PRODUCTION OF OMEGA-3FATTY ACID EGGS BY FEEDING HENS WITH LAYERS MASH MIXED WITH CONOPHOR NUT (*Tetracarpidium conophorum*) PASTE

BY

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CERTIFICATION

This is to certify that this project work was carried out by Olotu Olubunmi Deborah in the department of Food Science and Technology of the Federal University of Technology, Akure and that this project has not been submitted elsewhere for the award of any degree.

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DEDICATION

This project is dedicated to the Almighty God, the author and finisher of my success. Also to my father; retired warrant officer Samuel Olotu.

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I appreciate the Almighty God for his grace, presence, wisdom and strength he has granted me to ensure that this project work is successful.

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ABSTRACT

The research involves feeding of hens at the point of laying with *layers feed mixed with conophor nut paste (Tetracarpidium conophorum)* to modify the nutrient composition of eggs by increasing the level of Omega-3fatty acids in the eggs and to provide a substitute for other expensive Omega-3 rich foods such as fish, shellfish and sea foods.

Twelve birds were housed in a commercial type laying cage. Three hens were accommodated in each cage.Three of the hens were fed with layers mash to serve as control [A=100%feed] while the remaining nine birds were fed with layers mash mixed with conophor nut paste in varying ratio [B=90:10%], C [80:20%] D[70:30%].

On weekly basis, three eggs from each treatment [A,B,Cand D] were randomly selected to evaluate the average egg weight(g),yolk index, albumen index, shell thickness(mm), total fat(g),cholesterol level(mg) and egg fatty acid profile.

The egg weights have values ranged from 159.40 to 178.40g with treatment B[90:10] having the least value and treatment C and D having the highest values. The average weight of the eggs measured revealed that egg weight increases with increasing level of conophor nut paste, however the egg weights of hens fed control diets [A=100%feed] decreases with values ranged from 166.70 to 164.70g.

The yolk index ranged between 0.37 and 0.48 with treatment A having the least and treatment B,C and D having higher values than the control. The albumen

index ranged between 0.086 and 0.089 with the control having the least and treatment B having the highest value. Result of the egg qualities parameters yolk and albumen index indicate that there is no mark variations observed and the values were not significantly different among treatments. This shows that the varying inclusion levels of conophor nuts paste had no effect on these egg qualities.

Moreover, the egg shell thickness (mm) ranged between 32.25 and 32.59mm with the control having the least and treatment C having higher value than control.

The total fat content of the eggs increased with increasing weeks of feeding as well as increasing level of conophor nuts paste. The values obtained ranged from 10.50 to12.61g with treatment A [100%feed] and B[90:10%] having the least and the treatment C[80:20] and D[70:30] having the highest values. This suggests that conophor nuts increased the yolk of the egg.

In addition, mixing conophor nuts paste with layers mash significantly decreased cholesterol level from 187mg to 183mg while the decreasing effect was not shown with hen fed a control diets 187mg to 190mg.

Results of fatty acid profile showed that both saturated and unsaturated fatty acids were present in the eggs with hens fed conophor nuts having higher percentage of polyunsaturated fatty acid than the control.

In summary, hens fed with a diet containing layers mash and 20% conophor nuts paste consistently produce more Omega-3 enriched eggs.

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CHAPTER ONE

INTRODUCTION

Omega-3 fatty acids are a family of unsaturated fatty acids that have in common a carbon- carbon double bond in the Omega-3 fatty acid position; that is the third bond from methyl end of the fatty acid (Conner,2000). Important nutritionally essential Omega˙3 fatty acids are :α–Linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The human body cannot synthesize Omega˙3 fatty acids but it can form 20- and 22- carbon unsaturated Omega˙3 fatty acids, from the eighteen carbon Omega-3 fatty acid, α-linolenic acid (Laucet,2007).

Synthesis of the longer w-3 fatty acids from linolenic acid within the body is competitively slowed by the n-6 analogues(Carter,1975).The alpha –linolenic acid (LNA) c[18:3,(n-3] representative of the Omega-3 fatty acid (FA) family is found in considerable quantities in some oil seeds such a canola, flaxseed walnut, soyabean oil, hempseed, conophor nuts e.t.c (Dziezak, 1987) while the most widely available source of EPA and DHA is cold water National Institutes of Health recently published recommended daily intakes of fatty acids. Specific recommendations include 650mg of

EPA and DHA, 2.22g/day of α- linolenic acid and of 4.44g/day of linoleic acid.

Omega-3 fatty acid supplements are available in capsule form. They are also available in concentrates. The best supplements are those that are of pharmaceutical grade and those that have been molecularly distilled of heavy metals in particular mercury, nickel arsenic and cadmium as well as other contaminants (PCBs, furans, dioxins).

1.2 Health Benefits of Omega-3 Fatty Acids

Recent research has suggested that people will be healthier and have fewer heart problems if they increase their consumption of Omega-3 fatty acid (Caston and Leeson, 2006). Several clinical and research studies have shown that the dietary intake of Omega-3 fatty acids can lower blood cholesterol level in people who have hyper cholesteremia (high blood cholesterol). (Cherian and Sim, 1991; Van Elswyk, 1997). Other studies have claimed that those fatty acids can reduce blood pressure, tumor growth, arthritis, headaches, and the formation of blood clots (Carter,1975). It is also considered to be good for brain development especially for growing children and help strengthen immune function. (Manitoba Agriculture and Food, 1999).

The health benefits reported from several studies had prompted the fortification of foods with Omega-3 fatty acids.

Traditionally, most of the Omega-3fatty acids in the diet have come from fish and fish oil but many people eat only limited amount of these foods. In an attempt to increase the amount of these fatty acids in the diet, a food item such as egg consumed by every generation from childhood to late life was fortified with Omega-3 fatty acids (Jiang *et al*,1991). Eggs are a good and inexpensive source of protein and a 2 to 1 ratio of unsaturated fats to saturated fats (Simopoulous, 2002).

Simopoulous(2006)reported that egg is an excellent source of iron, phosphorous and other minerals and contains all the vitamins with exception of Vitamin C.

1.3 Previous Work/ Research on the Project

Many researchers have succeeded in fortifying eggs with Omega-3 fatty acids by altering the diets of laying hen using different plant sources of Omega-3 fatty acids like flaxseed, canola oil, Pearl millets and hemp seed. A publication in Manitoba Agriculture and Food (2006) reported that the level of Omega-3 fatty acid in the eggs can be increased by including 10% flaxseed in the layers feeds. The eggs produced from hens on this feed formular are higher in Omega`3 fatty acids.

Amini and Ruiz- Feria. s(2008) developed Omega`3 fatty acid enriched eggs using pearl millet grain, low level of flaxseed and natural pigment.It was reported that pearl millet diets with levels as low as 6% of flaxseed and low level of natural pigment (0.1%) can be used to produce Omega`3 fatty acid enriched eggs, preserving egg qualities and restoring yolk colour and maintaining hen health and productive performance.

Cherian and Sim (1997) subjected nine men and three women with normal blood cholesterol level and blood pressure to a diet of eggs enriched with Omega-3 fatty acids in addition to their normal diet for four weeks. It was discovered that the eggs with Omega`3 fatty acids did not cause blood cholesterol levels to increase; while they decreased the amount of triglyceride (fat) in the blood and decreased blood pressure as well. It was concluded that eggs enriched with Omega`3 fatty acids may be more healthful than regular eggs. In this study, the nutrient composition of eggs will be modified by altering the diets of laying hens using conophor nuts.

1.4 Justification of the Research

Eggs have been reported as one of the diet foods rich in cholesterol (lopez *et al.*, 1967). It has also been well documented that the cholesterol level increases with age (National Institute of Health consensus conference, 1993; Pang *et al.*,1998). These facts have prevented fat and old age people from taking eggs. It is generally agreed that high plasma level of cholesterol is associated with artheroscelerosis, the underlying cause in most of degenerative cardiovascular diseases. This health effect has drastically reduced the consumption pattern of eggs despite its good source of protein and some minerals.

In an attempts to increase the egg consumption rate of the whole populace, health authorities have recommended that the proportions of saturated fatty acids particularly polyunsaturated fatty acid (PUFA) be preferentially increased at the expense of saturated fat (Trust well *et a*l., 1992).Vegetable oils such as walnut, linseed, canola oil and conophor nuts oil are now attracting more attention towards improving the nutritional properties of eggs (Caston and Leeson, 1990; Cherian and Sim, 1991). Ogunsua and Adebona (1983) reported that dietary conophor nuts is the highest linolenic containing plant oil which has been shown to have 66% linolenic acid. The iodine value of the oil was very high (204). This value proved that the oil contain high amount of unsaturated fatty acids. Thus, it may be possible to produce Omega-3 fatty acids enriched eggs using conophor nuts.

1.5 Aims and Objectives

(1) To increase the level of Omega-3 fatty acids contain in the eggs by altering the diets of laying hens using conophor nut in varying ratio of 10%, 20%, and 30% .

(2) To provide a substitute for other expensive Omega˚3 rich foods such as fish, shell fish and sea foods.

1.6 Expected Contribution to Knowledge

This study among other things will explore the possibility of increasing the utilization of conophor nut oil in

(1) Producing functional foods such as Omega-3 fatty acid enriched eggs by altering hen's diet through incorporation of conophor nut paste.

(2) Reducing the cholesterol level in eggs,

(3) Producing alternative use for conophor nuts now largely used as snacks.

