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EFFECTS OF FEEDING FLAXSEED AND PROBIOTIC SUPPLEMENTATION TO LAYERS ON EGG CHOLESTEROL AND FATTY ACID COMPOSITION

By

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A thesis submitted to the faculty of Graduate studies and Research in partial fulfilment of the degree of master of Science

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Suggested short title.

Flaxseed and probiotics on egg composition

DEDICATION:

DEDICATED TO MY LATE BROTHER,
JERRY T.PHEKO

Master of Science **Animal Science**

ABSTRACT

Lieketseng Gladys Pheko

Effects of feeding flaxseed and probiotic supplementation to layers on egg cholesterol and fatty acid composition

A study was conducted to examine the response to feeding flaxseed (FS) and probiotics on plasma and egg yolk cholesterol and plasma fatty acid profile in laying hens. A total of 576 Single Comb White Leghorn hens (SCWL) at 26 weeks of age were fed diets containing 15% FS and a corn -soybean meal diet (C) supplemented with and without ferlac 25 (F25) or *Lactobacillus acidophilus* (LA). Feed consumption was significantly ($p<0.05$) increased by FS supplemented with both F25 and LA. Dietary treatments had no significant ($p>0.05$) effect on feed conversion. Egg production was significantly ($p<0.05$) higher among the FS probiotic supplemented diets and significantly ($p<0.05$) lower for dietary FS supplemented with LA . However, egg yolk weight did not respond to dietary treatments ($P>0.05$). Body weight was significantly low for all FS fed groups. Shell thickness was not statistically significant ($p>0.05$) among experimental treatments, while haugh units were significantly ($p<0.05$) higher for FS group supplemented with LA. Probiotic supplementation significantly ($p<0.05$) reduced plasma cholesterol (CHL) from 161mg/dL in C diet to 117mg/dL in probiotic supplemented groups. Plasma triglycerides (TG) were significantly ($p<0.05$) reduced from 2.8g/dL in FS to 1.9g/dL in probiotic supplemented groups while egg yolk CHL was significantly ($p<0.05$) reduced by probiotic supplementation on FS treatments from 14.04mg/g in FS fed group to 10.16 mg/g in

LA supplemented FS group. Fatty acids profile in the egg yolk responded significantly ($p<0.05$) to dietary treatments. FS treatments with and without probiotic supplementation significantly reduced C18:1 ω-9 by 12%; while the PUFA families (ω-3 and ω-6) were significantly ($p<0.05$) increased in FS fed group by 93% and 18%, respectively. C20:4 , ω -6 was significantly reduced from 6.6mg/g in the C group to 3.96mg/g in Fs groups. The long chain products of C18:3, ω -3 (C20:5,ω-3 EPA and C22:6, ω-3 DHA) were significantly ($p<0.05$) increased from 0.0 mg/g in C to 0.24 mg/g in FS groups, and C22:6 ω-3 was increased from 2.49 mg/g in C group to 6mg /g in FS fed diets.

Maîtrise en Sciences **Zootechnie**

RÉSUMÉ

Lieketseng Gladys Pheko

Effets de l 'alimentation de graines de lin et de la supplémentation probiotique pour pondeuses sur le taux de cholestérol de l'oeuf et sa composition en acides gras saturés

Une étude a été menée afin de vérifier les effects de l'alimentation de graines de lin (GL) et de probiotiques sur le taux de cholestérol plasmique et du jaune d'oeuf, ainsi que les taux d'acides gras chez les poules en ponte. Un total de 576 Leghorn blanches à simple crête (SCWL) âgées de 26 semaines ont reçu une ration contentant 15% GL et une moulée à base de mais et soya (MS) supplémentée ou non avec du ferlac 25 (F25) et du *Lactobacillus acidophilus* (LA), en comparaison avec une diète à base de MS. La GL additionnée de LA a significativement ($p<0.05$) réduit la production d'oeufs. La consommation volontaire a significativement ($p<0.05$) augmenté pour la GL additionnée de F25 et de LA. Les différentes rations n'ont eu aucun effet significatif ($p>0.05$)sur la conversion alimentaire. La production d'oeufs a été significativement ($p<0.05$) supérieure avec les diétes avec GL et probiotiques. Par contre le poids du jaune n'a pas été influencé par les différentes rations ($p>0.05$). La masse corporelle a été significativement ($p<0.05$) plus petite pour tous les groupes GL. L'épaisseur de la coquille n 'a pas été statistiquement plus élevée pour les groupes GL alors que les unités Haugh étaient significativement plus élevées pour les groupes GL supplémentés avec LA. L'addition de probiotique a significativement réduit le cholestérol plasmique de 161mg/dL de la diète C à

117mg /dL pour les groupes supplémentés avec des probiotiques. Les triglycérides plasmiques ont été significativement réduits de 2.8g/dL pour GL à 1.9g/dL pour les groupes supplémentés avec probiotiques, alors que le cholestérol du jaune a été significativement ($p<0.05$) réduit par l'addition de probiotiques pour les groupes GL, de 14.04mg/g pour les groupes nourris avec GL à 10.16mg/dL pour le groupe GL supplémenté avec LA. Le profil d'acides gras dans le jaune a été affecté significativement ($p<0.05$) par les rations. Les diétes GL avec ou sans probiotique ont significativement réduit C18:1 n-9 de 12%, alors que les familles d 'acides gras polyinsaturés (ω-3 et ω-6) ont été significativement ($p<0.05$) augmentées de 93% et 18% respectivement pour les groupes alimentés avec GL. C20:4 ω-6 a été significativement réduit de 6.6 dans le groupe MS à 3.96mg /g dans les groupes GL. Les produits à longues chaînes de C18:3 (C20:5 ω-3, EPA et C22:6 ω-3, DHA) ont été significativement ($p<0.05$) augmentés de 0.0 pour MS à 0.24mg /g dans les groupes GL, et C22:6 ω-3 a été augmenté de 2.49 pour MS à 6mg/g pour les diètes GL.

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Finally may the glory be to God the almighty for it is by his **love** and **will** that the mission is accomplished for " who ever wants to boast must boast of what the Lord has done" (Corin.1:1:31)

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SECTION 1

GENERAL INTRODUCTION

Animal producers , nutritionists, and geneticists are acutely aware of the challenges to the animal production industry. Often the response to a challenge can turn into an opportunity. In a highly competitive market those who are first to respond successfully gain an advantage, their growth in sales through marketing success creating the potential to be better equipped to meet subsequent challenges (William, 1997). Poultry and poultry products have undergone a remarkable increase in consumption since 1960. Much credit can be given to the nature of the products , high in nutrition and low in calories with minimal fat. Over the last 15-20 years there has been an explosion of interest in cholesterol and its relation to coronary heart disease (CHD); as a result, per capita consumption of eggs has been negatively affected. Cholesterol has been found the major contributing factor of atherosclerotic plaque causing coronary heart diseases. Recommended daily cholesterol intake is 250-300mg or less. Therefore, to remain within these limits, consumption of eggs containing more than the recommended levels should be reduced or prohibited (Hargis and VanElswyk, 1988). Many of the CHD risk factors respond to dietary changes particularly to fat modifications in the diet. This responsiveness was demonstrated by a number of clinical and epidemiological studies including a classic study by Dyenberg and Bang (1975) who found that, despite their high intake of dietary fat, Eskimos rarely suffer from heart diseases. The researchers explained this phenomenon by the unique fatty acids composition of the Eskimo's diet, which is based primarily on fish marine mammals high in ω-3 fatty acids. A typical 60g hen's egg contains about 6g of fat, of which 1.2g is

polyunsaturated (PUFA), 2.7g is monounsaturated and only 2.1g is saturated fatty acids. Polyunsaturated fatty acids consist of two distinct families , ω-3 fatty acids and ω-6 fatty acids. High dietary fat levels with ω-6 fatty acids encourage platelet aggregation through production of pro- inflammatory eicosanoids, which can be reduced by diets containing high levels of ω-3 fatty acids (Harris,1989, Krumhout,1992). Unlike the Eskimo diet, the typical western diet currently has a 25:1 ratio ω-6 to ω-3 fatty acids while the recommended ideal ratio should be about 5:1. The recent high increase in heart diseases due to high ratios of ω-6 to ω -3 fatty acids and elevated fish prices have created opportunities for poultry producers to offer their products enriched with high levels of ω -3 fatty acids. Of the major constituents of the egg , only its lipid component is easily changed by dietary manipulation of the laying hen. As early as 1934, Cruikshank demonstrated that feeding a ration containing 28% linseed / flaxseed oil increased the levels of linoleic acid in the egg five fold over that observed on a low fat basal diet. Recent studies by Caston and Leeson (1990) and Hargis(1993) demonstrated that flaxseed is rich in linolenic acid (C18:3 ω -3) which is the parent compound of the long chain metabolites such as: eicosapentaenoic acid (EPA), docasapentaenoic (DPA) and docosahexaenoic acid (DHA). It has been found that concentrations of ω-3 FA increase with an increase in dietary flaxseed. The best results on the increased concentration of ω -3 and n-6 fatty acids are achieved when flaxseed is incorporated in the diet at the range of 10% -20%, (Caston and Leeson1990, Hargis,1993). Recent studies showed stronger opposition to the use of antibiotics as therapeutic agents because of the intestinal upset which follows oral treatments and the public health concern over possible antibiotic residual effects. The possibility of ceasing the use of antibiotics as growth stimulants for farm animals and the concerns about their side effects as therapeutic agents have created a

climate in which both consumers and manufacturers are looking for alternatives. Hence probiotics are being considered to fill this preferential role, to antibiotics (Fuller, 1989). Probiotics supplementation is increasingly being used in poultry diets to enhance growth rate, improve feed utilization, control intestinal infection and finally to enhance a decrease in cholesterol concentration in the plasma and egg yolk. Species of *Lactobacillus* and *Streptococci* are the commonly used groups as probiotics. However, *Lactobacillus* strains have been proposed as likely dietary adjuncts, and *Lactobacillus acidophilus* is widely regarded as the most suitable candidate organism (Harvenaar at al.1992). Probiotics can be given to animals in various forms, the type of preparation depending on the specific use intended. They can either be included in pelleted feed or prepared capsules, paste, powder or granules which can be used for dosing the animals directly through their feed. Studies have shown that *Lactobacillus* supplementation at 100- 150mg/kg of layer feed significantly improved the performance of chickens and reduced plasma and egg yolk cholesterol. The enhancement of poultry meat and eggs with 20 carbon ω - 3 fatty acids as potential sources of the proposed healthful fatty acids, together with probiotic supplementation will be an alternative to consumption of relatively costly fish and fish products. The objectives of this study were:

a - To evaluate the effect of probiotics and flaxseed supplementation on the performance of the birds.

b - To study the influence of probiotics supplementation and feeding flaxseed on plasma and egg cholesterol.

c - To study the effects of dietary flaxseed and probiotics supplementation on fatty acid composition of the eggs.

