*(11), 1597-1602.

93. Grobas, S., Mendez, J., Lazaro, R., De Blas, C., and Mateo, G. G. (2001). Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. *Poultry Science*, *80*(8), 1171-1179.

94. GSS, G., and Macro, I. C. F. (2008). Ghana demographic and health survey 2008. Accra, Ghana: Ghana Statistical Service, Ghana Health Service, and ICF Macro.

95. Guter, M.M., and Low, E.M. (2008). The British Egg Marketing Board 1957-71- a reassessment. *Journal of Agricultural Economics*, 22(3),247-265.

96. Gyening, K. O. (2006). The future of the poultry industry in Ghana. *Ghana Veterinary Medical Association, Accra*.

97. Harris, W. S., Mozaffarian, D., Rimm, E., Kris-Etherton, P., Rudel, L. L., Appel, L. J., and Sacks, F. (2009). Omega-6 fatty acids and risk for cardiovascular disease

a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation*, *119*(6), 902-907.

98. Haugh, R. R. (1937). The Haugh unit for measuring egg quality.

99. Herron, K. L., and Fernandez, M. L. (2004). Are the current dietary guidelines regarding egg consumption appropriate? *The Journal of nutrition*, *134*(1), 187-190.

100. Hjalmarsdottir, F. 2016. 17 Science-Based Benefits of Omega-3 Fatty Acids. [https://authoritynutrition.com/17-health-benefits-of-omega-3/]. (Assessed 30 July 2016).

101. Holman, R. T., Johnson, S. B., and Hatch, T. F. (1982). A case of human linolenic acid deficiency involving neurological abnormalities. *The American Journal of Clinical Nutrition*, *35*(3), 617-623.

102. Holub, B. J. (2002). Clinical nutrition: 4. Omega-3 fatty acids in cardiovascular care. *Canadian Medical Association Journal*, *166*(5), 608-615.

103. Horniakova, E. (1997). Effect of different content of fat source on production and indices of egg quality. In *Aktualne problemy pri vyrobe a vyuziti priemyselnych krmiv a premixov krmnych aditiv vo vyzive zvierat, Ivanka pri Dunaji (Slovak Republic), 4-5 Nov 1997*. Vyskumny Ustav Krmivarsky.

104. Houston, D.K., Ding, J., Lee, J.S., Garcia, M., Kanaya, A.M., Tylavsky, F.A., Newman, A.B., Visser, M.,and Kritchevsky, S.B. (2011). Dietary fat and cholesterol and risk of cardiovascular disease in older adults: The Health ABC Study. *Nutrition, Metabolism and Cardiovascular Diseases* 21, 430–437.

105. Howe, P. R. (1997). Dietary Fats and Hypertension Focus on Fish Oil. *Annals of the New York Academy of Sciences*, *827*(1), 339-352.

106. Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G., and Willett, W. C. (2001). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *New England Journal of Medicine*, *345*(11), 790-797.

107. Jacqueline P. J., D.R. Miles and F. B. Mather, (2009). PS24/ps020: Egg quality. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, 32611. (http://edis.ifas.ufl.edu.)

108. Jacqueline, C., Amador, G., Batard, E., Le Mabecque, V., Miègeville, A.F., Biek, D., Caillon, J. and Potel G. (2011). Comparison of ceftaroline fosamil, daptomycin and tigecycline in an experimental rabbit endocarditis model caused by methicillin-susceptible, methicillin-resistant and glycopeptide-intermediate *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy,* Vol.66, No.4, pp. 863-866.

109. Jensen, L.S., Allred, J.B., Fry, R.E. and McGinnis, J. (1958) Evidence for an unidentified factor necessary for maximum egg weight in chickens. *Journal of Nutrition*, 65, 219– 223.

110. Jiang, Z., Fenton, M. and Sim, J.S. (1991b) Comparison of four different methods for egg cholesterol determination. Poultry Science, 70, 1015– 1019.