(4) Promoting the diversification of regular or classic egg by offering consumers a greater choice.

CHAPTER TWO

LITERATURE REVIEW

2. Experimental Diets

A commercial layer feeds was used. It is formulated for chickens that are producing eggs for hatching. These feeds contain slightly more protein and fortified with extra vitamin for proper chick development and hatching.

Layers use nutrients to satisfy their maintenance requirements and their egg production requirements. The larger the products devoted to production, the better the economic result. Feeds are formulated and manufactured for chicken to meet their nutritional needs at specific ages and production characteristics. For example, starter feeds are fed to chicks from hatch to few weeks of age. Grower and developer feeds are fed to "adolescent" growing chickens, while layer or breeder feeds are fed to chicken that are producing eggs.

Table 1.0: The percentage composition of the layer mash

Raw materials	Composition (kg)
Groundnut cake	12
Palm kernel cake	12
Bone meal	5
Limestone	6.62
Lysine	0.85
Methionine	0.13
Maize	44.50
Fish meal	2.50
Layers premix	0.10
Rice bran	7.80
Wheat offal	0.02
Soya bean meal	3.0

Source; Amini and Ruzia-feria(2007)

2.1 Layer Feeds

Feeds are formulated and manufactured for chickens to meet their nutritional needs at specific ages and production characteristics.The raw materials used in poultry feeding are agricultural products (cereal) agro-industrial by-products (oil cakes, miller's and rice chandler's wastes, molasses and brewer's grain) and chemical industrial products (minerals, trace elements, synthetic vitamins and amino acids, additives).To ensure maintenance of life and the synthesis of organic substances, feeding must supply energy requirement, body building requirement and requirement for functions.

(a) The energy requirements are carbohydrates and lipids. The carbohydrates are chiefly represented by starch, sugar and cellulose.

Carbohydrates and fats give energy to the animal allowing it to maintain its vital functions, movements, feeding and generation of heat. They also give the energy which allows synthesis of organic substances that is production of meat and eggs.

(b) Body-Building Element-- This is in the form of proteins and minerals high in oil cakes and meat meal from 40 to 50% and very high in fish meal up to 70% (Ralph, 1987)

.2 Importance of the Components of the Feeds.

.2.1 Maize-- It contains little cellulose and a relatively high proportion of fat which classes it among the best energy sources. However, it contains few nitro-compounds. Its utilization is limited only by the need to maintain the energy protein balance and utilization rates are commonly between 60% and 70%

Yellow maize, the colour of which is due to Xanthic pigments has the property of turning the skin and fat of poultry yellow and colouring the egg yolks. Use of white maize leads to decoloration of the egg yolks.

.2.2. Wheat offals:- It is a satisfactory content of nitro-compounds and a fairly high level of cellulose which limits their energy value. So their use is only possible if the other constituents of the ration are energy rich (Ralph,1987).

2.3 Rice bran:- The bran from hulling or crushing can only be included in feed stuff if it has been winnowed and free from most of the lighter husks. It is a by-product of mediocre nutritive value, rich in cellulose and silica (Cherian.*et al.*, 1991).

2.4 Soyabean Meal:- They are raw-materials very rich in fats and well supplied with nitro-compounds, but the excess of fats limit their use.

In practice, proportions of fats are less than 10% in growth ration and less than 80% in laying rations. Its beans contain relatively low levels of fats (17%) and many nitro-compounds (36%) which are particularly rich in lysine.

2.2.5 Groundnut cake

The cellulose content of this cake is moderate (5-7%).The residual oil content is variable according to the technique of preparation. Nitro-compounds content are fairly high (45%-50%). If it is guaranted harmless ,it can be extensively used up to 30% in feed for pullets and layers

2.2.6 Palm kernel cake

They have low nitro-compound contents from 19 to 23%.They are deficiency in lysine and their cellulose content varies from 9 to 24%. Their use for growing poultry is limited by their low energy value, However, they may be freely used with layers up to 2% of the diet Jiang *et al* (1992)

2.2.7 Fish meals-- Are very variable raw materials, because their source may range from whole fish (anchioves to fish not eaten by man). Fish meals commercially prepared from whole fish have excellent total nitro-compound values from 65 to 70% and are sought after because of their high lysine content which represent 7 to 8% of the total nitro-compounds and their good content of sulphated amino acid and tryptophan. Fish meals can enter into the composition of the ration up to 3-5% for layers Jiang *et. al* (1992).

2.8 Layer premixes

These are supplements added to the feed to give what is lacking that is minerals vitamins, and trace elements. Examples are nitro-compound and vitamin supplement (N.C.V.S) and mineral and Vitamin supplement (M.V.S).

When cereals and cakes are available at 10% N.C.V.S can be brought which will give minerals, vitamins trace elements and essential amino acids to balance the ration. M.V.S. is incorporated into the feed depending on its composition at 3, 5, or 7% in the feed. (Ralph,1987)

2.9 Bone meals-- These by-products are made up of bones and fat in various proportions. The amount of nitro-compound vary from 35 to 70%, those of fats from 2 to 13% and those of minerals from 3 to 40% (Ralph, 1987).

2.3 Storage Of Feeds

Factors which shorten the length of time for which feed still remains wholesome are heat, humidity, amount of fats and mealiness. In warm, humid tropical conditions, the length of storage of a mealy feed should not exceed one week and that of a pellet feed one month. (Ralph, 1987).

2.4 Polyunsaturated fatty acids, their presence in plants, animals and their importance

Polyunsaturated acids differ greatly with respect to chain length and double-bond characteristics (number, location and geometrical configuration). The usual arrangement in food fats is a configuration with a methylene group between the double bonds ($- CH = CH - CH_2 = CH -$). Most Polyunsaturated fatty acids are in the $C_{16} - C_{22}$ range, although some may have as many as 24 or 26 carbons. Examples are linoleic, linolenic and Arachidonic acids. (Owen,1976).

Linoleic acid is present at high concentration in many fats and oils. It is a dominant acid in safflower- seed oil (60-80%), cotton seed oil (45-50%) sunflower seed oil (30-70%), and corn oil (40-60%).Linolenic acid comprises 50-60% of the fatty acid in linseed oil. It is present in soy bean oil at a level of 8-10%, and this poses problems when the oil is used unless it is modified by hydrogenation and other processing(Fennema,1976).

Arachidonic acid is a C20 tetraenoic acid which occurs primarily in animal sources. It is a component of many phospholipids and may be an important element in membranes of organ or neural cell tissues. It is a precursor of one of the prostaglandins (Alais and Linden,1999) . Oleic acid is found in almost all fats.It is a monounsaturated fatty acid and a dominant component of Olive oil in which it is present at levels up to 75%, in cocoa butter at levels up to 40%, and in bovine and ovine fats at levels up to 35-40%. Oleic acid can be transformed into its trans isomer, known as elaidic acid by nitrogen oxides or selenium and to some extent by the condition present during catalytic hydrogenation. (Fennema ,1976).

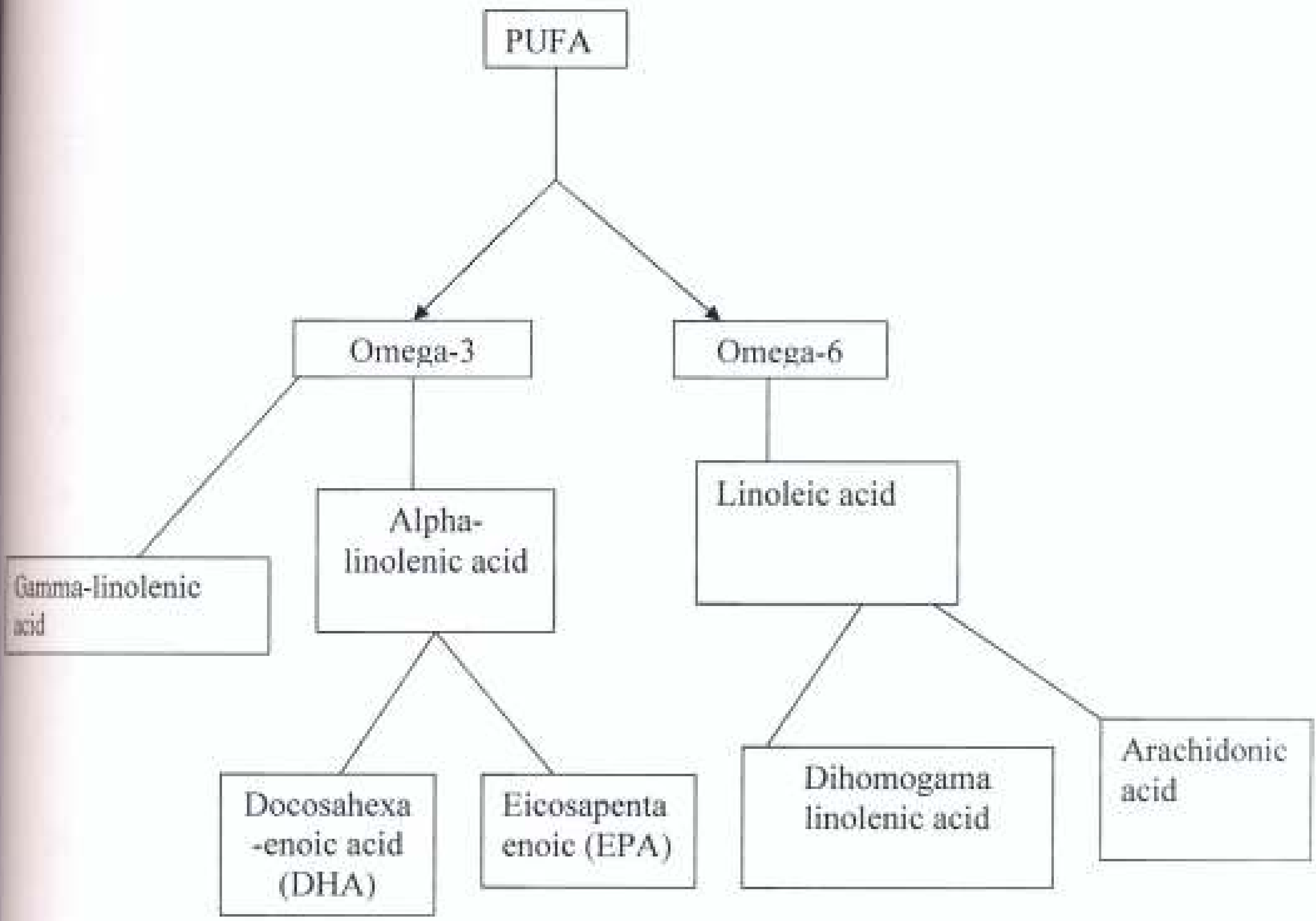

Fig. 1: Schemes for the types of poly unsaturated fatty acid

Adapted from Mayes et al., (2000)