SECTION II

LITERATURE REVIEW

2.1: EGG CHARACTERISTICS

Consumption of eggs by human has a long history, but the development of industrial egg production took place in the mid- twentieth century. The egg production industry developed first, and became most sophisticated, in Western Europe and North America. Its development was accelerated by scientific developments in areas such as genetics, nutrition, health and marketing. In industrialized economies, eggs have become more and more integrated into the total food production and distribution system; hence there has been a corresponding increase in the use of egg products and components as part of prepared foods, food service supply and a variety of other outlets. It is perhaps interesting to note that , among all animal agriculture, the egg is subject to the least restriction as food in terms of culture, religion or other restraints in its use.

2:1:1. Egg composition

The egg has extensively been researched because it has great potential for changes in its composition through dietary changes. A normal egg composition is as follows (Table 1,Romanoff and Romanoff, 1949)

Table 1: Major egg components (Romanoff and Romanoff 1949)

	Yolk	Albumen	Shell
Protein %	16.6	10.6	3.3
Carbohydrates %	1.0	0.9	0.0
Fat %	32.6	trace	trace
Minerals %	1.1	0.6	95.1

From: Romanoff and Romanoff (1949)

2:1:2. Biological value of eggs

Eggs are one of the few foods that not only taste good but are also of highest nutritional quality. Chicken eggs are the most complete and nourishing of natural foods available. This fact should not be surprising since eggs completely support the early growth and development of life in the embryonic chick. Since eggs contain all essential amino acids, all necessary vitamins except vitamin C, and many useful minerals required by humans, they are efficiently metabolized into human tissue. Therefore eggs have a relatively high "biological value" which is the percent of the food retained in the body and metabolized into tissues. Chicken eggs with a biological value of 96% are second to human milk at 98%.

2: 2. EGG QUALITY

The ultimate objective in marketing eggs for human consumption is to supply the consumer with a product that has retained its highest original quality. Egg quality includes the visible physical characteristics as well as flavour and odour. Providing the consumer with higher quality eggs not only increases the demand for eggs, but helps the producer to obtain the highest market grade and price (Moreng and Avens, 1985). Egg quality is determined by exterior and interior factors, however, nutritional value ,flavour, odour, yolk colour, taste, and appearance are quality factors that are not easily determined scientifically. Quality for individual shell eggs is determined on the basis of interior quality factors such as the condition of the white and yolk and size of the air cell, and exterior factors such as cleanliness and soundness. Five factors that influence the quality of eggs prior to laying have been well studied and are presented below.

2:2:1. Genetics

Individual birds exhibit differences in egg shell such as colour, size, shape and texture and in albumen and yolk quality depending on their genetic makeup. For example, there are some differences in the thickness of shells from different breeds, white shells are usually somewhat thinner. Broiler breeders tend to produce much larger eggs than layer breeders.

2:2:2 Nutrition

Rations fed to layers influence the quality and to some extent the nutritional value of the eggs. Minerals such as calcium and phosphorus, and vitamin D influence shell quality; yellow pigmented feed ingredients such as corn influence yolk colour; and many feed ingredients influence the flavour of eggs. Incorporation of flaxseed in layer diets was reported to bring in

eggs a fishy or fish-product related flavour such as "cod liver oil" or tuna flavour (Jiang et al. 1991). Increased protein intake by layers results in larger eggs, but this effect does not occur if the diet is deficient or low in energy, because protein would then be utilized for energy rather than synthesis of egg material. Vitamin A and K deficiency in layer diets results in increased incidence of blood spots in the eggs.

2:2:3 Disease

Disease such as Newcastle disease, infectious bronchitis and infectious hepatitis can have devastating effects on egg production, by causing misshapen eggs or by causing physiological disturbances that result in yolk moulting, thin albumen and decreased egg size.

2:2:4 Age

The quality of albumen declines throughout the normal egg production cycle. Shell quality declines a few months after the onset of egg production and in subsequent production years.

2:2:5 Environment

High temperatures in the laying house decrease albumin quality, reduce shell strength and decrease shell thickness. This may be due in part to decreased feed consumption in hot weather. Egg size decreases at temperature above 29^{0}C. Eggs are highly perishable unless properly handled and stored. Thus from the time the egg is laid through processing and marketing, the major objective is to preserve as much of the original quality as possible until it reaches the consumer. There is no known method for making an egg better in quality once it is laid, hence it is important to create all the conditions to preserve as much as possible of this degree of

quality. The following management practices contribute to keep the highest egg qualities from the point of production to market.

2:2:5:1 Temperature

Eggs should be cooled immediately after being laid. Temperatures above 29^{0}C cause a rapid deterioration in quality. High temperature permits the mucin fibres in the albumen to break down and liquefy and hence it flattens out upon breakage of the shell for cooking. A beneficial gas, CO_2 is lost from the egg while quick cooling drives the heat out quickly and retards loss of CO_2 (North and Bell, 1990). Eggs should be kept at 12 -14° C at the farm, and at 4° C at grading stations and stores.

2:2:5:2 Moisture

Eggs should be kept in relative humidity of 75% or higher. Low humidity in egg holding rooms causes moisture loss resulting in enlarged air cell (North and Bell, 1990).

2:2:5:3 Absorption of odours and flavours

Eggs readily absorb odours, which may or may not be lost during cooking. Care should be taken to keep eggs away from filth, decaying vegetables, disinfectants, or any other substances possessing a disagreeable odour (North and Bell 1990)

2:3 EGG CHOLESTEROL

Eggs provide humans the nutrient cholesterol; herein lies a well recognized and continuing controversy. Cholesterol is a nutrient that is utilized in, necessary for, and even synthesized by the human body. Egg yolk cholesterol is synthesized in the liver of laying hens, and transported to the developing follicles via plasma very low density lipoproteins (VLDL) where it is deposited by receptor mediated endocytosis (Nimpf and Schneider, 1991). Eggs are therefore high in cholesterol because of its importance in sustaining the developing embryo. Indeed, cholesterol is a structural component of cell membranes, and a precursor for adrenal and sex hormones, vitamin D and bile salts (Leeson and Summers, 1997). Since young chicks do not have the enzymes necessary for cholesterol synthesis, they rely entirely on the cholesterol deposited in the egg. An egg contains considerable amounts of cholesterol: a 60g egg contains 198-208mg cholesterol which is 35% less than egg cholesterol values reported in the older literature (250-300mg) but still high in relation to most foods (Leeson and Summers, 1997).

2:3:1 Factors affecting egg yolk cholesterol

Attempts to reduce the cholesterol content of egg yolk have been many and varied. While some modest changes may result from nutritional manipulation, the fact remains that most commercially produced eggs contain approximately 200mg of cholesterol. The synthesis of cholesterol is a highly dynamic process subject to many controlling factors. Several variables

were shown to affect the rate controlling enzymes of the cholesterol biosynthesis pathway, including nutritional status, levels of dietary fat and cholesterol, genetic component and age and production stage of the bird (Hargis and VanElswyk,1988).

2:3:1:1 Body weight and hen's energy and fat intake

Dietary fat does not seem to be a factor although in most instances high fat diets imply high energy diets. The ability of the hen to absorb dietary cholesterol is highly dependent upon the nature of dietary oil. This is supported by many studies in the literature including a study by Sim and Bragg (1977) who found a significant reduction in cholesterol levels in both serum and egg yolk (ranging from 16-33% depending on the dietary lipid) when 2% soysterols were added to diets containing saturated or unsaturated oil with or without cholesterol. The influence of dietary energy and body weight of the hen on egg cholesterol is mediated through the size of the egg. Thus reducing energy intake in order to achieve measurable reduction in cholesterol concentration has the disadvantage of adversely affecting egg production profitability.

2:3:1:2 Age and hen's production stage

In general yolk cholesterol concentration has been reported to decrease during the first year of production (Hurnik et al.,1977, Ambrosen and Rotenberg, 1981, Ingr et.al 1987). This is because cholesterol comprises 23% of the lipid in the plasma VLDL in immature birds, but 7.4 % in mature hens (Grifin et al., 1982).; the change in lipoprotein metabolism occurs only 2 to 3 days immediately before the onset of lay (Grifin and Hermier,1988). Plasma lipoprotein with the relatively high cholesterol concentrations characteristics of immature birds may

contribute to the yolk rapidly developing in the ovary just before the onset of egg production, and this explains the high yolk cholesterol concentration of early eggs. The proportion of lipoprotein characteristic of immature and mature birds depends upon the position of the developing yolk in the follicular hierarchy, with the first yolk to be ovulated and laid containing a higher proportion of lipoprotein characteristic of immature bird than the second and so on. Hence this will result in decreased yolk cholesterol concentration in consecutive eggs until all those yolk undergoing rapid growth around the onset of sexual maturity have been ovulated and laid; then subsequent yolk cholesterol concentration is relatively stable (Hall et al.,1992).

2:3:1:3 Genetic component

Egg cholesterol levels vary with species, breeds, or strain of bird (Cunningham, 1977; Sheridan et al., 1982 ; and Simmons and Somes, 1985). The heritability estimates for yolk cholesterol, ranging from 0.21 to 0.26 (Ansah et al., 1985), indicate the potential to change egg cholesterol levels by genetic selection. These estimates also indicate that the amount of genetic progress may vary between populations of birds. They also reported that selection for lower yolk cholesterol in a White Leghorn population was successful in decreasing egg cholesterol levels by 5.4% (or 9-10 mg/egg) in the third generation.

2:3:2 Egg consumption and public health

Poultry products consumption has increased since 1960. However, egg consumption has significantly decreased during this period due to concerns over the consumption of animal fat and incidence of heart disease in humans. Consumers show a continued interest in reducing their dietary fat and cholesterol (Scott et.al.1993). In recent years, per capita cholesterol intake

in the U.S has declined to an average intake of 220 to 260mg /day by women and 360mg /day by men following the repeated recommendations by medical professionals to lower cholesterol intake, hence decreasing per capita egg consumption (United States Department of Agriculture, 1994). The Canadian Consensus Conference on Cholesterol (1988) recommended that the agriculture and food industry be encouraged to maintain and increase its efforts to produce foods that will achieve lower levels of blood cholesterol in the Canadian population. Restaurants, fast food outlets, industrial and school cafeterias and other caterers should be encouraged to offer meals that are low in fat and cholesterol. Consumers should also have better and more explicit information about the nutrient content of foods to help them select a healthy diet. As a result of consumers demand for food products of superior health quality, there is a renewed interest in modifying the lipid composition of poultry meat and eggs (Hargis and VanElswyk, 1993).

2:4 FLAXSEED

Flaxseed is one of the world's oldest crop which has been grown for many thousand years to produce linen, linseed oil, and more recently, as grain for bread and laxative. Canada is the world leading producer and exporter of flaxseed. The three main provinces producing flaxseed are Manitoba, Saskatchewan and Alberta (Canada Grain Council Statistics 1996). More studies on the general effects of flaxseed have been done mainly in poultry.