111. Johnson, V. (2014). Omega-3 for Optimal Health at Every Age. *Accessed 2016 June 1*

112. Kannel, W.B., Castelli, W.P. and McNamara, P.M. (1969), "Serum lipid fractions and risk of coronary heart disease. The Framingham Study", *Minnesota Medicine*, 52, (8),1225-1230.

113. Kelley, D. S. (2001). Modulation of human immune and inflammatory responses by dietary fatty acids. *Nutrition*, *17*(7), 669-673.

114. Kluger, J. (2010). What's So Great About Organic Food? *Time* 176 (9), 30-40.

115. Koelkebeck, K. W., Parsons, C. M., Leeper, R. W., Jin, S., and Douglas, M. W. (1999). Early postmolt performance of laying hens fed a low-protein corn molt diet supplemented with corn gluten meal, feather meal, methionine, and lysine. *Poultry Science*, *78*(8), 1132-1137.

116. Kris-Etherton, P. M., Harris, W. S., Appel, L. J., and Nutrition Committee. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, *106*(21), 2747-2757.

117. Laca, A., Paredes, B., and Díaz, M. (2009). Quality characteristics of n:3 polyunsaturated fatty acid-enriched eggs. *Journal of Animal Feed Science, 18*, 101-112.

118. Layman, D. K., and Rodriguez, N. R. (2009). Egg protein as a source of power, strength, and energy. *Nutrition Today*, *44*(1), 43-48

119. Lazos, E. S. (1986). Nutritional, fatty acid, and oil characteristics of pumpkin and melon seeds. *Journal of Food Science*, *51*(5), 1382-1383.

120. Leeson, S., (2007). Metabolic Challenges: Past, Present, and Future. *The Journal of Applied Poultry Research 1*6, 121-125.

121. Leizer C, Ribnicky D, Poulev A, Dushenkov S, Raskin I. *(*2000). The composition of hemp seed oil and its potential as an important source of nutrition. *Journal of Nutraceuticals, Functional & Medical Foods* 2*(*4*):*35–53*.*

122. Leskanish, C.O. and Noble, R.C. (1997). Manipulation of the n:3 polyunsaturated fatty acid composition of avian meat. *World's Poultry Science Journal*, 53: 156-182.

123. Lewis, N. M., Seburg, S., and Flanagan, N. L. (2000). Enriched eggs as a source of n:3 polyunsaturated fatty acids for humans. *Poultry Science*, *79*(7), 971-974.

124. MacLean, C. H., Newberry, S. J., Mojica, W. A., Khanna, P., Issa, A. M., Suttorp, M. J., and Morton, S. C. (2006). Effects of omega-3 fatty acids on cancer risk: a systematic review. *Jama*, *295*(4), 403-415.

125. Mahfouz, M. M., and Kummerow, F. A. (1989). Effect of magnesium deficiency on Δ6 desaturase activity and fatty acid composition of rat liver microsomes. *Lipids*, *24*(8), 727-732.

126. Malcolmason, L. J., Przybylski, R., and Daun, J. K. (2000). Storage stability of milled flaxseed. *Journal of the American Oil Chemists' Society*, *77*(3), 235-238.

127. Manohar L., Robert J. Blake, Ron BH Wills, and Edward H. Clayton. (2007). Macadamia nut consumption modulates favourably risk factors for coronary artery disease in hypercholesterolemic subjects. *Lipids* 42 (6), 583-587.

128. Marangoni, A. G. (2004). *Fat crystal networks* (Vol. 140). CRC Press.

129. March, B.E. and McMillan, C. (1990) Linoleic acid as a mediator of egg size. *Poultry Science*, 63, 634– 639.

130. Mazalli, M. R., Faria, D. E., Salvador, D., and Ito, D. T. (2004). A comparison of the feeding value of different sources of fat for laying hens: 2. Lipid, cholesterol, and vitamin E profiles of egg yolk. *The Journal of Applied Poultry Research*, *13*(2), 280-290.