Table 2: List of unsaturated fatty acid

	Trivial name	Systematic name	Melting point ^{O}C	Boiling point ^{O}C
14	Myristoleic	9-		
14	Myristoleic	9-Tetradecenoic	0	
16	Palmitoic	9-Hexadecenoic	0	
18	Oleic	9-Octadecenoic	16.3	
18	Vaccenic	11-Octadecenioc	39.50	
20	Gadoleic	9-Eicosenoic	23.50	
18	Linoleic	9,12 Octadecadienoic	-5	202
20	Arachidonic	5,8,11,14-Eicosatetraenoic	-49 -50	
18	Linolenic	9,12,15-Octadecatrienoic	-11	157

2.4.1 Chemistry of Omega-3 fatty acids in diets.

Omega-3 fatty acid is polyunsaturated fatty acid which is formed when a double bond first occurs at the third carbon from the methyl (CH_3) end of the carbon chain.

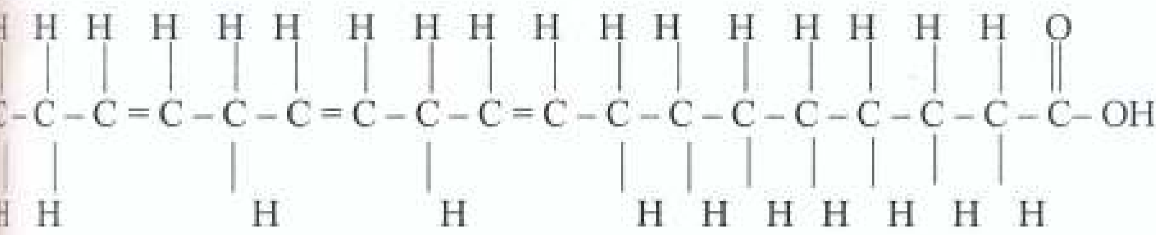

Fig 2: Polyunsaturated fatty acid (Alpha-linolenic acid, w-3; C18:3)

2.4.2 Types of Omega-3 fatty acid.

Important nutritionally essential Omega-3 fatty acids are

(a) $\propto$ - linolenic acid (ALA)

(b) Eicosapentaenoic acid (EPA)

(c) Docosahexaenoic acid (DHA)

The human body cannot synthesize Omega-3 fatty acid but it can form 20- and 22- carbon unsaturated Omega-3 fatty acids from the eighteen carbon Omega-3fatty acids, alpha linolenic acid. This means that Omega-3fatty acids are metabolized into eicosapentaenoic and docosahexaenoic acid in the body. These three polyunsaturated have either 3,5 or 6 double bonds in a carbon chain of 18,20 or 22 carbon atom respectively (Nester, 2000).

Mayes *et al.,* (2000) discovered that the accumulation of long chain Omega-3fatty acid in tissues is more effective when they are obtained directly from food or when competing amounts of Omega-6 analogs do not greatly exceed the amount of Omega-3.

2.4.3 Dietary sources of Omega-3fatty acids.

The primary dietary sources of Omega-3fatty acids are fish, plant, nut oil,krill and algae. Bernard et al.,(2000),Jill Haris (2008)reported foods that contain appreciable amounts of alpha linolenic acid (ALA) which are; canola oil, English walnuts, flax oil, Conophor nut, flaxseed, hempseed oil, hemp seed/ hemp nut (ground), Olive oil, leaf green vegetable, pumpkin seeds, soybean walnut oil, soybean oil and supplements.The most widely sources of eicosapentaenoic acid and Docosahexaenoic acid is cold water oily fish such as Salmon, Mackerel, Anchioves,Halibut, Sardine, Tuna & Herring.

The National Institutes of Health (Mayes *et al.)* recently published recommended daily intakes of omega-3fatty acids. Specific recommendations include 650mg of eicosapentaenoic acid and

Docosahexaenoic acid, 2.22gram/day of alpha- linolenic acid and 4.44g/day of linoleic acid (Conner, 2000.,Aroson *et al.*, 2001) pointed out that consumers of oily fish should be aware of the potential presence of heavy metals and fat solid pollutants like polychlorinated biphenyls and dioxins which may accumulate up the food chain. In addition, the United States Food and Drug Administration (2005) recommends that pregnant or nursing women and young children should avoid eating types with higher levels of mercury such as mackerel, shark sword fish(Harris *et al.*, 2008).

2.4.4 Health benefit of Omega-3 fatty acids

Clinical studies suggest that Omega-3 fatty acid may be helpful in treating a variety of health conditions. The evidence is strongest for heart disease and problems that contribute to heart disease, but the range of possible uses for Omega-3 fatty acids are;

2.4.4.1 High Cholesterol

Arita *et al.* (2005) reported that consumption of high amount of Omega-3fatty acids from fatty fish tend to have increased high density lipoprotein cholesterol and decreased triglycerides (fatty material that circulate in blood). In addition, fish oil supplements containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported in several clinical studies to reduce low density cholesterol and triglycerides levels.

2.4.4.2. High blood pressure

An analysis of 19 clinical studies using fish oil supplements found that supplementation with 3 or more grams of fish oil, daily can lead to significant reduction in blood pressure in individuals with untreated hypentension(Conner,2000).

2.4.4.3 Heart disease

Conner (2000) discovered that one of the best ways to help prevent and treat heart disease is to eat a low fat diet and to replace food rich in saturated and trans fat with those that are rich in monounsaturated and polyunsaturated fats. Clinical evidence suggests that Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) found in fish oil help reduce risk factors for heart disease including high cholesterol and high blood pressure. There is a strong evidence that these substances can help prevent and treat atherosclerosis by inhibiting the development and treat of plaque and blood clots each of which tends to clog arteries. (Conner, 2000).

2.4.4.4 Diabetes.

Individuals with diabetes tends to have high triglycerides and low high density lipoprotein (HDL) levels. Herber *et al.*, (1999) suggested that people with diabetes may have lower triglycerides and apoprotein (markers of diabetes) and raise HDL by taking foods or supplements that contain DHA and EPA.

2.4.4.5 Weight loss

Many individuals who are overweight suffer from poor blood sugar control diabetes and high cholesterol. Clinical studies (Conner , 2000) suggest that overweight people tend to achieve better control over their blood sugar and cholesterol levels from fish rich in Omega-3 fatty acid such as salmon, mackerel and herring which are staple in their low- fat diet.

2.4.4.6 Arthritis

Several articles reviewing the use of Omega-3fatty acid supplements for inflammatory joint condition conclude that Omega-3fatty acid supplement reduce tenderness in joints, decrease morning stiffness and allow for a reduction in the amount of modification needed for people with rheumatoid arthrisis (Arita *et al.*, 2005). 2.4.4.7

.4.4.7 Osteoporosis

Clinical studies suggest that Omega-3fatty acids such as Eicosapentaenoic acid help increase levels of calcium in the body, deposit calcium in the bones and improve bone strength. Conner (2000). demonstrated this in a study of women over 65 with osteoporosis, those given EPA and GLA (an omega-6-fatty acid) supplements experienced significantly less bone loss over 3 years than those who were set in control.

.4.4.8 Depression

Depression occurs when nerve cells do not communicate with each other effectively. This is as a result of insufficient intake of Omega-3fatty acids. The Omega-3fatty acids are important component of nerve cells which communicate and this is an essential step in maintaining good mental health. (Conner, 2000).

.4.4.9 Attention deficit/ hyperactivity disorder (ADHD)

Children with attention deficit/ hyperactivity disorder (ADHD) may have low levels of certain essential fatty acids (including EPA and DHA) in their bodies (Conner,2000). In a clinical study of nearly 100 boys, Conner (2000) reported that those with lower levels of Omega-

3fatty acids demonstrated more learning and behavioral problems such as temper tantrums and sleep disturbances.

2.4.4.10 Eating disorder

Arita *et a*l. (2005) suggests that men and women with anorexia nervosa have lower optimal levels of polyunsaturated fatty acids and this can be prevented with polyunsaturated fatty acid rich foods such as fish and organ meats which include Omega-3 fatty acids.