2:4:1 General performance of laying hens

Incorporation of flaxseed in layer rations has not shown major detrimental effect on the general performance of the birds. Caston et al. (1994) reported no statistical difference in performance between birds that were on a control and flaxseed diets. Scheideler and Froning (1996) observed a marginal decrease in feed consumption, weight gain, and egg weights by feeding flaxseed at 5%, 10% and 15%. Their results however, were not consistent with the results reported by Caston et al. (1994) where egg production, egg shell formation and yolk weight were not affected ($p > 0.05$) by level of ground flaxseed in the diet ($p > 0.05$). Scheideler and Froning, (1996) also reported a concomitant decrease in metabolizable energy ($p < 0.01$) as ground flaxseed was increased in the diet. They also observed that birds fed 10% and 20% flaxseed were significantly lighter than control fed birds at 51wk of age ($p < 0.01$), and by 72 wk of age, that the birds fed 20% flaxseed were significantly lighter than those fed 10% dietary flaxseed ($p < 0.01$). Scheideler and Froning, (1996) reported that up to 15% flaxseed can be safely added to layer diets without any detrimental production effects, if the rations are properly balanced for energy and protein.

2:4:2 Flaxseed and egg quality

Many attempts such as use of drugs and genetic selection were made to reduce egg cholesterol content with only little practical success(Naber 1993, and Noble 1987). However, dietary fatty acids modification has proven to be a viable method of adding value to poultry products for the health conscious consumer (Hargis and VanElswyk, 1993). Numerous recent

publications describe products high in ω-3 fatty acids that can be incorporated into laying hen rations and their subsequent effects on total egg yolk lipid composition, the cholesterol concentration of the egg yolk and the sensory qualities of the egg (Hargis and VanElswyk 1988, Caston and Leeson 1990, and Jiang et al.1992).

2:4:3 Flaxseed and egg cholesterol

The cholesterol lowering and ameliorative effects against atherosclerosis of dietary ω -3 fatty acids are of particular interest to egg producers, consumers and researchers. These much needed ω -3 fatty acids could be readily incorporated into the yolk lipids by feeding laying hens diets containing flaxseed, which is rich in these fatty acids. As a result, flaxseed was tested in a variety of experiments ranging from its effects on blood cholesterol, blood glucose levels to cancer risk. Omega- 3 fatty acids were reported to lower blood cholesterol (Herold and Kinsella 1986). They mainly reduce plasma triglycerides (TG) and cholesterol levels by reducing levels of VLDL and chylomicrons (Cunnor 1986), and have only a minor and variable effect on low density lipoprotein (LDL) (Cunnor and Cunnor, 1990). High density lipoprotein (HDL) increases as TG levels fall (Manniven et al.1992) ; but LDL levels may actually increase with the consumption of large amounts of the ω -3 fatty acids especially in hypertriglyceridemic subjects (Sullivan et al.1988). These fatty acids may influence atherogenesis by mechanisms other than their effects on lipoprotein, most likely by altering thrombosis or reducing the immune response (Leaf and Weber 1988).

2:4:4 Flaxseed and Off flavours

In order to realize their health benefits, customized poultry products must meet consumer expectations of sensory qualities if their consumption is to be sustained. The use of either menhaden oil or flaxseed in layer diets for production of eggs rich in ω -3 fatty acids may have drawbacks; highly unsaturated fatty acids are subject to oxidation and potential production of off - flavours. Because of the association of off-flavours of poultry meat products with fish oils, several studies examined the use of plant sources of ω -3 fatty acids on the fatty acid profile of eggs and meat. Jiang et al. (1991) reported that 33% of the panelists were able to detect a fishy or fish product related flavour such as" cod liver oil" or tuna flavour in eggs from hens fed flaxseed. Contrary to these results, Farrell and Gibson (1991) reported that eggs produced by hens fed diets containing fish oil , canola oil or flax oil were indistinguishable from those fed a control diet. The fishy flavour of egg or meat from flaxseed - fed chickens reported in many studies is due to the high level of ω - 3 fatty acids in flaxseed which is also found in fish. "Fishy" odour is due not only to one or two volatile compounds, but rather to a range of these substances, the levels of which rise and decline during storage life as oxidative deterioration progresses. Fishy flavours appear to arise from both lipid and non- lipid substances as well as their combination. It is well established that fatty acids have an increasing tendency to auto oxidation with increasing unsaturation (Dahle et al.1962, and Rhee et al. 1988). Thus the highly polyunsaturated fatty acids in fish oil are very susceptible to oxidative deterioration. Furthermore, the long chain ω -3 fatty acids are associated more with the phospholipid than the triacylglycerol fraction, making flaxseed a rich source of these fatty acids as well as their oxidative products.

2:4:5 Polyunsaturated fatty acids (PUFA)

Polyunsaturated fatty acids consists of two distinct families, the ω -6 and ω -3 fatty acids which are found in vegetable oils. A dietary balance between the ω -6 and ω -3 fatty acids is important to maintain human health (Farrell 1996). A typical western diet has a ratio >25:1 while the ideal ratio is about 5:1. Fortunately altering fat saturation in the yolk is much easier than influencing cholesterol level. By feeding predominantly unsaturated fatty acids to the bird the fatty acid profile of the egg can be moved favourably towards increased unsaturated fat content. More recently there has been interest in the association between linolenic C18: 3 ω -3) acid intake and coronary heart disease (Leeson and Summers 1997). The chicken has the somewhat unique ability to divert increased quantities of linolenic acid into the egg when its diet contains high levels of this fatty acid. Unfortunately most layer diets do not contain high levels of this fatty acid; yet there is a feed ingredient high in this fatty acid, flaxseed (Leeson and Summers 1997). Preliminary studies done by Leeson and Summers (1997) showed that fatty acid profile of the egg changes with the level of flaxseed incorporated in the diet (Table 2).

Table 2 The effect of dietary flax on egg fatty acid composition%

Fatty acid	pretest	10% Flax	30% Flax
16:0 (Palmitic)	31.7	29.2	26.8
18:0 (Stearic)	8.3	8.3	8.3
18:1 (Oleic)ω-9	42.2	37.3	33.5
18:2 (Linoleic) 18:2ω-6	13.9	15.6	14.8
18:3 (Linolenic)18:3ω-3	0.3	6.2	13.3
20:4 (Arachidonic)	1.7	0.9	0.7
22:6 (Docosahexaenoic)22:6ω3	0.5	1.0	1.1

From: Leeson and Summers (1997)

Sim and Charian, (1991) who fed laying hens full fat flaxseed at 8% and 10% and canola at 16% compared to a control diet reported that the prominent ω- 3 fatty acid in the yolk was linolenic acid , with pronounced concentrations of EPA, DPA and DHA in the yolk of hens fed ground flax or canola seed. Flax and canola treatments had similar increases in linoleic acid compared to the control diets. Similar results have also been observed by Caston and Leeson (1990). In their study they fed flaxseed at 10% and compared it to a control diet. They reported that flaxseed - fed birds produced egg yolk with a ratio of ω -6 to ω -3 fatty acids of 3:1, while eggs from birds fed control diet exhibited ω -6 to ω -3 fatty acids ratio of 37:1. Experiments on feeding flaxseed were also done in other animal species. Ganguli et al., (1990) fed young

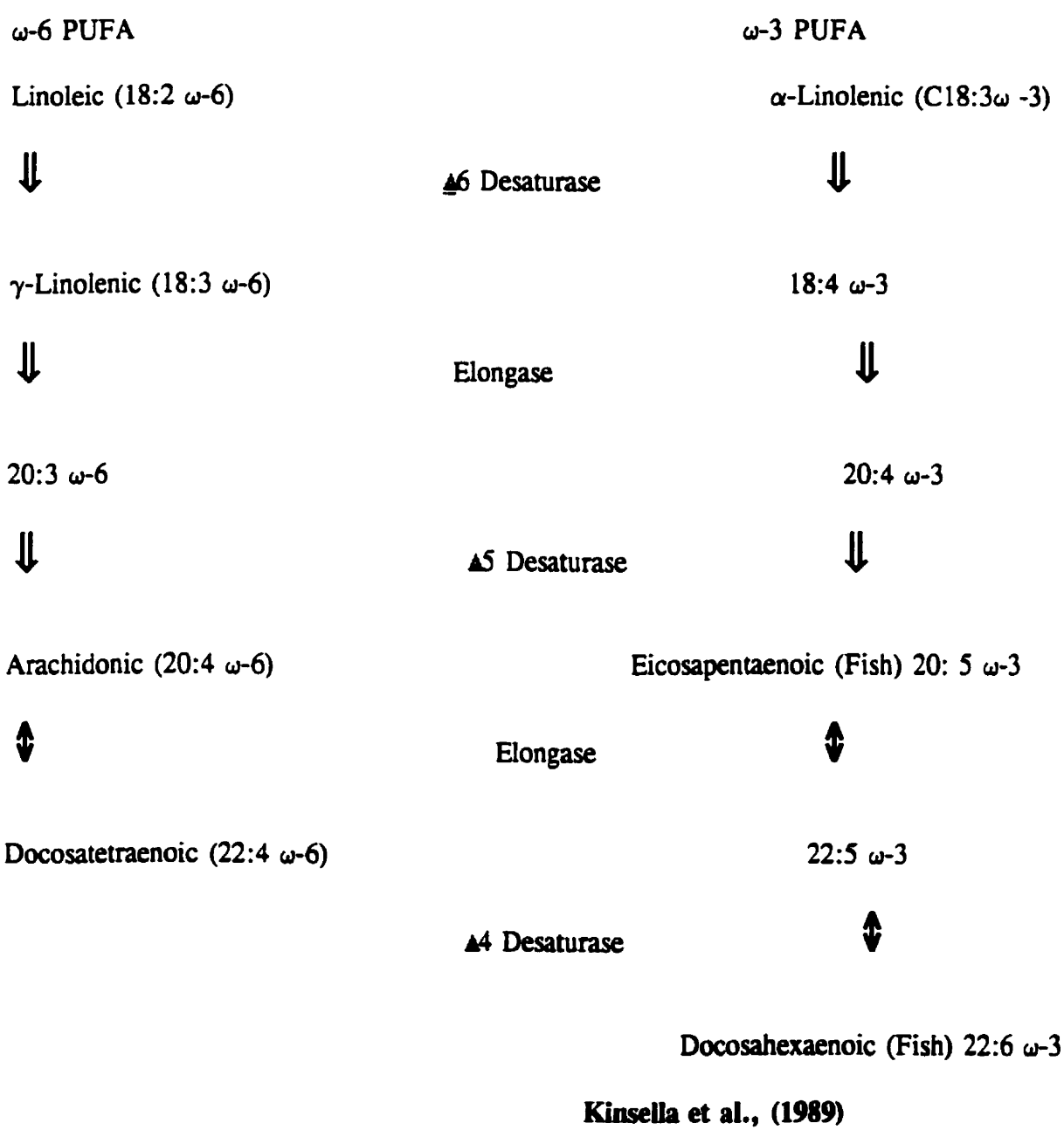
growing pigs a creep feed containing 5% flaxseed for eight weeks. They observed a significant increase in total ω -3 fatty acids in the liver, heart and kidneys,however, there was a significant decrease in total ω -6 fatty acids in the flax - fed pigs. Similar observations were reported by Olomu and Barcos, (1991). In their study, they fed flaxseed oil to broilers. There was an increase in ω -3 fatty acids in the skeletal muscle lipids. Increased amounts of desaturation and elongation products (C20:3, C20:5, C22:5 and C22:6) of ά linolenate (18:3ω-3) were observed in the sartorius muscle of chicks fed flaxseed oil. On the contrary, the amounts of ω -6 fatty acids (C20:3,and C20:4) were significantly depressed in the muscle lipids after 21 days of feeding flaxseed oil.