131. Mburu, B. M. and Ondwasi, H. O (2005). Discover hidden treasure in local chicken. Manual on local chicken production. *Kenya Research Institute*, 8-10

132. Meluzzi, A., Sirri, F., Manfreda, G., Tallarico, N., and Franchini, A. (2000). Effects of dietary vitamin E on the quality of table eggs enriched with n:3 long-chain fatty acids. *Poultry Science*, *79*(4), 539-545.

133. Mensah-Bonsu, A., Birol, E., Asare-Marfo, D., Ayele, G., Ndirangu, L., Okpukpara, B., and Yakhshilikov, Y. (2010). Investigating the role of poultry in livelihoods and the impact of HPAI on livelihoods outcomes in Africa: evidence from Ethiopia, Ghana, Kenya and Nigeria. In *African Association of Agricultural Economists (AAAE) AAAE Third Conference/AEASA 48th Conference*.

134. Mercier, S., Villeneuve, S., Moresoli, C., Mondor, M., Marcos, B., and Power, K. A. (2014). Flaxseed-Enriched Cereal-Based Products: A Review of the Impact of Processing Conditions. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 400-412.

135. Meyer, B. J., Mann, N. J., Lewis, J. L., Milligan, G. C., Sinclair, A. J., and Howe, P. R. (2003). Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids*, *38*(4), 391-398.

136. Ministry of Food and Agriculture (2002) Food and Agriculture Sector Development Policy (F.A.S.D.E.P.), pp. 45 – 50. Government of Ghana, Accra, Ghana.

137. Ministry of Food and Agriculture, SRID. (2011).Assessment of the maize situation in Ghana. Prepared by the Statistical Research and Information Directorate, Accra.

138. Ministry of Trade and Industry. (2011). Exports of poultry feed ingredients. Ghana

139. Mohan, I. K., and Das, U. N. (2001). Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. *Nutrition*, *17*(2), 126-151.

140. Monira, K. N., Salahuddin, M., and Miah, G. (2003). Effect of breed and holding period on egg quality characteristics of chicken. *International Journal of Poultry Science 2*(4), 261-263.

141. Morris D. (2003). Flax: A health and nutrition primer. Winnipeg: Flax Council of Canada, 79

142. Morris, H.M., (2007). Flax: A health and nutrition primer. Flax Council of Canada, Winnipeg, Canada, pp: 140

143. Mountney, G. J. (1976). Poultry Product Tecnology. The Avi Publishing, Company Incorporated., Westport, Connecticut.

144. Mukhtar, U., 2012. Economic Analysis of Poultry-Egg Production in Bauchi Local Government Area, Bauchi State, Nigeria. Ahmadu Bello University, Zaria.

145. Najib, H., and Al-Khateeb, S. A. (2004). The effect of incorporating different levels of locally produced canola seeds (Brassica napus, L.) in the diet of laying hen. *International Journal of Poultry Science*, *3*(7), 490-496.

146. Nakamura, Y., Iso, H., Kita, Y., Ueshima, H., Okada, K., Konishi, M., and Tsugane, S. (2006). Egg consumption, serum totals cholesterol concentrations and coronary heart disease incidence: Japan Public Health Center-based prospective study. *British Journal of Nutrition*, *96*(05), 921-928.

147. Narahari D., (2003) Health-promoting and therapeutic uses of egg. Poult. Int., 42, 10, 45-47.

148. National Food Survey (2001), "Consumption of selected household foods, 1942 to1996", available at: https://statistics.defra.gov.uk/esg/publications/nfs/default .asp (12th July, 2015).

149. Neuringer, M., Anderson, G. J., and Connor, W. E. (1988). The essentiality of n:3fatty acids for the development and function of the retina and brain. *Annual Review of Nutrition*, *8*(1), 517-541.