2.4.4.11 Burns

Essential fatty acids have been used to reduce inflammation and promote wound healing in burn victims (Conner ,2000). He reported an animal research that indicates the importance of Omega-3 fatty acids in promoting a healthy balance of proteins in the body.

2..4.4.12 Macular degeneration.

Macular degeneration is a serious age related eye condition that can progress to blindness. A clinical study comparing people with macular degeneration to 500 without the eye disease found that those with a healthy dietary balance of Omega-3 and Omega-6 fatty acids and higher intake of fish in their diet were less likely to have this particular eye disorder (Conner ,2000)

2.4.4.13 Colon cancer

Maillard *et al.*,(2002) reported that consuming significant amounts of foods rich in Omega-3fatty acids appears to reduce the risk of colorectal cancer. Animal and laboratory studies have found Omega-3 fatty acids and daily consumption of eicosapentaenoic acid and Docosahexaenoic acid prevent worsening of colon cancer.

2.4.4.14 Breast cancer

In addition, the risk of dying from breast cancer may be significantly less for those who eat large quantities of Omega-3fatty acids from fish and brown kelp sea weed (Maillard et al., 2002).

2.4.4.15 Prostate cancer.

Laboratory and clinical studies (Conner,2000) indicates that Omega-3 fatty acids specifically, DHA and EPA may inhibit the growth of prostate cancer. He suggests that a low-fat diet with the addition of omega-3 to omega-6 fatty acids from fish or fish oil help prevent the development of prostrate cancer.

2.4.4.16 Asthma

Clinical research suggests that Omega-3 fatty acids supplements in the form of perilla seed oil which is rich in ALA may decrease inflammation and improve lung function in adult with asthma. This was demonstrated in a well designed clinical study of 29 children with asthma.Those who took fish oil supplements rich in eicosapentaenoic acid and Docosahexaenoic acid for 10 month had improvement in their symptoms compared to children who took a placebo pill (Conner , 2000)

2.4.5 Effect of deficiency of Omega -3 fatty acids.

If humans fail to consume enough Omega-3fatty acids, the following symptoms are seen;

Depression

- Cardiovascular disease
- Type 2 Diabetes
- Fatique
- Brittle hair and nails.
- Inability to concentrate.(**Amini and Ruiz-Feria,2000).**

2.4.6 Fortification of foods with Omega-3 fatty acids

Due to the nutritional importance and potential health benefits (especially in children) many foods are now being fortified with Omega-3s.Omega-3 fatty acids supplementation in food has been a significant trend in food fortification with global food companies

X

launching Omega-3 fatty acid fortified bread, mayonnaise, pizza yoghurt, orange juice ,children's pasta, milk, egg confections and infant formular (Amini and Ruiz-Feria,2007).

The structural formula of some unsaturated fatty acids

Linoleic acid

$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$

Cis-cis-9-12 octadecadienoic.

Linolenic acid

$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$

Cis-cis-cis-9-12-15 octadecatrienoic

Arachidonic acid

$CH_3(CH_2)_4CH=(CHCH_2CH)_3=CH(CH_2)_3COOH$

Cis-cis-cis-cis-5-8-11-14 eicosatetraenoic

Oleic acid

$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$

Cis-9-octadeceonic

Elaidic acid

$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$

Trans-9-octadeceonic.

Table 3: Food Fortified with Vegetarian Omega-3 Fatty Acids

Foods	Process
Eggs	It is produced by feeding hens with layer feeds mixed with flaxseed.
Soya milk beverages	It is usually from flax seed
Grain bread	Mixing wheat flour with flax seed.
Margarine	Some brands use a flax/ Olive oil mix
Milk	Some milks (like Beatrice brand are fortified with vegetarian sourced (Alpha linolenic acid
Yoghurt	Astro brand bio best Omega-3 yoghurt is fortified with flax oil and it's free from gelatin
Salad dressing	Usually uses a combination of oils (flax, olive and canola oil)

Source: Amini and Ruiz-feria (2007).

2.5 Egg Composition and Structure

There are two main constituents of egg

(1) An external mineral constituents of egg

(2) Organic constituents: The egg yolk and Albumen

2.5.1 Egg shell

The egg shell is composed of

(1) A matrix of interwoven protein fibers and

(2) Interstitial calcite (calcium carbonate) crystals

The matrix consists of two regions

(a) The mamillary matrix and

(b) The spongy matrix

Microscopic studies have shown that the matrix fibers pass through calcite instead of simply surrounding them. Undoubtedly, the matrix has a significant influence on shell strength. The matrices are made up of protein-mucopoly saccharides complexes. The poly-saccharides contain chondroitin sulfates alpha and Beta galactosamine, glycosamine, galactose, mannose fucose and sialic acid (Owen,1976).

According to Romanoff and Roman off (35) the elemental composition of the egg shell is 98.2% calcium, 0.9% magnesium and 0.9% phosphorous (present in the shell as phosphate). Jiang and

Sim(1991) indicated that shell hardness increases with increases in magnesium content.

The water insoluble protein cuticles form a protective coating (about 10-30mm thick) on the entire surface of the egg. The cuticle is made up of mucoproteins with hexosamine, galactose mannose and fucose as constituents of the polysaccharides.

2.5.2 Shell membrane

Two membranes with thickness of 48Nm and 22Nm resided between the inner surface of the shell and the albumen. The membranes are composed of protein –polysaccharides fibers. The outer shell membrane is attached firmly to the shell with numerous cones on the inner shell surface and by fiber associations. (Owen,1976).

2.5.3 Albumen

Albumen is made up of four distinct layers

(a) Outer fluid (thin) albumen

(b) Viscous albumen (thick)

(c) Inner –fluid albumen (thin)

(d) A chalazeferous layer(Owen,1976).

The wide range in the proportion of layers has been attributed to breed, environmental conditions, size of egg and rate of egg production.

The major constituents of all albumen layers are water, which decreases some what in amount from the outer to the inner albumen layers. The water content of mixed albumen ranges from 87 to 89% and is dependent on the strain and age of the hens

Proteins are the major constituents in albumen solids. The variability of protein content (9.7-1.6%) in the albumen can be attributed primarily to the age of the bird. The amount of lipid in albumen (about 0.03%) is negligible compared to that of yolk. The carbohydrate in albumen is in combined (with protein) and free forms and can reach a level of about 1%. Approximately 98% of the free carbohydrate (about 0.5%) of the albumen is glucose. The elemental composition of the ash of albumen is presented in table below. (Owen,1976).

Table 4: Elemental composition of albumen and yolk

Element	Percentage	Percent in yolk
Sulphur	0.195	0.016
Potassium	0.145-0.167	0.112-0.360
Sodium	0.161-0.169	0.070-0.093
Phosphorus	0.018	0.543-0.980
Calcium	0.008-0.02	0.121-0.0262
Magnesium	0.009	0.032-0.128
Iron	0.0009	0.0053-0.011

Source: Owen (1976).

Table 5: Fatty acid components of lipid fraction of yolk and dietary lipids(g).

FATTY ACIDS	CRUDE LIPID	TRIGILYCERIDES	LECITHIN	CEPHALIC
16.0	23.50	22.5	37.0	21.6
16.1	3.80	7.30	0.60	TRACE
18.0	14.0	7.50	12.40	32.50
18.1	38.40	44.70	31.40	17.30
18.2	16.40	15.40	12.0	7.00
18.3	1.40	1.30	1.0	2.00
20.4	1.30	0.50	2.7	10.20
20.5	0.4	0.2	0.8	3.0
22.5				
22.6	0.8	0.6	2.1	6.4

From table 5 above, it can be deduced that a regular egg has high percentage of oleic acid follow by palmitic (16.0), linoleic acid (18.2) and stearic acid with low percentage of linolenic acid (18.3)

2.5.4 Yolk

The solid content of yolk is in the vicinity of 50%. Yolk from fresh eggs has a solid content of 52-53% but the yolk solids drop about 2% when eggs are stored at refrigerated temperature for 1-2 weeks because water migrates into the yolk from the albumen. Protein and lipids are the major constituents of yolk, with minor amounts of carbohydrate and minerals also present. The protein content of yolk is about 16%, with limited variability. The amount of lipid varies between about 32 and 36% and the variation can be attributed to the strain of bird rather than to the diet. The yolk lipid fraction contains approximately 66% triglycerides, 28% phospholipids, 5% cholesterol and minor amounts of other lipids (Amini and RuizFeria,2007).

The fatty acid composition of yolk lipid is influenced by the types of fatty acids in the diets of the hen. The total amount of saturated fatty acids, primarily palmitic and stearic, does not change with an alteration of dietary fatty acid composition.(Simopoulous,2002).In the fatty acid content of yolk lipid fraction palmitic and stearic acids amount to about 30% of the triglycerides, whereas they amount to about 49% in lecithin and 54% in cephalin.(Baucell *et al.*,2000).

The amount of carbohydrate (combined and free) in yolk may be as high (1%) as that in albumen. The major elements in yolk ash (1.1%) are calcium, potassium and phosphorous.

2.5.5 Egg storage and preservation

Methods for preserving whole eggs aim at maintaining the original water content and carbon dioxide in the egg for as long as possible; as well as to retard microbial activity.

2.5.5.1 **Dry packing:** This involves the packing of eggs in material such as sand, chaff and saw dust. This method offers no protection against microbial storage (Van Elswyk,1997).