2:5 BIOCHEMISTRY OF PUFA

The essential fatty acids follow a number of metabolic pathways which include β-oxidation in the mitochondria to generate ATP (Schulz, 1991), desaturation and chain elongation leading to the long chain polyunsaturated fatty acids and incorporation into glycerolipids (Tijburg et al. 1989). Total dietary fat and type and amounts of essential fatty acids influence metabolic use. The liver desaturase and elongase enzymes are influenced to some extent by changes in diet and hormones. During the conversion of essential fatty acids to polyunsaturated fatty acids, the rates of elongation are generally faster than those for desaturation (Sprecher,1982). Dietary polyunsaturated fatty acids are derived from two distinct structural families, the ω-6 linoleic family which is abundant in seed oils (Fig.1) and the ω -3 linolenic family which occurs in abundance in the lipids of green vegetable leaves where it accounts for >50% of the fatty acids; modest amounts of the ω -3 fatty acids family occur in soybean meal oil (7%) and canola

oil (10%) (Kinsella, 1989). Linolenic acid is the preferred substrate for the elongated products EPA an DHA which occur in sea foods and fish oils (Hwang et al. 1988). Dietary linolenic may be slowly converted to EPA and DHA in humans, the extent depending on the linoleic acid intake (Adam et al. 1986).

Fig. 1 Major two families of PUFA.



Kinsella et al., (1989)

Dietary linoleic acid is metabolized , mostly in the liver, via the rate limiting Δ6 desaturase enzyme, to γ-linolenic (18:3 ω-6), which is converted to arachidonic acid(AA) (20:4 ω-6) by desaturase and elongation (Holman, 1986). Regulation of Δ6 and Δ5 desaturase by dietary and hormonal controls was described in mammals (Poisson and Cunnane, 1991). Several effectors of Δ6 desaturase activity influence the Δ5 desaturase as well. The concentration of arachidonic acid in tissue pools is influenced by the amount of dietary linoleic acid and the activity of Δ6 desaturase enzyme. Generally, increasing dietary linoleic acid intake up to 12% tends to increase AA concentration in tissues (Mathias et al. 1985) whereas intakes > 12% cause little further increase or result in some reduction in tissue arachidonic concentration because of inhibition of the Δ6 desaturase (Lands, 1989). Experiments in rats on the Δ6 desaturase showed that decreased temperature, a fed state, and essential fatty acids deficiency activate the enzyme, but fasting, low dietary protein, linoleic acid and arachidonic acid inhibit the activity of the enzyme. Insulin stimulates Δ6 desaturase but increases in temperature, glucagon, epinephrine, glucocorticoids and adrenocorticotropin depress its activity (Mandon et al. 1987). The literature describing the enzymatic control of desaturation and elongation of essential fatty acids in mammalian models greatly exceeds the understanding of essential fatty acids metabolism in poultry. In poultry, it is presumed that Δ6 desaturase is the rate limiting enzyme for polyunsaturated fatty acids formation as it is in mammals, but it is not clear to what extent essential fatty acids are converted to polyunsaturated fatty acids in tissues other than the liver (Bruce, 1995). It is unclear if the enzymes of polyunsaturated fatty acids formation follow a diurnal rhythm, and if age or genetic heritage influence enzyme activity in poultry (Berdanier and Boltzell 1986, Blond et al.1989).

2:6 HEALTH BENEFITS ASSOCIATED WITH ω -3 PUFA

High fat intakes in humans is associated with increased incidence of hypercholesterolemia. As medical research continues to unravel the relationship between linolenic acid and coronary heart disease and other high fat related diseases, it is likely that this fatty acid will attract more attention in the near future especially from those consumers at risk from diseases, as well as from the growing number of people concerned about their nutritional habits (Leeson and Summers, 1997). The Canadian government through Health and Welfare Canada (1990) adopted the recommendation that ω -3 PUFA are essential nutrients and that a dietary supply of at least 0.5% of energy in the diet should be supplied as linolenic acid so that a ratio of ω -6 to ω -3 PUFA of 6:1 should be achieved.

2:6:1 Cardiovascular disease

Saturated fat and cholesterol intake are positively correlated with plasma cholesterol and risk of cardiovascular disease (CVD) (Canadian Concensus Conference on Cholesterol 1988). Interest in increasing fish consumption as a possible mechanism of preventing CVD and other CVD related diseases developed from the epidemiological studies conducted in Greenland Eskimos by Dyerberg and Bang (1975) which led to the hypothesis that marine oils rich in n-3 fatty acids have antiatherogenic properties. This hypothesis was derived from the observation that CVD was uncommon among Eskimos despite their distinctively high intake of animal fat (Connor et al.1992). On the other hand the Danes who also ate a high animal fat based diet had much higher incidence of CVD. The dietary fat of the Eskimo was different, comprised mainly of sea animals, and contained substantial amounts of ω -3 fatty acids

(EPA and DHA). Conversely, the Danes dietary fat was mainly saturated from land animals. Eskimos also exhibited lower plasma lipids, both cholesterol and TG. From this study, it was concluded that amongst the unsaturated fatty acids consumed by humans, two should receive a particular attention with respect to their ameliorative effects on CVD and other related diseases. These two fatty acids are EPA and DHA commonly found in fish oils and vegetable oils. Polyunsaturated fatty acids can lower the risk of CVD directly or indirectly. Patients with high blood cholesterol levels are at risk for CVD hence, most studies looked at lowering the risk of CVD either through cholesterol lowering mechanisms or directly. Early clinical trials reported 12-18% decrease in cholesterol levels of selected sample of adolescents and adults with average blood cholesterol levels following dietary adjustments such as lowered saturated fat (about 10% of total energy) increased polyunsaturated fatty acids (about 20% of total energy) (Turpeinen et al. 1979). Singer et al (1985) reported that feeding mackerel and herring diets to patients with mild essential hypertension decreased total TG and cholesterol levels after 2 weeks by some 24% and 0.07% respectively, while HDL levels rose by 10%, with a concomitant decrease in systolic blood pressure. Other human studies with dietary supplementation of fish oil showed a marked decrease in the ratio TG to LDL levels (Mortensen et al. 1983) and increase in HDL levels and a decreased platelet aggregation, resulting in decreased risk of thrombosis (Herold and Kinsella, 1986). The daily consumption of 0.3-1.0g long chain ω -3 polyunsaturated fatty acids appears to prevent CVD (Harris, 1989, Duthie and Barlow 1992). For humans in a clinical situation, it was stated that a higher level of 2- 5 g EPA and DHA may be needed to generate positive effects (Barlow et al., 1990, and Duthie and Barlow 1992).

2:6:2 Inflammatory disease

Besides their effects on CVD, ω -3 polyunsaturated fatty acids have been implicated in the alleviation of symptoms of a number of diseases including cancer. A major justification for decreasing dietary fat has been the anticipated reduction in breast, colon and prostate cancer (NRC, 1989). The primary support for the proposed link between dietary fat and cancer is that countries with low fat intake experience low rates of these cancers. The correlations with cancer were seen primarily with animal fat and meat consumption rather than with vegetable fat consumption (Prentice et al. 1990). The hypothesis that greater fat intake increases breast cancer risk is supported by many animal studies. However, much of the effect of dietary fat appears to be due to an increase in total energy intake, which profoundly increases mammary tumour incidence in animals (Welsch, 1992). In a study involving patients with mild rheumatoid arthritis, fish oil was able to replace a non steroidal anti- inflammatory drug treatment (Belch et al. 1988). High levels of ω -3 polyunsaturated fatty acids intake showed antipromotional effects on some types of cancer (e.g. breast and pancreatic cancers) in human and animal studies (Brit. Nutr. Found. 1992). The effects on cancer may be mediated for example by reduction in the formation of certain prostaglandins and thromboxanes. At high levels, fish oil supplementation in cancer patients prevented the loss of body condition which naturally occurs in cancer as well as proving to be more effective than a conventional drug in inhibiting tumour growth. These effects were shown to be mediated primarily by EPA, the most potent known inhibitor of platelet aggregation (Leeson and Summers, 1997).

2:6:3 Plasma lipids

The study of the effects of ω -3 fatty acids on plasma lipids was initiated from the findings of Dyerberg and Bang (1975) who observed that levels of cholesterol and LDL cholesterol were significantly lower and that levels of HDL were higher among Eskimos than Danes in all age groups of both sexes. Dietary polyunsaturated fatty acids depress serum VLDL lipoprotein and LDL lipids and cholesterol in the following manner, where different polyunsaturated fatty acids families show varying efficacies (Grundy, 1989). The polyunsaturated fatty acids of vegetable oils which contain mostly linoleic acid (ω -6 fatty acids) are effective in counteracting effects of dietary saturated fatty acids but the ω -3 polyunsaturated fatty acids of fish oils and flaxseed may be equally or more hypolipidemic (Bonanome and Grundy, 1988). Earlier studies indicated that even though they contain cholesterol, fish oils were effective in reducing plasma lipids (Avery-Nelson, 1972). More recent epidemiological studies showed low plasma lipids in populations consuming sea food and fish, indicating the effectiveness of dietary fish in reducing plasma lipids, especially TG and their association with a decreased incidence of atherosclerosis, thrombosis and coronary infarctions (Leaf and Weber 1988). Dietary ω -3 fatty acids can modify lipids and lipoprotein metabolism (Kris-Etherton et al. 1988) Most clinical studies with human subjects consistently showed decreased VLDL, TG, and in some decreased plasma cholesterol (Kinsella, 1989). Connor and Connor (1992) reported the preferential depression of serum TG and a slight decrease in the plasma cholesterol in normolipidemic subjects after consumption of salmon oil compared with vegetable oil. Sanders et al. (1989) reported that a daily intake of 20g fish oil plus EPA (5 g ω -3 / d) significantly reduced plasma TG in humans whereas cholesterol reduction was observed in those subjects with high initial plasma cholesterol. They

also observed an insignificant drop in LDL and a significant increase in HDL.