150. North, M. O. (1984). Commercial chicken production manual. *Commercial Chicken Production Manual.* (Ed. 3).

151. Novak, C. and Scheideler, S.E. (2001) Long-term effects of feeding linseed-based diets. 1. Egg production parameters, components, and eggshell quality in two strains of laying hens. *Poultry Science*, 80, 1480– 1489.

152. NRC, National Research Council (1994). Nutrient Requirements of Poultry. 9th ed. *National Academy Press, Washington, DC.*

153. Ogbonna, P. E. (2013). Floral habits and seed production characteristics in "Egusi" melon (Colocynthis citrullus L.). *Plant Breeding and Crop Science*, *5*(6), 137-140.

154. Ogunremi, E.A. (1978). Effect of nitrogen on melon (Citrullus lanatus) at Ibadan, Nigeria. *Expl Agric Journal* 14,351-365.

155. Ojieh, G. C., Oluba, O. M., Ogunlana, Y. R., Adebisi, K. E., Eidangbe, G. O, and Orole, R. T., (2008), Compositional studies of C. lana (Egusi) melon seed. *The internent Journal of Wellness* 6 (1)

156. Okantah, S. A., Aboe, P. A. T., Boa-Amponsem, K., Dorward, P., and Bryant, M. J. (2003). Small-scale chicken keeping in peri-urban Accra and Kumasi (No. R7631). A Technical Report on the DFID (LPP) Project.

157. Okita, M., Yoshida, S., Yamamoto, J., Suzuki, K., Kaneyuki, T., Kubota, M., and Sasagawa, T. (1995). N:3And N:6 Fatty Acid Intake and Serum Phospholipid Fatty Acid Composition in Middle-Aged Women Living in Rural and Urban Areas

in Okayama Prefecture. *Journal of Nutritional Science and Vitaminology*, *41*(3), 313-323.

158. Okoli, B.E. (1984). Wild and cultivated cucurbits in Nigeria. Econ. Bot. 38(3):350-357

159. Olaofe, O., Adeyemi, F. O., and Adediran, G. O. (1994). Amino acid and mineral compositions and functional properties of some oilseeds. *Journal of Agricultural and Food Chemistry*, *42*(4), 878-881.

160. Ollis, M., Herman, H., and Singh, S. (1999). Analysis and design of panoramic stereo vision using equi-angular pixel cameras. Carnegie Mellon University, The Robotics Institute.

161. Oluba, O. M., Ogunlowo, Y. R., Ojieh, G. C., Adebisi, K. E., Eidangbe, G. O., and Isiosio, I. O. (2008). Physicochemical properties and fatty acid composition of Citrullus lanatus (Egusi Melon) seed oil. *Journal of Biological Science*, *8*(4), 814-817.

162. Oomah, B. D. (2001). Flaxseed as a functional food source. *Journal of the Science of Food and Agriculture*, *81*(9), 889-894.

163. Oyolu, C. (1977). Quantitative and qualitative study of seed types in Egusi (Colocynthis citrullus). *Tropical Science* 24(2),93-98.

164. Palmquist, D. L., Lock, A. L., Shingfield, K. J., and Bauman, D. E. (2005). Biosynthesis of conjugated linoleic acid in ruminants and humans. *Advances in Food and Nutrition Research*, 50, 179-217.

165. Pandalai, P.K., Pilat, M.J., Yamazaki, K., Naik, H., Pienta, K.J. (1996). The effects of omega-3 and omega-6 fatty acids on in vitro prostate cancer growth. Anticancer Res. Mar-Apr;16(2):815–820.

166. Pappas, A.C., Acamovic, T., Sparks, N.H.C., Surai, P.F. and McDevitt, R.M. (2005) Effects of supplementing broiler breeder diets with organic selenium and polyunsaturated fatty acids on egg quality during storage. *Poultry Science*, 84, 865– 865.

167. Paris, H. S. (2001). History of the cultivar-groups of Cucurbita pepo. *Horticultural Reviews-Westport then New York-*, *25*, 71-170.