2.5.5.2 **Immersion in liquid**: Eggs are immersed in lime water and glass water (aqeous sodium silicate). The high pH of these solution retard microbial growth and the pores of the egg are sealed by deposits of calcium carbonate and calcium silicate (Owen,1997).

2..5.5.3 **Cool storage:** In this process, the intact egg is held at the lowest possible temperature above its freezing point of -20^{O}C. The low temperature will retard loss of carbon dioxide and water from the egg as well as the diffusion of water from albumen to yolk.

2.5.5.4 **Shell- sealing treatments:** This involves dipping egg into materials such as agar, rubber, soap, gelatin, sulphuric acid and oil. Oiling in conjunction with storage at about 1OC can preserve eggs almost unchanged for period of more than six months (Leeson and Summers,2005).

2.5.6 Egg quality evaluation

Changes which occur during the storage of shell eggs include

- Loss in weight due chiefly to loss of moisture from the albumen but also partly due to loss of carbon dioxide.
- Increase in air cell size as a result of moisture loss.
- Decrease in specific gravity due to increase in air cell size.
- Mottling on the surface of the shell due to uneven moisture distribution.
- Decrease in the amount of thick white due to breakdown of the fibrous glycoprotein ovomucin
- Increase in the size of the yolk because of the movement of water from the albumen to the yolk
- Loss of carbon dioxide and
- Increase in pH particularly in the albumen which increases from approximately pH 7 up to 10 or 11 as a result of carbon dioxide loss

(Ihekoronye and Ngoddy, 1985).

2.5.7 Importance of eggs in the diets.

Eggs make a useful contribution to the daily intake of vitamin D, retinal, riboflavin, iodine, iron and protein in the average diet and for the elderly they can be a particularly important source of protein, iron, vitamin B_{12} and vitamin D.(Ihekoronye and Ngoddy,1985).

2.6 . Tetracarpidium Conophorum- Morphology and Uses

The conophor plant *(Tetracarpidium conophorum*), commonly called the african walnut, is a perennial climbing shrub found in the moist forest zones of sub-sahara Africa (Enujiugha, 2003). It is indigenous to South-western parts of Nigeria and is also grown in the western Cameroon where it is known as Kaso or Ngak. This exotic, perennial wild fruit is grown in the traditional farming system of the lowland humid tropics of Nigeria between latitude $4^{O}15^{1}$ and 8^{O}N of the equator (Okafor, 1983; Adebona *et al.*,1988). *Tetracarpidium conophorum* is a plant that usually grows wild. The fruit is a four-winged and ribbed capsule, containing sub globe seeds with thin brown shell and yellowish kernel (Adesioye, 1991). Flowering and fruiting takes place in the months of March, April and May. Heavy crop is harvested between June and September and light one in December (Adebona *et al.*, 1988).

Among the most commonly eaten oil seeds in Nigeria is conophor nuts (Ige et al; 1984). It is grown in Nigeria for its fruits which bear some oil rich nuts. The only known use of the nuts is as a snack although Ogunsua and Adebona (1983) reported that the crop has a great potential as a source of food. The matured unshelled conophor nuts are cooked in pots at atmospheric pressure for 1-2hours depending on the intensity of the heat from the firewood (Adesioye, 1991). After cooking, the nut are cracked to remove the shell and then consumed as snacks along with boiled corn of the nut.

Ogunsua (1987) reported the use of defatted oil seeds in composite flour for bread making. It was reported that substitution of wheat flour with up to 10% defatted conophor flour produced the best rheological characteristics but gave loaves with the loaf volumes and loaf scores. Similarly, Adebona *et al., (*1988) incorporated conophor flour into wheat and minimize based biscuits. He found that substitution at 12% level produced good quality products similarly, Adesioye (1991)successfully extended the shelf life of the traditionally processed nuts which is not more than 3days to at least one month through a combination of heat processing,brining,freezing and frozen storage.

2.6.1 Chemical composition of conophor nut (*Tetracarpidium conophorum*).

The proximate chemical composition of *Tetracarpidium conophorum* reveals that the fresh shelled nut contains about 50% moisture and 17% free lipid on a wet weight basis. The nut contains about 5.9% crude fibre and about 2.8% ash on a dry weight basis. Table 8 shows the proximate composition of conophor nut (Ogunsua and Adebona, 1983). The nut contains on a dry weight basis about 46.49% fat (Ogunsua and Adebona, 1983; Ige et al; 1984; Ogunsua 1987; Adebona et al; 1988; Adesioye, 1991; Enujiugha, 2003; Odoemelam, 2003). The oil is pale yellow and clear and may turn brown if the solvents used for lipid extraction is not removed at low temperature or under inert atmosphere (Ogunsua and Adebona, 1983). The fatty acids analysis of the oil by gas liquid chromatography showed that linolenic acid constituted 66% of the fatty acids. Polyunsaturated fatty acid accounted for over 80% of the fatty acid (Ogunsua and Adebona, 1983). The iodine value is very high about 204 indicating the presence of a high degree of unsaturation. The specific gravity and refractive index at 28.5°C are 0.9325 and 1.4708 respectively (Table 8)

Table 6: Proximate composition of Tetracarpidium conophorum

	Percent on dry weight basis
Fat	50.0
Ash	2.80
Protein	23.50
Fibre	5.90
Nitrogen free-extractive by difference	17.80

Source: Ogunsua and Adebona (1983)

Table 7: GLC of the Fatty Acid Methyl Esters and characteristics of oil Extracted from *Tetracarpidium Conophorum*

Fatty acid	Amount in percentage (%)
Palmitic acid	1.3
Stearic acid	2.3
Oleic acid	14.4
Linoleic acid	15.8
Linolenic acid	66.3
Iodine value	204
Specific gravity	0.9325
Refractive index at $28.5^{O}C$	1.4708

% of total methyl esters

Source : Ogunsua and Adebona (1983)

Conophor nut is highly nutritious containing high levels of protein (20-24%) on a dry weight basis (Ogunsua and Adebona, 1983; Ige *et al.*, 1984, Ogunsua, 1987; Adebona *et al.*, 1988; Adesioye, 1991; Odoemelam, 2003). After defatting, the resulting cake is grey in colour and has a characteristic conophor nut flavor. This residue has about 35-40% protein content (Ogunsua and Adebona, 1983; Ogunsua 1987). The non-protein nitrogen is about 9.2% of the total nitrogen (Ogunsua and Adebona, 1983).

The amino acid composition of the conophor nut protein has also been investigated. The total sulphur amino acids of the nut was high- methionine 2.62% ,tryptophan 4.45% and cystine 4.90%.Glutamic aspartic and arginine acid were abundant (Oyenuga *et al.*, 1971). Similarly, Ogunsua (1988) reported that conophor nut contains all the essential amino acids in adequate levels for nutrition except methionine. Two unusual amino acids $\propto$ – amino butyric acid and gamma- amino butyric acid were also found. Table 10 gives relative amounts of amino acids in conophor nut flour.

Table 8: shows FAO/WHO requirements for amino acids in conophor Nut flour (in moles)

Amino acids	**By ion-exchange**	**By GLC**
Ala	9.7	10.4
Gly	27.9	27.9
Val	11.5	12.6
Thr	11.2	11.2
Ser	13.7	14.0
Leu	13.1	11.0
Ile	9.0	10.1
Pro	10.4	9.6
(CYS)	ND	6.9[a]
Hypro	ND	2.1
Met	1.8	2.89
Asp	21.8	23.3
Phen	4.6	4.4
Glu	17.9	14.3
Lys	7.6	3.69
Tyr	6.4	7.6[a]
Arg	14.0	7.5
His	2.9[a]	11.1
$(CYS)_2$	2.4[a]	___
Tryp	ND	___

Average of triplicate analysis

Source: Ogunsua, (1988).

Table 9: Essential Amino Acid Analysis of Conophor Flour (mg/gN)

Amino acid	FAO/WHO Reference	Conophor nut flour
Try	90	184
Phe	180	194
Met	140	68
Lys	270	354
Thr	180	343
Leu	300	441
Isoleu	270	304
Val	270	345

Source: Ogunsua (1998)

The carbohydrate content of the nut is about 15% (Ogunsua and Adebona, 1983; Enujiugha, 2003; Odoemelam, 2003. The total sugar content was about 2% (Ogunsua and Adebona, 1983). Thin layer chromatography of the ethanolic extract revealed that sucrose was the only detected sugar.

2.7 Cholesterol

High plasma level of cholesterol is associated with atheros cleroris and coronary heart diseases. It is one of a group of compounds called sterol. It is related to such sterols as sex hormones and the hormones of the adrenal cortex- cholesterol is a compound of all mammalian cells and occurs either as free cholesterol or it may be combined with fatty acids as cholesterol ester (cholesterides). It can be activated by U-V light to form (vitamin D) .It is an essential element of cell membranes and it is found in nerve tissues, body fluids, adrenal cortex and the ovaries. The main site of its synthesis is the liver (Wilson *et al.*, 1975).

The body sources of cholesterol are :

(i) Diet- foods rich in cholesterol e.g eggs, butter cream, meats (liver, kidney, brain (Lopez *et al.*, 1967).

(ii) Synthesis in tissues from acetyl coenzymes A (acetyl coA). About 1.5-2.0grams of cholesterol is synthesized daily in the body, this is far in excess of that normally consumed (Bell *et al.,* 1963).

Cholesterol has some useful functions

(a) Transportation of unsaturated fatty acids.