2:6:4 Early development

The essentiality of dietary ω -3 fatty acids in the development of brain tissue is well documented. (Anderson and Connor,1988). DHA and EPA are biosynthesized from α- linolenic acid in humans and animals and can also be obtained from dietary sources. Animals, however, lack the ability to synthesize linolenic acid de novo, and the ω -3 and ω -6 fatty acids are not interconvertible (Neuringer et al., 1988). DHA is the major ω -3 fatty acid in the brain. Diets deficient in ω -3 fatty acids resulted in inferior learning abilities in rats (Lamptey et al., 1976) and neurological disorders in humans (Holman et al. 1982). Hiramitsu et al. (1997) who fed mice palm oil, lard, perilla oil, sardine oil and salmon oil found that plasma DHA levels were higher in fish oil diet groups than in the perilla oil diet group. The plasma AA level of salmon oil diet was lower than that of the sardine oil group, while both fish diets showed a low level of linoleic acid. They also observed a significant increase in brain DHA levels and decreased levels of arachidonic acid and DPA in the mice fed perilla oil, sardine oil and salmon oil. DHA level was higher in the salmon oil diet group followed by the sardine oil group and the perilla group. Similar effects were observed by Sim and Charian (1992), who reported significant elevated concentrations of EPA, DPA and DHA in the brain tissues of newly hatched chicks from eggs enriched with high levels of flaxseed and canola, compared with the soybean meal control diet and a sunflower enriched diet. The effect of increased ω -3 fatty acids in the brain tissues was most pronounced with respect to DHA which is the major PUFA in chick brain.

2:7 PROBIOTICS

Intensive production systems inflict considerable stress on livestock, adversely affecting the health status of birds. To achieve a high level of economic efficiency, due mostly to capital costs, poultry are raised under intensive production systems in densely populated colonies or flocks (Jin 1997). However, intensive production systems reduce the production of the individual birds but not the flock productivity. As a result, chickens are stressed by various factors such as transportation to the growing site, overcrowding, vaccination, chilling and or overheating; these tend to create an imbalance in the intestinal microflora and lowering of body defense mechanisms. Under such circumstances, antimicrobial feed additives such as antibiotics and synthetic antimicrobial agents are often used to suppress or eliminate harmful organisms (Jin, 1997). Over the years there has been a reaction against the use of antibiotics as therapeutic agents because of the intestinal upset that follows oral administration of these agents , and the public health concern over possible antibiotic residual effects. Hence, probiotics are being considered to fill this role and already some farmers are using them in preference to antibiotics (Fuller, 1989).

2:7:1 Definition

Over the years the word probiotic was used in several different ways. It was originally used to describe substances produced by one protozoa which stimulated another (Lilly and Stillwell, 1965). Later on, Parker (1974) described probiotic as an animal feed supplement which had a beneficial effect on the host animal affecting its gut flora. Fuller (1989) considered the definition by Parker to be broad because it included not only cultures, cells and metabolites but also antibiotic preparations. He therefore redefined probiotic as a live microbial feed supplement

which beneficially affects the host animal by improving its microbial balance. Havenaar et al (1992) broadened Fuller 's definition of probiotic as a mono or mixed culture of living micro-organisms which (when applied to animal or man) beneficially affects the host by improving the properties of the intestinal microflora.

2:7:2 Mode of action

The beneficial effects of probiotics may be mediated in three ways: suppression of viable count, alteration of microbial metabolism,and stimulating immunity (Fuller,1989).

2:7:2:1 Suppression of viable count

Continuous probiotic feeding to animals was found to maintain the beneficial microflora in two ways: by competitive exclusion and antagonistic activity towards pathogenic bacteria(Jin,1997).

Competitive exclusion

Lactic acid bacteria are known to be associated with the gut wall of chickens (Fuller and Turvey 1971) and pigs (Barrow et al 1980). Competitive exclusion in chicken can be produced with material which remains attached to the caecal wall after washing four times in buffered saline (Stavric et al. 1987). Nurmi et al., (1973) demonstrated this property in their study by oral inoculation of 1-2 day old chicks with 1 : 10 dilution of normal intestinal contents from healthy adult birds one day prior to oral challenge with *Salmonella* sp resulting in 77% of birds being free of infection compared to 100% infection rate in control or untreated birds. However, results under field conditions have been more variable (Stavric and D'Aoust 1993). Defined

cultures are less effective than undefined cultures under laboratory conditions (Stavric and D'Aoust. 1993). The potency of defined cultures decreases gradually during cold storage and during repeated laboratory manipulations of the bacterial isolates(Gleeson et al. 1989; Stavric et al.1991).

Antagonistic activity

In vitro studies demonstrated that lactic acid bacteria can inhibit the growth of poultry pathogens (Chateau et al. 1993). This was supported by Oyarzabal and Conner (1995) where three commercial strains (*Lactobacillus acidophilus, Lactobacillus casei and Streptococcus faecium)* inhibited the growth of six *Salmonella* serotypes. Jin et al., (1996) also reported that all *Lactobacillus* isolates studied had the ability to inhibit the growth of five *Salmonella* serotypes and three serotypes of *E. Coli.*

2:7:2:2 Alteration of microbial metabolism

Gut microflora is beneficial to the nutrition of the host because it increases the digestion of nutrients, especially in the lower intestines (March, 1979). Sisson et al (1989) and Jin et al. (1996) reported that twelve *Lactobacillus spp.* isolated from chickens were found to secrete amylase, proteases and lipases, either extracellularly, intracellularly or both. They also observed that amylase activity in the small intestine increased when *Lactobacillus* cultures were fed to broilers but there was no effect on lipolytic and proteolytic activities. These results are inconsistent with those by Collington et al. (1990) who observed that inclusion of *Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus casei and Streptococcus faecium* in a basal diet fed to pigs resulted in a significant high carbohydrate digestive activity in the intestinal mucosa.

2:7:2:3 Stimulating immunity

Immunity resulting from gut exposure to a variety of antigens, such as pathogenic bacteria and dietary protein, is important in the defense mechanism of young animals against enteric infection (Perdigon et al. 1995). *Lactobacillus* could be important in the development of immune competence in young animals, particularly when protection must be acquired against antigens likely to cause gut inflammatory reactions (Perdigon et al., 1990). Kato et al., (1981) reported that *Lactobacillus* can also be used to prevent the growth of tumours; their importance in cancer prevention has been suggested but to date there are no clinical studies to support this hypothesis. Similarly, Dunham et al (1993) observed that birds treated with *Lactobacillus reuteri* exhibited longer ileal villi and deeper crypts, which is a response associated with enhanced T cell function and increased production of anti- *Salmonella* antibodies (IgM). The different findings on the systemic effect on immunity do indicate that probiotics have the potential not only to affect the balance of the gut flora, but to influence the pathogenesis of disease which occurs in tissues remote from the intestinal tract (Fuller, 1989).

2:8 PROBIOTIC EFFECTS ON PERFORMANCE

There are many types of probiotic preparations in the market today. Many studies were conducted to test their efficacy on animal growth and performance, both in broilers and layers.

2:8:1 Broilers

Supplementation of broiler rations with mono cultures or mixtures of *Lactobacillus* and other

bacteria gave variable results. Kim et al., (1988) observed increased weight gain of chickens fed a diet containing 10% mouldy maize supplemented with *Lactobacillus sporegenes* at 2 and 6 weeks of age. Similar effects of probiotics were observed by Mohan et al., (1995) in their experiment where they fed broilers diets supplemented with either probiotics or antibiotics. They observed increased weight gain of 8.4% for the antibiotic supplemented group and 9% gain for the probiotic - fed group while the control group showed 7.3% weight gain. Inconsistent with these results , Yeo and Kim, (1997) reported that feeding a diet containing probiotics (*Lactobacillus casei*) significantly increased average weight gain during the first 3 weeks of age ($p < 0.05$) but did not show further influence in weight gain during the weeks 4 to 6 of growth. They explained the increase in weight during the first 3 weeks to be partly due to increased feed intake.

2:8:2 Layers

A number of bacterial preparations were also tested in laying birds. Their effects on the general performance of birds are more consistent than those reported in broilers. Tortuero and Fernandez, (1995) reported that supplementation with mixed cultures of *Lactobacillus acidophilus* and *Lactobacillus casei* improved hen-day egg production, feed conversion ratio (kg feed/doz eggs or /kg eggs), egg weight and albumin quality. These results however, were not consistent with the results obtained by Abdulrahim (1996) in which incorporation of *Lactobacillus acidophilus* significantly improved egg production and feed conversion but did not affect egg weight, feed consumption (g/hen/day) and shell thickness. Besides their improvement in the general performance of the birds, probiotics were also used successfully in lowering plasma cholesterol of broilers and layers. However, studies on lowering the lipids and TG in the

egg yolk were not very successful. This is supported by the study done by Mohan et al (1995) where serum cholesterol levels in groups supplemented with a probiotic mixture ranged from 120.5 mg/ dL to 131.4 mg/dL while the control group had 164.7 mg/dL. In their study they were also successful in lowering yolk cholesterol from 14.69 mg/g yolk in control to 11.37 mg/g yolk in the probiotic supplemented groups. They established that cultures needed to exceed a concentration of 1x 10^6cfu/g (colon forming units) of diet in order to be effective.

SECTION III

MATERIALS AND METHODS

3:1:1 Experimental animals and management

A total of 576 Single Comb White Leghorn Shaver pullets were purchased from a commercial breeder[1] at 19 weeks of age and allocated in an environmentally controlled double - deck cage battery house with four birds in each cage (45cm x 45cm). Birds were subjected to a 14 hour light regime per day and room temperature was maintained at 20^0 C. Feed and water were provided ad libitum upon arrival, all the birds received a commercially prepared corn soybean meal diet until 26 weeks of age.

3:1:2 Experimental diets and feeding procedure

Two isonitrogenous, isocaloric layer experimental diets were purchased from a branch of the Co-opérative fédérée de Québec[2]. The composition of the corn soybean meal diet (control) and the diet supplemented with 15% flaxseed is presented in Table 3:1. Two commercially prepared probiotics supplements were given, chr. Hansen *Lactobacillus acidophillus*[3] and Ferlac 25[4]. The latter probiotic supplement is a cocktail of 5 different bacteria: *Lactobacillus acidophilus* (4%), *Lactobacillus rhamnosus* (65%), *Streptococcus faecium* (25%), *Streptococcus thermophilus* (5.9%) and *Lactobacillus bulgaricus* (0.1%)

The two dry cultures were mixed weekly in distilled cold water and kept at 4^0 C. They were then hand mixed with feed daily at 1g/ 100 birds, to provide about 10^6cfu/g feed. The

supplemented feeds with and without probiotics were fed to layers starting at 26 weeks of age for a duration of 20 weeks. The experimental facilities had 2 rows of cages each side of the room. For this study the experimental design consisted of 6 pens, (with 3 pens in each block) each pen having 4 rows of cages (24 hens/row), each row being an experimental unit. The treatments were grouped as follows (Table 3.2): C, C + F-25, FS, FS + F-25 and randomly distributed among the rows in each of the first 4 pens (2 pens on each block). C + LA and FS + LA were randomly assigned to the rows in the last 2 pens (with 1 pen on each block) hence there were 4 replicates of each of the 6 treatments.

3:1:3 Feed sample analysis

A representative sample from each batch of experimental feed for five study periods and the flaxseed used in the 15% flaxseed diets were collected for proximate analysis (Table 3:1) Each sample was freeze - dried using a virtis freeze dryer[5]. Absolute dry matter was determined using a vacuum oven dryer[6]. Samples were finely ground for the determination of crude protein using a nitrogen analyzer[7]. ADF was determined by the method of Van Soest and fat was ether extracted. Calcium content was measured using flame atomic absorption spectrophotometry[8], while phosphorus was determined by alkalimeter ammonium molybdate method (AOAC,1984), the optical density read with a spectrophotometer [9] at 400nm.