168. Patterson, P.H., Koelkebeck, K.W., Bell, D.D., Carey, J.B., Anderson, K.E. and Darre, M.J. (2001) Egg marketing in national supermarkets: Specialty Eggs-Part 2. *Poultry Sciience*, 80, 390–395.

169. Paul, A. A., and Southgate, B. A. T. (1980). Mucance and Widdowson's composition of food. *Hermajesty's stationary office, London, UK*, 227-228.

170. Payet, M., Esmail, M. H., Polichetti, E., Le Brun, G., Adjemout, L., Donnarel, G and Pieroni, G. (2004). Docosahexaenoic acid-enriched egg consumption induces accretion of arachidonic acid in erythrocytes of elderly patients. *British Journal of Nutrition*, *91*(05), 789-796.

171. Pengilly, N. L. (2003). 13 Traditional food and medicinal uses of flaxseed. *Flax: the genus Linum*, 252.

172. Philip, T.A. (1977). An Agricultural Notebook (With special reference to Nigeria). Longman Group Ltd. London. P. 312

173. Plourde, M., and Cunnane, S. C. (2007). Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Applied Physiology, Nutrition, and Metabolism*, *32*(4), 619-634.

174. Pomeranz, A., and Clifton, D. (1981). Properties of defatted soybean, peanut, field pea and pecan flours. *Journal of Food Science*, *42*, 1440-1450.

175. Raeini-Sarjaz, M., Vasoukolaei, M. N., Sayahzadeh, H., Ansari, Z., and Jones, P. H. J. (2006). The effects of plant sterols on serum lipid profiles of laying hens. In *EPC 2006-12th European Poultry Conference*.

176. Raes, K., Huyghebaert, G., De Smet, S., Nollet, L., Arnouts, S. and Demeyer, D. (2002) The deposition of conjugated linoleic acids in eggs of laying hens fed diets varying in fat level and fatty acid profile. *Journal of .Nutrition*, 132, 182–189.

177. Raji AO, Aliyu J, Igwebuike JU, Chiroma S (2009) Effect of storage methods and time on egg quality traits of laying hens in a hot dry climate. ARPN *Journal of Agriculture and Biological Science,* 4(4):1–7

178. Ramakrishnan, U., Imhoff-Kunsch, B., and DiGirolamo, A. M. (2009). Role of docosahexaenoic acid in maternal and child mental health. *The American Journal of Clinical Nutrition*, *89*(3), 958S-962S.

179. Ray, V. H. and Roy L. D. (1991). The Agriculture Dictionary (Second Edition). Published by Delmar Publishers Inc. Albany, New York

180. Reddy, R. V., Lightsey S. F., and Maurice D. V., (1991). Effect of feeding garlic oil on performance and egg yolk cholesterol concentration. *Poultry Science*, 70:2006–2009.

181. Roberts, J. R. (2004). Factors affecting egg internal quality and egg shell quality in laying hens. *The Journal of Poultry Science*, *41*(3), 161-177.

182. Rose, D. P., and Connolly, J. M. (1999). Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacology and Therapeutics*, *83*(3), 217-244.

183. Rose, D.P. J, (1997) Effects of dietary fatty acids on breast and prostate cancers: evidence from in vitro experiments and animal studies. *American Journal of Clinical Nutrition.* Dec;66(6 Suppl):1513S–1522S.

184. Rowghani, E., Arab, M., Nazifi, S. and Bakhtiari, Z. (2007) Effect of canola oil on cholesterol and fatty acid composition of egg-yolk of laying hens. *International Journal of Poultry Science*, 6(2), 11–114.

185. Rymer, C., and Givens, D. I. (2005). n− 3 fatty acid enrichment of edible tissue of poultry: a review. *Lipids*, *40*(2), 121-130.