(b) Production of sex (Steroid) hormones e.g most of the adrenal cortex hormones are derivatives of cholesterol

(c) Synthesis of bile salt and bile acid. These substances are passed into the intestine for emulsification of digested fat (i.e fatty acid and glycerol) thus facilitating their absorption into the villi of small intestine where they are recombined into neutral fats. But it can be separated from bile, giving rise to gall stones (Neil, 1961). Some of this cholesterol is excreted in faeces.

Plasma cholesterol is elevated in diabetes, obesity, excess caloric intake, high fat diet e.g. saturated fats, dietary cholesterol (Coons, 1959). Its level varies with nutritional and physiological functions, the degree of physical activity and the amount of stress an individual experience (Lopez et al., 1967). Normal blood cholesterol level for man aged 25yrs is 100-250mg per 100ml. It increases with age from 150-370mg per 100ml at 55yrs (Keel and Neil, 1963). The plasma level tends to be higher in men than in women. The excessive amount in the blood following diets containing much animal fat can be dramatically reduced by consumption of some unsaturated fatty acids such as linoleic acid (Datta and Ottaway, 1962).

Increase in cholesterol consumed by people has little effect on the amount of cholesterol synthesis or the rate of its conversion to bile acids (Anon,1970). It is also suggested that there appears to be a threshold for absorption of cholesterol in the gut beyond which the excess is excreted in faeces (Wilson *et al.,* 1975).

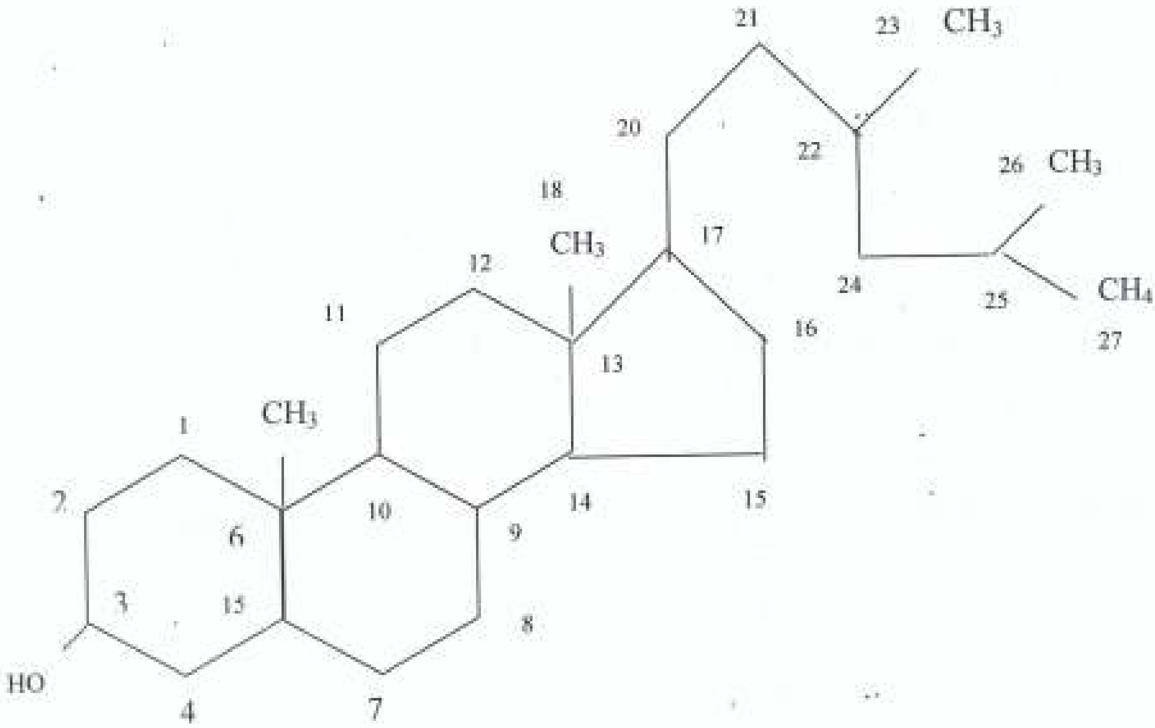

Fig. 3: The structural formular for cholesterol
Adapted from Owen (1976).

CHAPTER THREE

3 MATERIAL AND METHODS

3.1 **Source of samples used;** conophor nuts were purchased from owena market In Osun state

3.2 Sample preparation.

The unshelled nuts were washed and cooked at $100^{O}C$ for 2hrs. it was dried for 12hrs in an oven at $60^{O}C$ to a moisture content of 20%. Shelling of fruit was by hand. The shelled fruits were further dried at $50^{O}C$ for 48hrs to a moisture content of 12%. Dried fruits were milled in a hammer mill for fine particle size reduction. The paste obtained was mixed with layers mash to feed the hens.

Moisture content determination

Apparatus: Gallenkamp air-oven, aluminum dishes and oheuns weighing balance.

The aluminum dishes were dried and weighed. The unshelled nuts were dried for 12hours and weighed until the moisture content was about 20%. The shelled fruits were similarly treated and dried at 50^{o} for 2days until the moisture reached 12%.After milling a paste was observed instead of powder indicating a high percentage of fat in the seed.

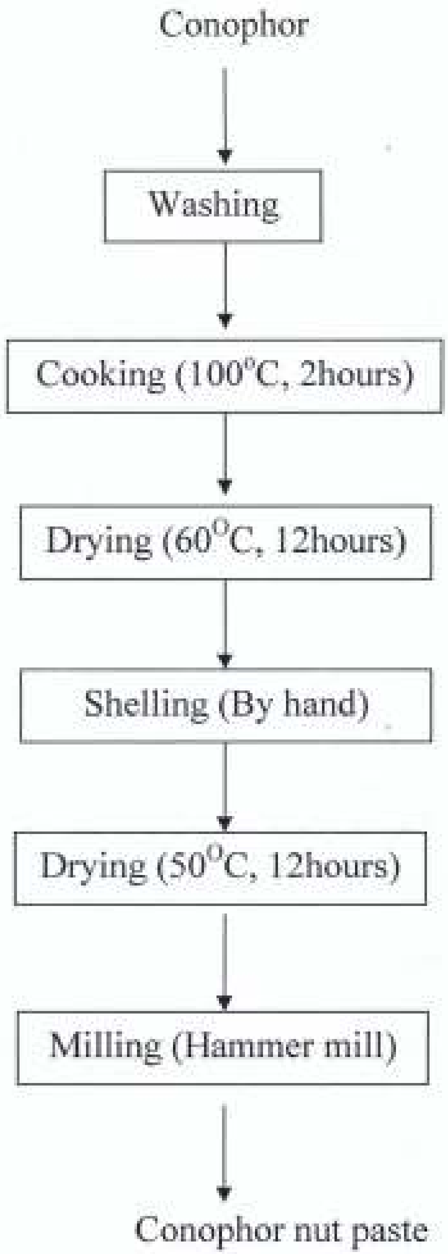

Fig. 4: Scheme for the production of conophor nut paste

Adapted from Ogunsua (2000)

3.3 Animals and Treatments

3.3.1 Site of the project

This project was carried out at a private poultry farm at Leo junction in Akure, Ondo State.

3.3.2 Birds and management

A total of 12 seventeen week old commercial layers were used for the experiment. They were brought from a private poultry farm. The birds were randomly distributed into four feeding regimes. Each regime consisted of three birds, individually caged . Each cage was taken as a replicate. The cages were previously cleaned and disinfected. The birds were analyzed under intensive care and hygiene. Water troughs were washed regularly and filled with fresh and clean water. Birds in the same treatments were fed together and carefully separated such that they could not have access to feed from other treatments. Feeds were protected from any contamination by closing the bags every time. Droppings were collected every week and the poultry house swept regularly.

The poultry house was an open sided type expected for tropical environment, adequate ventilation was provided for the birds. Three of the birds were fed with layer feeds while the remaining nine birds were fed with layers mash mixed with conophor nut paste in varying ration (9:1, 8:2, 7:3).During the feeding period anti-stress drug called vitalight was administered for 3-5days consecutively for every interval of two weeks. Once in every two weeks a vaccine (lasota) against New castle disease was given to the hen by dissolving it in drinking.

3.3.3 Flock performance

During the experiment three eggs per cage replicate was randomly selected to evaluate the following parameters:

- Average egg weight
- Average yolk index
- Average Albumen index
- Shell thickness
- Egg fatty acid profile

3.3.3.1 Average egg weight

Weight of eggs obtained from chickens were measured immediately after laying using 0.01g sensitive electronic balance. The values obtained were expressed in grammes.

3.3.3.2 Yolk index and albumen index

The eggs collected were broken on a table with a glass cover in order to measure yolk and albumen height using spirometer and yolk and albumen width using a measuring ruler. The value obtained was expressed in millimeters.

$$\text{Yolk index} = \frac{\text{height of the yolk}}{\text{width of the yolk}}$$

$$\text{Albumen index} = \frac{\text{height of the albumen}}{\text{width of the albumen}}$$

3.3.3.3 Shell thickness

The shell thickness of the eggs collected was determined by separating the egg shell from the membrane and shell thickness was later measure using micrometer screw gauge accurate to 0.01mm.

3.3.3.4 Fatty acid analysis

Two eggs were collected from each treatment for fat analysis using Soxhlet apparatus according to the method described by AOAC (1990). The fat extracted from the egg yolk was later subjected to chemical analysis for fatty acid determination using Gas liquid chromatography as described below.