From each representative feed sample, two duplicate aliquots were prepared for fatty acid profile analysis using gas - liquid chromatography[11]. Fatty acid profile of the feed is presented in Table 3.4

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[1]Shaver (Couvoir Boire et Frères, Inc. Wickham, QC. Canada)

[2]Coopérative fédérée de Québec,Montréal, QC, Canada

[3]Chr. Hansen's Laboratory, Inc. Milwaukee USA

[4]Institut Rosell Inc. Montreal QC, Canada

[5]Virtis Freeze Dryer # 278341, Gardinor, New York, USA

[6]National Appliance Company, Illinois, USA

[7]Leco FP -428, Leco Corporation, St- Joseph, MI , USA

[8]Number 1241, Parr Instrument Company, Moline, Illinois, USA

[9]Model 2380, Perkin Elmer, Norwalk, CT60521 USA

[10]Model Du -20, Beckman Fullerton, CA 92713 USA

[11]Hewlett Packard Series II 5890, Hewlett -Packard Company, USA

Table 3:1 Proximate analysis of experimental diets and flaxseeds based on DM

Diets	Period	DM	Fat	CP	ADF	Ash	Ca	P	G.E.Kcal
Control	1	90.11	3.35	19.56	2.68	10.34	3.75	0.79	3,104
15%FS	1	91.04	7.81	20.06	3.47	11.23	3.90	0.84	3,343
Control	2	90.46	4.89	20.44	2.66	12.35	4.20	0.84	3,390
15%FS	2	91.84	7.01	19.18	3.36	14.67	4.68	0.85	3,390
Control	3	89.93	7.36	18.66	2.87	9.42	2.79	0.69	3,406
15%FS	3	90.44	10.44	16.85	3.86	11.36	3.47	0.60	3,703
Control	4	90.31	7.42	19.84	2.58	14.33	4.50	0.93	3,391
15%FS	4	90.52	10.59	19.98	3.46	12.02	3.42	0.78	3,460
control	5	90.23	6.91	20.75	2.62	15.26	4.63	0.83	3,376
15%FS	5	90.12	11.71	19.90	3.72	12.72	3.70	0.81	3,687
Flax. In	5	94.29	40.05	24.50	7.71	4.09	0.23	0.69	5,538

Flax. In == Flaxseed ingredient

Table 3:1a Composition of experimental diets on as fed basis

Ingredients	Control	15% Flaxseed
Corn	53.47	55.32
Oats	5.07	-
Flaxseed	-	15.00
Soybean meal	20.46	14.30
Meat and bone meal	7.00	4.33
Animal tallow	3.88	1.00
Oyster shell	5.00	5.00
Dicalcium phosphate	1.53	1.51
Limestone	2.59	2.54
Salt	0.35	0.35
DL Methionine	0.20	0.20
Vit. E. Premix	0.25	0.25
Vit/Min Premix	0.10	0.10
Choline chloride	0.05	0.05
Grit	0.05	0.05

Nutrient Analysis (%)
Protein calculated; 18.00
ME. Kcal /kg; 2,800
Calcium calculated; 4.00
Crude fibre; 2.84

Table 3:2 Experimental diets

Diet #	Diet description
1	Control (C)
2	Control + ferlac 25 (C+ F25)
3	Flaxseed (FS)
4	Flaxseed + ferlac 25 (FS + F25)
5	Control + LA
6	Flaxseed + LA

LA: *Lactobacillus acidophilus*

Table 3:3 Fatty acid profile of experimental diets, flaxseed and soybean ingredients

Feed/In	C18:1	C18:2	C18:3
Control	35.30	28.18	2.60
15%FS	22.16	37.46	34.04
FS/In	19.02	14.29	56.89
SB/In	17.68	55.07	11.23

Feed/ In : Feed/ ingredient

A: LAYER PERFORMANCE

i. Measurements

Eggs were collected and recorded daily. On the last 2 days of each 28 - day period, eggs were collected to measure weight, egg yolk weight, haugh units and shell thickness which was measured by taking two measurements on each half of the dry shell using a micrometer. Feed consumption, feed conversion ratio (kg feed /doz eggs) and percent hen day- egg production were determined on five four - week periods. Mortality was also calculated for each period. Finally body weight changes were determined by weighing birds individually at the start and at the end of the study period (20 weeks)

ii. Statistical analysis

Data were analyzed using PROC mixed repeated measurement statement fitting an autoregressive covariance structure by SAS (1992). The dependent variables were egg production, feed consumption, feed conversion ratio, egg weight, yolk weight, shell thickness and haugh units. Data on hen -day egg production was log transformed prior to analysis. The following model was used for data analysis.

$$Y_{ijkl} = \mu + trt_i + blk_j + prd_k + pen_l + trt * blk_{ij} + trt * prd_{ik} + e_{ijkl}$$

where;

Y_{ijkl} = Observations

μ = Overall mean

trt_i = Effect of the i^{th} treatment where i = 1,2,3,4,5 or 6

blk_j = Effect of the j^{th} block where j =1 or 2

prd_k = Effect of the k^{th}period where k = 1,2,3,4 or 5

pen_l = Effect of the l^{th}pen where l = 1,2,3,4,5 or 6

trt * blk = The interaction of the i^{th}treatment and the j^{th} block

trt * prd = the interaction of the i^{th} treatment and the k^{th} period

e_{ijkl} = The error term associated with the i^{th} treatment, j^{th}block , k^{th}period and l^{th}pen

There was no effect of block and pen hence they were dropped from the model. Body weight was taken only at the beginning and the end of the experiment, it was analyzed using proc mixed without repeated measurement statement.

B: PLASMA AND EGG YOLK CHOLESTEROL

i. Measurements

At the end of each "period" 3 birds were chosen at random from each treatment replicate, after 11:00 h to avoid interference with oviposition. A 3ml blood sample was taken from the branchial vein with heparinized 5cc syringe. The samples were centrifuged at 3000rpm (2,000"g" force) and plasma collected. Duplicate aliquots from each sample were prepared for cholesterol and triglyceride analysis using procedures for Abbott - VP Discrete Autoanalyzer[10]. Three eggs from each treatment replicate were pooled on the last day of each period for cholesterol determination by Gas - liquid chromatography[11] described by Washburn and Nix (1974). All samples were kept at -20^0 C pending analysis.

ii. Statistical analysis

Data were subjected to analysis of variance using the General Linear Model procedure of SAS (1992). The statistical model included the effects of treatments, block, experimental period, pen and the interactions. The dependent variables were plasma cholesterol, TG, and egg yolk cholesterol. The design was a factorial with 6 treatments and 4 replicates per treatment. Five pairs of contrasts were planned, hence it was appropriate to use a simple T test because they were less than the number of treatments.

Contrasts
C vs C +F25
FS vs FS + F25
C vs C + LA
FS vs FS + LA
C vs FS

The following linear model was used for data analysis;

$$Y_{ijkl} = \mu + trt_i + blk_j + prd_k + pen_l + trt * blk_{ij} + trt_{il} * prd + e_{ijkl}$$

where;

Y_{ijkl} = Observations

μ = Overall mean

trt_i = Effect of the ^{ith}treatment where i = 1,2,3,4,5 or 6

blk_j = Effect of j^{th}block where j = 1 or 2

prd_k = Effect of k^{th}period where k = 1,2,3,4,or 5

pen_l = Effect of l^{th} pen where l is 1,2,3,4,5,or 6

trt * blk_{ij} = The interaction of the i^{th} trt and the j^{th}block

trt * prd_{ik} = The interaction of the i^{th} trt and the k^{th}period

e_{ijkl} = The error term associated with the i^{th} trt, j^{th} blk, k^{th}period and l^{th} pen

In the initial analysis of data, there was no effect of block and pen hence these terms were dropped from the model.

C: FATTY ACID ANALYSIS

i. Measurements

At the end of each 28 -day period 3 eggs were selected at random from each treatment replicate. The yolks were separated from the albumen, weighed, and fatty acids profile was assayed according to the methods described by Holub and Skeaff (1987) where FA methyl esters were prepared from pooled egg samples and fatty acid profile obtained by gas chromatography[11]

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ii. Statistical analysis

Fatty acid profile analysis was determined at the end of each period, data analysis was performed by PROC mixed using repeated measurement. An autoregressive covariance structure was fitted because it is the most appropriate fit for measurements that are taken at equal intervals. The statistical model included the effects of treatment, block, experimental period, pen and the interactions. The dependent variables were C18:1, C18:2, C18:3, C20:4, C20:5 or C22:6. The design was a factorial with 6 treatments and 4 replicates per treatment. The following linear model was adopted for yolk fatty acid data analysis.

$Y_{ijkl} = \mu + trt_i + blk_j + prd_k + pen_l + trt * blk_{ij} + trt * prd_{ik} + e_{ijkl}$

where;

Y_{ijkl} = Observation

μ = Overall mean

trt_i = Effect of the i^{th} treatment where i = 1,2,3,4,5,or 6

blk_j = Effect of the j^{th} block where j = 1 or 2

prd_k = Effect of the k^{th} period where k = 1,2,3,4,or 5

pen_l = Effect of the l^{th} pen where l = 1,2,3,4,5 or 6

$trt * blk_{ij}$ = The interaction of the i^{th} treatment and the j^{th} block

$trt * prd_{ik}$ = The interaction of the i^{th} treatment and the k^{th}period

e_{ijkl} = The error term associated with the i^{th} treatment, j^{th} block ,k^{th} period and l^{th} pen

In the initial analysis of these data , there was no effect of block and pen hence these terms and their interactions were dropped from the model.

SECTION IV

RESULTS AND DISCUSSION

A: LAYER PERFORMANCE

B: PLASMA AND EGG CHOLESTEROL AND PLASMA TRIGLYCERIDES

C: FATTY ACIDS COMPOSITION

A: Layer performance

Egg production among the probiotic supplemented diets was not significantly different ($p > 0.05$) from the C and FS groups (Table 4A.1), except that the FS diet supplemented with LA was significantly ($p > 0.05$) lower than the C, C + F 25 and FS groups. These results are not consistent with the report by Abdulrahim et al.,(1995) in which LA supplementation significantly ($p < 0.05$) improved egg production over the C fed group. The results on the effect of FS on egg production are in agreement with studies on 22 week old SCWL reported by Aymond and VanElswyk, (1995). Feed consumption (Table 4A.1) was significantly ($p < 0.05$) higher for the FS + LA and F+ F 25 diets compared to C diet and C+LA. These results are not consistent with work published by Scheideler and Froning, (1995) whereby birds fed different levels of FS (5 and 15%) exhibited statistically ($p < 0.05$) lower feed consumption. Even though there was a marginal difference between probiotic and non probiotic supplemented diets the probiotic supplemented groups exhibited a higher feed intake. Experimental diets had no significant effect on feed conversion per dozen eggs ($p > 0.05$) (Table 4A.1). These results are not in accordance with the report by Abdulrahim et al. (1996). Body weight gain (Table 4A.1) significantly ($p < 0.05$) decreased in response to feeding FS alone or FS supplemented with both F 25 or LA. These results are in agreement with the report by Caston et al., (1994) whereby increasing the level of FS from 10 to 20% resulted in lower weight gains than the C group. Supplementing FS with probiotics did not enhance weight gains. Contrary to these results, Mohan et al., (1995b) reported a marginal increase in body weight gain attributed to LA supplementation.