186. Samli, H. E., Agma, A., and Senkoylu, N. (2005). Effects of storage time and temperature on egg quality in old laying hens. *The Journal of Applied Poultry Research*, *14*(3), 548-553.

187. Sarwar, M. F., Sarwar, M. H., Sarwar, M., Qadri, N. A., and Moghal, S. (2013). The role of oilseeds nutrition in human health: A critical review. *Journal of Cereals and Oilseeds*, *4*(8), 97-100.

188. SAS Institute. (2012). JMP 10 Basic Analysis and Graphing. SAS Institute.

189. Scheele, C. W. (1997). Pathological changes in metabolism of poultry related to increasing production levels. *Veterinary Quarterly*, 19(3), 127-130.

190. Scheideler, S. E., and G. W. Froning, (1996). The combined influence of dietary flaxseed variety, level, form, and storage conditions on egg production and composition among vitamin E-supplemented hens. *Poultry Science*. 75:1221–1226.

191. Scheideler, S. E., Jaroni, D., and Froning, G. (1998). Strain and age effects on egg composition from hens fed diets rich in n:3fatty acids. *Poultry science*, *77*(2), 192-196.

192. Schmier, J.K., Barraj, L.M. and Tran, N.L. (2009). Single food focus dietary guidance: lessons learned from an economic analysis of egg consumption, Cost Effectiveness and Resource Allocation, 7(1)7.

193. Schreiner, M., Hulan, H. W., Razzazi-Fazeli, E., Böhm, J., and Iben, C. (2004). Feeding laying hens seal blubber oil: effects on egg yolk incorporation, stereospecific distribution of omega-3 fatty acids, and sensory aspects. *Poultry Science*, *83*(3), 462-473.

194. Schumann, B.E., Squires, E.J. and Leeson, S. (2000) Effect of dietary linseed, flax oil and n:3 fatty acid supplementation on hepatic and plasma characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. British Journal of Poultry Science, 41, 465– 472.

195. Scott, T. A., and Silversides, F. G. (2000). The effect of storage and strain of hen on egg quality. *Poultry science*, *79*(12), 1725-1729.

196. Scrafford, C. G., Tran, N. L., Barraj, L. M., and Mink, P. J. (2011). Egg consumption and CHD and stroke mortality: a prospective study of US adults. *Public Health Nutrition*, *14*(02), 261-270.

197. Scrafford, C; T. Nga; and B. Leila (2009). The impact of egg consumption on heart health using the NHANES III follow-up survey. *FASEB Journal*, 23:551.557

198. Shafey, T.M., Dingle, J.G., McDonald, M.W. and Kostner, K., (2003). Effect of type of grain and oil supplement on the performance, blood lipoproteins, egg cholesterol and fatty acids of laying hens. *International Journal of Poultry Science* 2, 200-206.

199. Shapira, N., Weill, P. and Loewenbach, R. (2008) Egg fortification with n:3 polyunsaturated fatty acids (PUFA): Nutritional benefits versus high n:6 PUFA western diets, and consumer acceptance. *Isreal Medical Association Journal*, 10, 262– 265.

200. Shini, S., Stewart, G., Shini, A., Bryden, W., (2006). Mortality rates and causes of death in laying hens kept in cage and alternative housing systems, In: 12th

European Poultry Conference. World Poultry Science Association, Verona, Italy, p. 601.

201. Shrimpton, D.H. (1987). The Nutritive Value of Eggs and Their Dietary Significance, In: R. G. Wells and C.G. Belyavin, (Eds) *Egg Quality—Current Problems and Recent Advances*, Pp. 11–25 (London, Butterworths).

202. Silversides, F. G., Twizeyimana, F., and Villeneuve, P. (1993). Research note: a study relating to the validity of the Haugh unit correction for egg weight in fresh eggs. *Poultry Science*, 72(4), 760-764.

203. Sim, J. S., and Qi, G. H. (1995). Designing poultry products using flaxseed. *Flaxseed in human nutrition*, 315-333.