3.3.3.4.1 Fat analysis

Procedure

The yolk of an egg was gently separated from the albumen. It was collected in a sterilized petri-dish. The moisture content of the working sample was reduced to the bearest minimum level by arranging them in an oven heated at $70^{O}C$.

Five grams of each dried sample (W1) obtained from each treatment was weighed into a previously weighed thimble (W2).About 500ml round bottom flask was weighed and filled with petroleum ether up to two third of the flask. The soxhlet extractor was fitted up with a reflux condenser so that the solvent boils gently for 5-6 hours. At the end of the extraction the condenser was removed and the thimble detached and transferred into the oven at $100^{O}C$ for 5min and later cooled in the dessicator and weighed again (W4). The percentage (%) of extracted lipid can be expressed as

$$\% \text{ fat} = \frac{W4-W3}{W1} \times \frac{100}{1}$$

W1=Weight of the sample

W2=Weight of the thimble

W3=Weight of the thimble+ sample before extraction

W4=Weight of the thimble+ sample after extraction

The extracted fat for each treatment was collected for fatty acid analysis

3.3.3.4.2 Fatty Acid Methyl Esters by Gas Chromatography.

The intact triacyglycerol and free fatty acids are not very volatile and are therefore difficult to analyse using gas chromatography (which requires that the lipid be capable of being volatized in the instrument). For this reason lipids are usually derivatized prior to analysis to increase their volatility. Triacyglycerols are first saponified.This breaks them down to glycerol and free fatty acids and are then methylated.

Procedure for Methylation of Fatty Acids

The methyl esters of the fatty acids are prepared directly by treatment of the fat with sodium methoxide and are then separated by gas chromatography.

Sodium methoxide(1M) was prepared by dissolving 1.15g sodium in 50ml methanol .0.25g of the sample (oil) was weighed into a small screw capped vial. Three milimeters petroleum ether was added, the cap replaced and vigorously shaken to dissolve the fat. The cap was later removed and 0.15ml sodium methoxide with a pipette was introduced into the vial containing the sample .Then the cap was replaced and shaken vigorously for a few seconds. The sample was left for 5min or more and later injected into a gas chromatograph in order to identify and quantify the fatty acids present.

Saponification followed by methylation increase the volatility of the lipids. The concentrations of different volatile fatty acid methyl esters (FAMES), present in the sample is then analysed using gas chromatography. The FAMES are dissolved in a suitable organic solvent that is then injected into a gas chromatography injection chamber. The sample is heated in the injection chamber to volatilize the FAMES and then carried into the separating column by a heated carrier gas. As the FAMES pass through the column they are separated in to a number of peaks on differences in their molecular weight and polarities.They are quantified using a suitable detector. Determination of the fatty acid profile allows one to calculate the type and concentration of fatty acids present in the original lipid sample (Pearson,1976).

3.3.4 Quantitative determination of cholesterol in egg yolk

Procedure

The yolk of an egg was separated from the egg white .It was then placed in a tarred beaker to determine the weight of the yolk. Exactly

25ml acetone was added to the yolk, stirred well and filtered with suction. It was later washed with five 10ml portions of acetone and combined with the extracts. The extract was quantitatively transfered into a 100ml volumetric flask, diluted with acetone to the mark and mixed thoroughly.Five millimeters of the solution was transferred into a tarred centrifuge tube and 25ml of digitonin solution was added. The same process was carried out in another tarred centrifuge tube with 5ml of the standard cholesterol solution.

Digitonin, a pentasaccharide plus a polycyclic compound, forms an insoluble complex with cholesterol. The precipitate formed was centrifuged at top speed for 15min, the supernatant was poured off and finally dried in an oven .After the experiment, the weight of the precipitates formed the weight of cholesterol in the standard cholesterol tube and the percentage of the precipitate (i.e. cholesterol) were determined and recorded.

The weight of cholesterol in 5ml of extract (X), the total weight of cholesterol in the egg yolk (X) (20), and the percentage cholesterol in the egg yolk was calculated and reported.(Zimmerman,1984).

Calculation.

$$\frac{0.015}{\text{Weight of standard ppt.}} = \frac{x}{\text{weight of Egg ppt.}}$$

$$\frac{(X)\,(20)\,(100)}{\text{Weight of egg yolk}} = \text{\% cholesterol in egg yolk}$$

KEY: wt. std. ppt = weight of standard %

: precipitate

Wt. of egg ppt. = weight of cholesterol in 5ml of extract..

precipitate

The diets were based on the following composition in Kilogram

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

RESULTS

Table 10: Average egg weight (g) produced by laying hens fed layer feeds mixed with different levels of conophor nuts (10,20 and 30%) and control..

Samples	Description	First week	Second week	Third week
A	Feed only	166.69b ± 0.02	165.60c ± 0.36	164.63b ± 0.27
B	F + c (10%)	162.05c± 0.06	161.30d± 0.06	159.42c± 0.036
C	F + c (20%)	171.06b± 0.04	170.6ob+ 1.05	170.00a±2.23
D	F + c (30%)	178.39a±6.09	176.66a±3.02	174.05a± 2.50

Values reported are means ± s.d of triplicate determinations.
Mean values within each row bearing a different superscript roman letter are significantly ($p < 0.05$, duncan multiple range) different

F – FEED

C – CONOPHOR NUTS

4.1 The effect of different level of conophor nuts paste mixed with layers feed on the egg weight.

Table 10 shows the effect of conophor nuts paste mixed with layer feeds on the egg weights. The result shows that diet treatment containing conophor nut paste have a significant effect on the egg weights. The values recorded signify an increase in egg weights with increasing level of conophor nut paste mixed with the layer feeds. There is an increase in egg weight with 20% and 30% inclusion level of conophor nuts paste compared to the egg weight obtained from hens fed a control diet except with 10%. This observation is in agreement with some previous findings (Mazalli *et al.,* 2004; Amini and Ruiz_Feria, 2008) which indicate that increasing level of 20% flaxseed gave a positive increase on the egg weight. The lower values of the egg weights in hens fed with F + c 10% may be traced to slow response of the birds to feeding when experimental diet (feed +10%) was newly introduced.

From Table 10, it can also be deduced that conophor nut paste may manifest a negative effect on egg weight after several weeks of use. Van Elswyk (1997) suggested that the reduction in egg weight when feeding flaxseed to hens could be mediated by a low oestradiol level in the blood, which may limit lipid availability for yolk formation and consequently egg weights.

Table 11: average egg yolk index produced by laying hens fed a layer feeds mixed with different levels of conophor nut paste and control

Samples	Description	First week	Second week	Third week
A	Feed only	0..48a ±0.05	0.37b ±0.04	0.41a ±0.04
B	F + c (10%)	0.48a ±0.05	0.45a ±0.04	0.48a ±0.05
C	F + c (20%)	0.47a ±0.05	0.45a ±0.04	0.46a 0.04
D	F + c (30%)	0.48a ±0.05	0.46a ±0.04	0.47a 0.05

Values reported are means ± s.d of triplicate determinations.
Mean values within each row bearing a different superscript roman letter are significantly ($p < 0.05$, duncan multiple range) different

F – FEED

C – CONOPHOR NUTS

Table 12: Average egg albumen index produced by laying hen fed a layer feeds mixed with different levels of conophor nuts and control.

Samples	Description	First week	second week	third week
A	Feed only	0.086b ± 0.01	0.089a ± 0.02	0.088a ±0.01
B	F +C (10%)	0.088a ±0.01	0.088a ±0.01	0.086b ±0.04
C	F + C (20%)	0.087a ±0.01	0.087a ±0.01	0.087a± 0.03
D	F +C (30%)	0.089a ±0.02	0.089a ±0.02	0.089a ±0.02

Values reported are means ± s.d of triplicate determinations.
Mean values within each row bearing a different superscript roman letter are significantly ($p < 0.05$, duncan multiple range) different

F – FEED

C – CONOPHOR NUTS

4.2 Effect of supplemented feed on the egg yolk and albumen index.

The effect of conophor nut paste mixed with layers feeds on egg qualities (yolk and Albumen index) is presented in Table 11 and 12. Egg yolk and albumen index are the quality parameters used to measure the value of an egg (Alkan *et al.*, 2008; Altan, and Akbas,1998). It is also mentioned that the higher the yolk and albumen index the higher the quality of the eggs (Carter, 1975; Amini and Ruiz Feria, 2008). The results indicate that there were no differences in yolk and albumen index due to the treatment when compared to control and the standard values. However, the yolk index of the eggs collected are within the range reported by other workers (Amini and Ruiz-feria, 2007 and Mazalli et al., 2004).These values were also comparable to the standard values of yolk index (0.33-0.50) and albumen index (0.090-0.120). The different inclusion level of conophor nut paste in the layer based diet did not affect these two quality parameters.

Table13: Average cholesterol content (mg) produced by laying lens fed layer feeds mixed with different levels of conophor nut paste (10, 20 & 30%) and control.