Table 4A.1 Effect of probiotic and flaxseed on hen-day egg production, feed consumption (FC), feed conversion per dozen eggs (FC/doz) and body weight gain of SCW Leghorns from 26 to 46 weeks of age.

Diets	Egg production (%)	FC (g /h /d)	FC /doz	Body wt gain (g)
Control	93.71[a]	120.04[b]	1.58[a]	221.41[a]
Control + F25	93.88[a]	120.92[ab]	1.59[a]	211.19[a]
Flaxseed	93.69[a]	122.53[ab]	1.60[a]	113.23[b]
Flaxseed + F25	92.94[ab]	123.76[a]	1.63[a]	95.84[b]
Control + LA	92.73[ab]	120.04[b]	1.59[a]	183.65[a]
Flaxseed + LA	92.27[b]	122.95[a]	1.58[a]	94.81[b]
SEM	0.20	0.86	0.02	7.87

[a-b] Means with different superscripts in the same column differ significantly ($P<0.05$)
FC (g /h / d) : Average feed consumption per hen per day during 5 28 day - period
FC / doz : Feed conversion ratio (kg feed consumption per dozen eggs)

Main dietary treatment (Table 4A.2), C and FS had no significant effect on egg weight ($p>0.05$). When FS was supplemented with either F25 or LA, it gave significantly higher egg weights than C with LA supplementation. The contrasts between the selected pairs of treatments was not significant ($p>0.05$). Similar to these results, Abdulrahim et al., (1995) reported no significant effect ($p>0.05$) due to LA supplementation. Contrary to these observations, Caston and Leeson (1990) reported a significant reduction in egg weight following feeding 15% FS. Yolk weight (Table 4A.2) responded to dietary treatments with a significant ($p<0.05$) decrease in size for FS vs C with LA. For this parameter, there was no significant difference between the two probiotics. Caston and Leeson (1990) also reported a significant decrease ($p<0.01$) in response to feeding FS at 5 and 15%. Dietary treatments had no effect on shell thickness except for FS + LA which exhibited a significantly ($p<0.05$) greater shell thickness over the C + F25 diet. A similar report was published by Abdulrahim et al.,(1995) whereby LA supplementation enhanced significantly stronger shell thickness. In general, LA supplementation gave significantly better shell thickness than F25. This suggests that lactic acid produced by LA might encourage better absorption of calcium and phosphorus from the digestive tract, hence it was anticipated that level of mineral deposition might increase with LA supplementation (Abdulrahim et al 1996.) Contrary to these observations Scheideler and Froning,(1996) found no significant effect ($p>0.05$) on shell thickness by feeding FS. Haugh units (Table 4A.2) did not respond to dietary treatments; only one treatment group, FS + LA were significantly ($p<0.05$ increased over C group. Jiang et al.,(1991) in studies with FS and sunflower seed feeding found no significant effect ($p<0.05$) of dietary FS on haugh units.

Table 4A.2 Effect of probiotics and flaxseed on egg weight, shell thickness and haugh units. Eggs were collected over 5 periods of 4 weeks each.

Diets	Egg weight (g)	Yolk weight (g)	SHTH.(mm)	Haugh units
Control	61.85^{ab}	16.47^{ab}	0.402^{ab}	75.85^{b}
Control + F25	61.40^{ab}	16.40^{ab}	0.401^{b}	78.13^{ab}
Flaxseed	61.74^{ab}	16.08^{b}	0.406^{ab}	76.86^{ab}
Flaxseed + F25	62.12^{a}	16.33^{ab}	0.406^{ab}	77.78^{ab}
Control + LA	61.34^{b}	16.84^{a}	0.405^{ab}	77.88^{ab}
Flaxseed + LA	62.10^{a}	16.30^{ab}	0.409^{a}	78.85^{a}
SEM	0.20	0.09	0.11	0.11

$^{a-b}$ Means with different superscript letter in the same column differ significantly ($p<0.05$)

SHTH - Shell thickness

Conclusion

The results from this study indicate that FS can be supplemented in layer diets without any serious detrimental effect on performance parameters when the ration is balanced to meet the metabolizable energy requirements of the birds. Probiotic supplementation showed to improve general performance of the birds, and prevented birds from gaining too much weight during laying period which may cause fatty liver syndrome. Although the effect of the two probiotics was not consistent across the parameters measured, supplementation with LA seemed more effective than supplementation with F25

B: PLASMA AND YOLK CHOLESTEROL AND PLASMA TRIGLYCERIDES

The average plasma cholesterol values per treatment are summarized by period in Table 4B.1. Probiotic supplementation did not show a significant effect ($p > 0.05$) in the first two periods of the study. However, in period 3, a significantly ($p < 0.05$) lower level was observed in the FS supplemented with F25 group than the FS group. This observation was also confirmed by the results from the contrasts in Table 4B.2 in which FS vs FS + F25 was significant ($p=0.0975$). In period 4, F25 supplementation on both C and FS significantly ($P < 0.05$) reduced cholesterol from 161.74 in C to 117.69 mg /dL in C + F25; and from 156.64 in FS to 108.35 mg /dL in FS+ F25 group. *Lactobacillus acidophilus* supplementation did not have a significant ($p < 0.05$) effect on C treatments but it significantly ($p < 0.05$) reduced cholesterol from 161.74 to 109.37 mg /dL in FS diets. The effects of treatment in period 4 were in accordance with selected pairs of contrasts in which F25 supplementation on both C and FS was significant ($p= 0.0056$ and 0.0025, respectively); LA supplementation effect was only significantly ($p= 0.0077$) different when supplemented to FS. During the last period of the study, F25 reduced cholesterol in C group from 158.28 mg/ dL to 113 mg /dL, while it also reduced cholesterol from FS group from 145.10 mg /dL to 101.89 mg /dL. LA supplementation also reduced cholesterol significantly ($p < 0.05$) from 158.28 from C group to 125.08 mg /dL, it also significantly ($p < 0.05$) reduced cholesterol in the FS group from 145.10 to 95.80 mg/dL. The results from period 5 were in agreement with the contrasts tested, which showed that probiotic supplementation reduced cholesterol significantly at the levels presented in Table 4B.2. Throughout the study there was no significant ($p > 0.05$) difference between plasma cholesterol values in C fed birds and FS fed

groups. This observation is also confirmed by the contrast C vs FS which showed no significant effect in all 5 periods. Mohan et al., (1995b) also reported that LA supplementation significantly ($p < 0.05$) lowered plasma cholesterol in chickens.

Table 4B:1 Effects of flaxseed and probiotics on plasma cholesterol in mg/ dL by period (28 days)in SCW Leghorns from 26 to 46 weeks of age.

Diets	Period 1	Period 2	Period 3	Period 4	Period 5
Control	148.83[a]	163.24[a]	178.85[a]	161.74[a]	158.28[a]
Control + F25	128.38[a]	164.24[a]	158.31[ab]	117.69[b]	113.67[cd]
Flaxseed	126.39[a]	150.26[a]	180.49[a]	156.64[a]	145.10[ab]
Flaxseed +F25	141.04[a]	159.29[a]	149.32[b]	108.35[b]	101.89[cd]
Control + LA	147.63[a]	136.25[a]	168.51[ab]	154.09[a]	125.08[c]
Flaxseed + LA	137.15[a]	156.13[a]	156.38[ab]	109.37[b]	95.80[d]
SEM	5.05	4.99	5.69	5.20	4.17

[a-d] Means within the same column with different superscript letter differ significantly ($p<0.05$)

Table 4B :2 Selected pairs of contrasts by period for plasma cholesterol

Contrasts	Period 1	Period 2	Period 3	Period 4	Period 5
C vs C +F25	0.2225^{ns}	0.9442^{ns}	0.2730^{ns}	0.0056**	0.0002***
FS vs FS +F25	0.3823^{ns}	0.5849^{ns}	0.0975*	0.0025**	0.0003***
C vs C+ LA	0.9485^{ns}	0.1485^{ns}	0.6206^{ns}	0.6605^{ns}	0.0119*
FS vs FS +LA	0.5655^{ns}	0.7504^{ns}	0.2498^{ns}	0.0077**	0.0002***
C vs FS	0.1819^{ns}	0.4380^{ns}	0.9297^{ns}	0.7435^{ns}	0.2540^{ns}

C = Control
F25= Ferlac 25
FS = Flaxseed
LA= *Lactobacillus acidophilus*

The results of the effects of treatment on plasma TG are presented in (Table 4B.3). During the entire period of the study, there was no significant ($p > 0.05$) difference in TG between the two main dietary treatments. The results from the first 3 periods agree with the contrasts tested for those periods (Table 4B.4). In the first period none of the contrast was significant, whereas in period 2 and 3 the contrast was significant for FS vs FS +LA at ($p = 0.0262$) and ($p = 0.0023$) respectively. In period 4 supplementing FS with F25 significantly ($p < 0.05$) reduced TG from 3064 to 2426mg /dL, while it did not show significant ($p > 0.05$) effect when supplemented to C. On the other hand, LA supplementation also significantly ($p < 0.05$) reduced TG in FS from 3064 to 2268mg /dL but did not show significant ($p > 0.05$) effect on C fed groups. These results are confirmed by the contrasts in which only FS vs FS + F25 and FS vs FS +LA were significant ($p = 0.0635$) and ($p = 0.00473$), respectively. In period 5, F25 supplementation reduced TG in FS from 2838 to 2121mg /dL. However, it did not show a significant ($p > 0.05$) reduction in TG when supplemented to C treatment. LA supplementation significantly($p < 0.05$) reduced TG in FS treatments from 2838 to 1941mg /dL, while no significant ($p > 0.05$) effect was observed when it was supplemented to C diet. The effects of probiotic supplementation during period 5 were in agreement with the contrast tested for this period which showed significant difference for only FS groups supplemented with probiotics; FS vs FS +F25 ($p = 0.0001$) and FS vs FS +LA ($p = 0.0023$). These observations were in agreement with the report by Mohan et al., (1995b) and Haddadin et al., (1996) who also observed the effects of probiotics on laying hen plasma TG after 10 and 40 weeks of supplementation, respectively.