204. Sim, J. S., and Sunwoo, H. H. (2002). Designer eggs: nutritional and functional significance. Iowa State Press: Ames, IO, 19-36.

205. Simopoulos, A. P. (2000). Human requirement for N:3polyunsaturated fatty acids. *Poultry science*, *79*(7), 961-970.

206. Simopoulos, A. P., Leaf, A., and Salem Jr, N. (1999). Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Annals of Nutrition and Metabolism*, *43*(2), 127-130.

207. Song, K. T., Choi, S. H., and Oh, H. R. (2000). A comparison of egg quality of pheasant, chukar, quail and guinea fowl. *Asian Australasian Journal of Animal Sciences*, *13*(7), 986-990.

208. Song, W.O. and Kerver, J.M. (2000), Nutritional contribution of eggs to American diets, *Journal of the American College of Nutrition*, 19(5), 556S-562S.

209. Sosin, E., Borowiec, F., Strzetelski, J. and Smulikowska, S. (2006). The effect of feeding regular or low a-linolenic acid linseed on the fatty acid composition of egg yolks. *Journal of Animal Feed Science*, 15, 641– 650.

210. Souza, J.G., Costa, F.P.G., Queiroga, R.C.R.E., Silva, J.H.V.,Schuler, A.R.P. and Goulart, C.C. (2008). Fatty acid profileof eggs of seamy-heavy layers fed feed containing linseed oil. *Revista Brasileira de Ciência Avícola*, 10(1), 1590– 1599.

211. Sparks, N. H. C. (2006). The hen's egg–is its role in human nutrition changing? *World's Poultry Science Journal*, *62*(02), 308-315.

212. Spurlock, M. E., and Savage, J. E. (1993). Effect of dietary protein and selected antioxidants on fatty liver hemorrhagic syndrome induced in Japanese quail. *Poultry Science*, *72*(11), 2095-2105.

213. Squires, E.J., Leeson, S., (1988). Aetiology of fatty liver syndrome in laying hens. *British Veterinary Journal* 144, 602-609.

214. Stadelman, W. J., and Schmieder, H. (2002). Functional uses of eggs—an overview. *Eggs and Health Promotion*, 3-8.

215. Sugano, J., Toshima, S. I., Hasegawa, A., Kurabayashi, M., Itabe, H., Takano, T., and Nagai, R. (2000). Circulating oxidized low density lipoprotein levels a biochemical risk marker for coronary heart disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *20*(10), 2243-2247.

216. Sugura, N., Horiike, S., Masida, Y., Kunou M. and Kokubu T. (1999). Bioavailability and commercial use of egg shell calcium, membrane proteins and yolk lecithin products. *Egg Nutrition and Biotechnology*.

217. Sumner, D. A., Rosen-Molina, J. T., Matthews, W. A., Mench, J. A., and Richter, K. R. (2008). Economic effects of proposed restrictions on egg-laying hen housing in California. *University of California Agricultural Issues Center*.

218. Temple, N. J. (1996). Dietary fats and coronary heart disease. *Biomedicine and Pharmacotherapy*, *50*(6), 261-268.

219. Tolimir. N. and Masic, B. (2000). The results of European egg production tests in 1997-1998. *Zivinarstvo*. 35(5): 66-68

220. Trautwein, E. (2001). n-3 Fatty acids—physiological and technical aspects for their use in food. *European Journal of Lipid Science and Technology*, *103*(1), 45-55.

221. Trott, K.A., Giannitti, F., Rimoldi, G., Hill, A., Woods, L., Barr, B., Anderson, M., Mete, A., (2013). Fatty Liver Hemorrhagic Syndrome in the Backyard Chicken: A Retrospective Histopathologic Case Series. *Veterinary Pathology.*

222. UFAW (1994). The Use of Animals in Scientific Procedures.

223. Umar, K. J., Hassan, L.G. and Garba H.J. (2005) Proximate and mineral compositions of M.miristica. *Chemclass Journal*, 3,81-84

224. Van Camp, J., and Huyghebaert, A. (1995). A comparative rheological study of heat and high pressure induced whey protein gels. *Food Chemistry*, *54*(4), 357-364.