Samples	Description	First week	Second week	Third week
A	Feed only	187a±0.30	188a±0.15	190a±0.07
B	F + c (10%)	187a±0.04	186b±0.02	185b±0.08
C	F + c (20%)	185ab±0.14	184c±0.15	183c±0.19
D	F + c (30%)	185b±0.02	185b±0.20	183c±0.5

Values reported are means ± s.d of triplicate determinations.
Mean values within each row bearing a different superscript roman letter are significantly ($p< 0.05$, duncan multiple range) different

F – FEED

C – CONOPHOR NUTS

4.3 Effect of conophor nuts supplementation in layers feed on cholesterol content of the egg.

It is well established that dietary polyunsaturated fatty acid can cause changes in cholesterol level of the eggs (Health Canada's Nutrition Recommendations 2005; Caston andLeeson,1990). The results presented in (table13) shows that the cholesterol level of eggs decreased with increasing level of conophor nut paste mixed with the layers feed, except at first two weeks when the cholesterol level of eggs produced by layers based diet containing 30% conophor nut paste were not significantly different. Moreover, it can also be deduced from the table that the average cholesterol content obtained from egg yolk produced by hens fed a control diet increased with increasing weeks of feeding. With conophor added diets, the conophor elicited a decrease of the level of cholesterol in the eggs with increase in time of feeding. The results of this present study compare favourably with those obtained by Noble *et al.*, (1990) and Mazalli *et a*l., (2004) which reported that Omega-3 fatty acid tend to reduce low density lipoprotein (LDL or bad) cholesterol and triglyceride levels thereby lowering total cholesterol and triglyceride in individual with high cholesterol levels.

Table 14: Average egg shell thickness (mm) produced by laying hens fed layer feeds mixed with different levels of conophor nut paste (10%, 20% & 30%) and control.

Samples	Samples	First week	Second week	Third week
A	Feed only	32.33	32.25	32.38
B	F +c (10%)	32.33	32.30	32.55
C	F + c (20%)	32.59	32.34	32.50
D	F + c (30%)	32.31	32.35	32.39

Where:

F= Feed (Layer Feeds)

C = Conophor Nuts

4.4 Effect of conophor supplementated diet on egg shell quality.

Table 14.depicts the result of the effect of conophor nuts mixed with the layers mash on the eggs shell quality. The values obtained show that different inclusion levels of conophor nuts in the layer based diets did not affect egg shell quality parameters which is in agreement with previous findings (Amini and Ruiz-feria, 2007), and with the result reported by Caston *et al.*, (1994) in which increasing level of 0,10% and 20%Conophor nuts did not have any significant impact on shell weight. Mazalli *et al.*, (2004) obtained comparable results after feeding 3% flax oil or 9% flaxseed to the birds.

Table 15:Fatty acids profile of methyl fatty acids) in the eggs produced by laying hens fed layers mash mixed with different levels of conophor nutspaste (10%, 20% & 30%) and the control diets (mainly layers mash).

S/N	number of carbon atoms	Fatty acids	Feed only	F+ c (10%)	F+ c (20%)	F+c (30%)
1	C16:0	Palmitic	25.13	18.92	13.24	17.08
2	C16:1	Palmitoleic	2.13	1.72	0.48	0.96
3	C18:0	Stearic	7.31	9.23	11.47	12.37
4	C18:1	Oleic	49.37	42.73	41.11	41.54
5	C18:2	Linoleic	13.62	22.38	25.11	22.33
6	C16:3	Linolenic	0.46	1.93	4.73	2.59
7	C20:0	Arachidic	1.98	3.07	3.85	3.11
		SFA	34.42	31.22	28.56	32.56
		MUFA	49.37	42.73	41.11	41.54
		PUFA	14.08	24.31	29.84	24.92
		Un SFA	6345	67.04	70.95	66.46

F – Feed
C – Conophor nuts
SFA – Saturated fatty acid
MUFA – Monounsaturated fatty acid
PUFA – Polyunsaturated fatty acid
UnSFA – Unsaturated fatty acid

4.4 Effect of conophor supplemented feed on the fatty acid profile of egg.

The fatty acid profile of eggs produced by laying hen's fed a layer mash mixed with different levels of conophor nut paste (10%, 20% & 30%) and the control diet (mainly layers mash) are shown in Table 15. The analysis carried out show that three types of fatty acid are present in the samples. These are saturated fatty acids; palmitic acid, stearic acid Arachidic acid while the second type is monounsaturated fatty acid; Oleic acid and finally polyunsaturated fatty acids which are Linoleic acids linolenic acids and palmitoleic acid.

Table 15 shows that egg obtained from laying hens fed with control diet(feed only) contain higher concentration of oleic acid(41.5-49.37%) a monounsaturated fatty acid than stearic acid(7.31-12.39%),palmitic acid (13.24 - 25.13%) and arachidonic acid(1.97-3.85%)saturated fatty acid. There are essential fatty acids which are required for growth, physiological function and body maintenance in the sample examined.These essential fatty acids are linoleic acid(13.62-25.11%),alpha linolenic(0.46-4.73%) and palmitoleic acid(0.48-2.13%).These results obtained compare favourably with those reported by (Leeson and Summers ,2005;Baucell *et al*.,2000;Mazalli *et al*.,2004).

From the table above ,it can also be deduced that the level of the linolenic acid increase from 1.98% to 3.85% in those hens fed with supplements 20% conophor nut paste.The result obtained agrees with findings of other workers who used high level of flax seed in the diets and observed incremental trends of omega-3fatty acid content of the eggs (Herber and Van Elswyk,1996;Van Elswyk,1997 and Amini-Ruiz- feria,2008). According to Leeson and Summer (2005), Baucell *et al.,* (2000), Mazalli *et al.,* (2004), egg contains low concentration of linolenic acid (1.4%) and higher concentration of oleic acid (38.4%).

As previously reported that dietary polyunsaturated fatty acids can cause major changes on egg fatty acid profile (Noble *et al.,*. 1990; Mazalli *et al.* 2004b). In the same study, it was found that Omega -3 fatty acids content of the eggs can be increased with increasing level of any vegetarian source of Omega-3 fatty acid like soybean , flaxseed, canola oil, conophor nut e.t.c.

included in the layers mash. (Amini and Ruiz-feria, 2007; Simopoulous , 2006). In this study, a dose –related improvement in the Omega-3 fatty acid content of the eggs in response to increasing levels of dietary conophor nut paste mixed with the layers mash was observed.

The value for polyunsaturated fatty acids (linolenic and linoleic acids) from Table 15 indicates that the concentration of poly unsaturated fatty acid especially linolenic acid can be doubled by mixing layers mash with 20% conophor nut paste as the total values are increased from 14.08 to 29.84% in the hens fed feed supplemented with conophor.The fatty acid profile of oil extracted from the egg yolk reveal that egg produced from the hens fed the experimental based diets (feed+conophor)have reduced saturated fatty acid and increased unsaturated fatty acid.It also gives increased polyunsaturated fatty acids especially omega-3fatty acid. From literature such omega-3fatty acid enriched eggs can help reduce the risk factors for heart diseases and high blood pressure.

Table 16: Average Total fat content (%) produced by laying hen fed layer feeds mixed with different levels of conophor nut paste (10,20 and 30%) and control

Sample	Description	First week	Second week	Third week
A	Feed only	10.75c	10.76c	10.77c
		±1.83	±1.86	±1.86
B	F+ c (10%)	10.50c	10.70c	10.79c
		±1.88	±1.86	±1.86
C	F+ c (20%)	11.64b	11.85b	11.90b
		±1.90	±1.95	±2.04
D	F+ c(30%)	12.32a	12.50a	12.61a
		±2.05	±2.01	±2.01

Values reported are means ± s.d of triplicate determinations.
Mean values within each row bearing a different superscript roman letter are significantly ($p < 0.05$, duncan multiple range) different

F – FEED

C – CONOPHOR NUTS

4.5 Effect of conophor supplemented feed on the total fat content of egg

The result in Table16 shows that there is an increase in the fat content in response to increasing level of both experimental and control diets. This positive effect is highly pronounced in experimental diets containing 20 and 30% conophor nut paste. In this study, the fat content deposited in the egg yolk increased with increasing level of conophor nut paste except treatment A and B when the total fat content of egg yolk produced by layer feeds (treatment A) and experimental diets containing10% conophor nut paste were not significantly different. This fact agrees with findings of other workers who used high level of flaxseed (10 and20%) in the layer feed and observed incremental trend in the total fat content of the eggs (Herber and Elswyk, 1996; Van Elswyk, 1997). This result also agrees with research finding and clinical studies reported by Ogunsua and Adebona (1983);Victor.N.Enujuigha(2003) and Okuyama(2000) which declared that conophor nuts contain a lot of oil and the dried seed contains 40% free lipid.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion.

In general, this finding confirm that for the purpose of Omega-3 fatty acids enriched egg production the use of inclusion level of 20-30% conophor nuts paste into layer based diets increase the egg weight.

The combination of different inclusion level of conophor nut paste and Layer mash in the experimental diets has no detrimental effect on egg quality parameters (i.e. yolk index, albumen index and shell thickness. This shows that conophor nut can also provide a good source of food to the hen.

Moreover, the use of high level of conophor nut paste inclusion in layer feed is associated with reduced cholesterol level. Therefore Omega-3 enriched eggs can be used as a diet for reducing the risk of high cholesterol level, high blood pressure and Heart diseases in individual with hypertension.

Finally, the lower concentration Omega-3 fatty acid eggs can be increased with the use of inclusion level of 20% conophor nuts paste.

5.2 Recommendation

Despite that global food companies had lunched Omega-3 fatty acid fortified bread, mayonnaise, pizza yoghurt, orange juice, children's pasta, milk, eggs, confections and infant formula there's still a great need to fortify more of our available and affordable local feeds with Omega-3 fatty acid. If this is achieved, then the whole populace will be a partaker of the health benefits of Omega-3 fatty acid.

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