225. Van Elswyk, M., Sams, A. R., and Hargis, P. S. (1992). Composition, functionality, and sensory evaluation of eggs from hens fed dietary menhaden oil. *Journal of Food Science*, *57*(2), 342-344.

226. Van Elswyk, M.E. (1997). Nutritional and physiological effects of flax seed in diets for laying fowl. *World's Poultry Science Journal*, 53, 253– 264.

227. Van Niekerk, M. (2014). *Agaat: roman*. btb Verlag.

228. Veterinary Services Directorateof the Ministry of Food and Agriculture. (2013). *Annual reports 2008 2009 2010.2011 2012.*

229. Virtanen, J. K., Mursu, J., Virtanen, H. E., Fogelholm, M., Salonen, J. T., Koskinen, T. T. and Tuomainen, T. P. (2016). Associations of egg and cholesterol intakes with carotid intima-media thickness and risk of incident coronary artery

disease according to apolipoprotein E phenotype in men: The Kuopio Ischaemic Heart Disease Risk Factor Study. *The American Journal of Clinical Nutrition*, *103*(3), 895-901.

230. Wahlqvist, M.L. (1998). Nutritional pathways to coronary heart disease. Atherosclerosis, 136(Suppl.1):28 vist,

231. Wahlqvist, M. L., Kouris-Blazos, A., Dalais, F., Kannar, D., and Wattanapenpaiboon, N. (1998). Phytochemical deficiency disorders: inadequate intake of protective foods. *Current Therapeutics*, *39*(7), 53.

232. Wang, B.W. (1996) Effect of safflower seed oil on productivity and polyunsaturated fatty acids and cholesterol in egg yolks. Chin. J. Anim. Sci., 32, 39–40.

233. Warner, K., and Gupta, M. (2003). Frying quality and stability of low-and ultra-low-linolenic acid soybean oils. *Journal of the American Oil Chemists' Society*, *80*(3), 275-280.

234. Weggemans, R. M., Zock, P. L., and Katan, M. B. (2001). Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. *The American Journal of Clinical Nutrition*, *73*(5), 885-891.

235. Widdowson, M. A., Sulka, A., Bulens, S. N., Beard, R. S., Chaves, S. S., Hammond, R., & Mead, P. S. (2005). Norovirus and foodborne disease, United States, 1991–2000. *Emerg Infect Dis*, *11*(1), 95-102.

236. Yashodhara, B. M., Umakanth, S., Pappachan, J. M., Bhat, S. K., Kamath, R., and Choo, B. H. (2009). Omega-3 fatty acids: a comprehensive review of their role in health and disease. *Postgraduate Medical Journal*, *85*(1000), 84-90.

237. Younis, J. S., Ben-Ami, M. O. S. H. E., Brenner, B., Ohel, G., and Tal, J. (2000). Activated Protein C Resistance and Factor V Leiden Mutation can be Associated with First-as well as Second-Trimester Recurrent Pregnancy Loss1. *American Journal of Reproductive Immunology*,*43*(1), 31-35.

238. Yuwono, M., and Indrayanto, G. (2009). On-column injection for GC. Encyclopedia of Chromatography. 3rd ed. Taylor and Francis, New York, NY, 1621-1625.

239. Zaheer, K. (2015). An Updated Review on Chicken Eggs: Production, Consumption, Management. Aspects and Nutritional Benefits to Human Health. *Food and Nutrition Sciences*, 6, 1208-1220.

240. Zellner, D. A., Loaiza, S., Gonzalez, Z., Pita, J., Morales, J., Pecora, D., and Wolf, A. (2006). Food selection changes under stress. *Physiology and Behaviour*, *87*(4), 789-793