Table 4B :3 Effects of probiotics and flaxseed on plasma triglycerides in mg /dL by period (28 -days) in SCW Leghorns from 26 to 46 weeks of age

Diets	Period 1	Period 2	Period 3	Period 4	Period 5
Control	3958.8^{a}	3684.1^{ab}	4791.1^{a}	2861.1^{a}	2457.2^{ab}
Control + F25	3319.8^{a}	3812.4^{ab}	4067.7^{a}	2426.3^{a}	2225.6^{bc}
Flaxseed	3373.3^{a}	3925.7^{a}	4632.8^{a}	3064.5^{a}	2838.9^{a}
Flaxseed + F25	3739.8^{a}	3259.7^{ab}	3843.2^{ab}	2399.3^{b}	2121.7^{bc}
control + LA	3909.7^{a}	3155.5^{ab}	3914.1^{ab}	2818^{a}	2243.3^{bc}
Flaxseed + LA	3897.3^{a}	2912.1^{b}	2897^{b}	2268.4^{b}	1941.1^{c}
SEM	163	125	159	109.6	68.1

$^{a-c}$ Means within the same column with different superscript letter differ significantly ($p<0.05$)

Table 4B: 4 Differences between the selected treatments for plasma triglycerides by period

Contrasts	Period 1	Period 2	Period 3	Period 4	Period 5
C vs C +F25	0.2380^{ns}	0.7499^{ns}	0.1467^{ns}	0.2226^{ns}	0.2600^{ns}
FS vs FS +F25	0.4404^{ns}	0.1003^{ns}	0.1135^{ns}	.0.0635*	0.0001***
C vs C +LA	0.9357^{ns}	0.2416^{ns}	0.1158^{ns}	0.9135^{ns}	0.3518^{ns}
FS vs FS +LA	0.3856^{ns}	0.0262*	0.0023**	0.0473*	0.0023**
C vs FS	0.2794^{ns}	0.5485^{ns}	0.7495^{ns}	0.5671^{ns}	0.1651^{ns}

The yolk cholesterol mean values are summarized by period in Table 4B.5. During the first and second periods there were no significant ($p>0.05$) effects on egg cholesterol due to diet or probiotic supplementation. The contrasts presented in Table 4B.6 for the two periods also show no significant difference. In the 3rd period, F25 supplementation significantly ($p<0.05$) reduced egg cholesterol in the FS group from 13.62 to 12.58 mg/g. However, F25 did not show significant ($p>0.05$) effect when supplemented to the C diet. LA supplementation however, reduced egg cholesterol significantly ($p<0.05$) in C diet from 11 in to 9.80mg/g, while it also reduced cholesterol significantly ($p<0.05$) in FS diet from 13.62 to 9.47mg/g. After the first two periods, cholesterol values were significantly ($p<0.05$) higher in FS diet than in C diet. These results are however, in accordance with the results from the contrast tested for this period; all the contrasts, except for C vs C +F25 were significant. During period 4, F25 supplementation showed significant ($p<0.05$) effect when supplemented to C but not to the FS groups. The effect of LA was only significant($p<0.05$) when supplemented to the FS but not to the C. The results were confirmed by the contrasts. In period 5, F25 did not have significant ($p>0.05$) effect when supplemented to either C or FS diets. However, LA supplementation significantly reduced egg cholesterol in the C diet from 11.78 to 8.17mg/g, while it also reduced cholesterol in FS diet from 14.04 to 10.16mg/g. These results during period 5 were tested with the selected contrasts; where C vs C+LA was significant ($p=0.0004$); FS vs FS +LA was significant ($p=0.0002$), and C vs FS was significant ($p=0.0086$).

Table4B :5 Effect of flaxseed and probiotics on egg yolk cholesterol in mg/ g by period (28-day) in SCW Leghorns from 26 to 46 weeks of age.

T/Diet	Period 1	Period 2	Period 3	Period 4	Period 5
Control	11.37^{b}	11.44^{a}	11.00^{c}	11.54^{b}	11.78^{bc}
C+ F25	11.39^{b}	11.43^{a}	10.16^{cd}	11.08^{c}	11.51^{bc}
Flaxseed	11.82^{ab}	11.40^{a}	13.62^{a}	12.80^{a}	14.04^{a}
FS+ F25	11.63^{ab}	11.06^{a}	12.58^{b}	12.51^{a}	13.12^{ab}
C + LA	12.15^{ab}	10.53^{a}	9.80^{d}	11.34^{b}	8.17^{d}
FS + LA	11.70^{ab}	10.87^{a}	9.47^{d}	11.04^{c}	10.16^{d}
SEM	0.08	0.17	0.31	0.21	0.39

$^{a-d}$ Means within the same column with different superscript letter differ significantly ($p < 0.05$)

Table 4B:6 Egg yolk cholesterol contrasts by period

Contrasts	Period 1	Period 2	Period 3	Period 4	Period 5
C vsC+F25	0.9423^{ns}	0.9776^{ns}	0.4521^{ns}	0.0283*	0.7402^{ns}
FS vsF+F25	1.4715^{ns}	0.5712^{ns}	0.0268*	0.6406^{ns}	0.2565^{ns}
C vsC+LA	0.1103^{ns}	0.1400^{ns}	0.0008***	0.7875^{ns}	0.0004***
FSvsFs+La	0.6745^{ns}	0.4346^{ns}	0.0001***	0.0121*	0.0002***
CvsFS	0.1803^{ns}	0.9522^{ns}	0.0001***	0.0103*	0.0086**

See Table 4B.1 for abbreviation

Conclusion

It was hypothesized that diet composition and probiotic supplementation would affect plasma and egg yolk cholesterol. This study proved that probiotic supplementation resulted in reduced plasma and egg yolk cholesterol as well as plasma TG in layers. However, long term inclusion of FS increased the egg yolk cholesterol, but when FS was supplemented with probiotics egg yolk cholesterol was reduced to the level observed when the C diet was fed supplemented with probiotics.This study has also demonstrated that the effectiveness of probiotic supplementation depends on a number of factors including duration of probiotic supplementation, and the microorganisms in the culture.

C: Egg fatty acid profile

A complete fatty acid profile of eggs from dietary treatments is presented in Table 4C. Although the other fatty acids make a significant component of the whole profile and since their content is not affected by treatment, this study focused mainly on those fatty acids whose content in the egg yolk are influenced by dietary manipulation. Hence, the discussion on the egg yolk fatty acid profile will mainly focus on the fatty acids summarized in Table 4C.a. Dietary FS is a major source of linolenic acid that has been shown to significantly increase the ω - 3 fatty acids content of eggs (Caston and Leeson, 1990; Jiang et al., 1991,1992). Flaxseed is one of the most concentrated source of linolenic available in natural plant feed - stuffs for poultry. It contains 40% ether extract, of which 56% is linolenic. Since the interaction between treatments and periods was not significant ($p > 0.05$) it was then appropriate to look at the main dietary effects. Dietary FS with and without probiotic supplementation significantly ($p < 0.05$) lowered C18:1 in the egg yolk by about 12%. There was no difference ($p > 0.05$) supplementing F25 or LA to the FS diet, although both of them were significantly ($p < 0.05$) higher in 18:1 content than those in the FS diet alone. Upon analysis of the difference between treatments for C18:1,(Table 4C.1) it was observed that in both comparisons, FS vs FS + F25 and FS vs FS + LA were significantly lower in C18:1, ($p=0.006$) and ($p=0.0151$), than FS without probiotics, ($p < 0.0001$) respectively; the difference between the C vs FS was more significant ($p=0.0001$) than any other contrast. The results showed that supplementing FS with F25 was more effective than supplementing with LA. The contrasts also reaffirmed that incorporating FS in the diets was highly beneficial. The purchased market ω -3 chicken eggs from a local supermarket in december 1997 had relatively lower C18:1 compared to the results presented here in.

Table 4C. Summary of the mean values of the complete fatty acid profile for the egg yolk fat eggs (%) from SCW Leghorns fed flaxseed and probiotics from 26 to 46 weeks of age

FA	C	C+F25	FS	FS +F25	C +LA	FS +LA
C14:0	0.31	0.31	0.26	0.28	0.31	0.28
C14:1	0.06	0.06	0.03	0.04	0.05	0.04
C16:0	24.67	24.50	22.88	24.45	26.45	24.31
C16:1	1.94	2.03	2.13	2.67	2.93	2.73
C17:0	-	-	-	-	-	-
C18:0	9.59	9.46	9.96	9.55	9.23	9.77
C18:1	47.51	46.56	41.60	43.47	50.50	44.49
C18:2	11.92	13.02	12.71	14.78	10.83	14.32
C18:3	0.42	0.43	4.86	5.07	0.79	4.64
C20:0	-	-	-	-	-	-
C20:1	0.33	0.32	0.23	0.24	0.32	0.26
C20:2	0.07	0.09	0.01	0.03	0.08	0.09
C20:3	0.20	0.20	0.16	0.22	0.05	0.23
C20:4	2.07	2.09	1.30	1.33	2.09	1.31
C20:3	-	-	-	0.01	-	-
C20:5	-	-	0.04	0.13	-	0.09
C22:0	-	-	-	-	-	-
C22:1	-	-	-	-	-	-
C22:6	0.92	0.93	1.86	1.98	1.08	2.03
C24:0	-	-	-	-	-	-
C24:1	-	-	-	-	-	-

FA = fatty acid

Table 4C:a Fatty acid profile of the egg yolk(%) from SCW Leghorns fed flaxseed and probiotics from 26 to 46 weeks of age

Diet	C18:1	C18:2	C18:3	C20:4	C20:5	C22:6
Control	47.13^{ab}	12.15^{b}	0.34^{b}	2.21^{a}	0.00^{c}	0.83^{b}
Control +F25	46.80^{b}	12.02^{b}	0.34^{b}	2.21^{a}	0.00^{c}	0.83^{b}
Flaxseed	41.37^{d}	14.51^{a}	4.97^{a}	1.32^{b}	0.05^{b}	1.99^{a}
Flaxseed +F25	42.66^{c}	14.41^{a}	5.48^{a}	1.27^{b}	0.08^{a}	1.95^{a}
Control +LA	47.73^{a}	12.65^{b}	0.33^{b}	2.21^{a}	0.00^{c}	0.86^{b}
Flaxseed +LA	42.64^{c}	14.89^{a}	5.06^{a}	1.34^{b}	0.07^{ab}	2.03^{a}
SEM	0.27	0.14	0.29	0.04	0.01	0.05

$^{a-d}$ Means within the same column with different superscript letters differ significantly ($p<0.05$)

Table 4C:b Fatty acid profile (%) of purchased market ω -3 eggs and duck eggs.

Sample	C18:1	C18:2	C18:3	C20:4	C20:5	C22:6
ω -3 chicken eggs	35.36	16.97	8.29	1.13	-	1.82
Duck eggs	51.91	8.08	0.38	4.09	-	1.49

Table 4C.1 Effects of feeding FS and probiotic supplementation to SCW Leghorns from 26 to 46 weeks of age on yolk C18:1

Diets	C18:1	Contrasts
Control	47.13^{ab}	C vs C+ F25 = 0.47^{ns}
Control + F25	46.80^{b}	FS vs FS + F25 =0.006**
Flaxseed	41.37^{d}	C vs C + LA=0.245^{ns}
Flaxseed + F25	42.66^{c}	FS vs FS + LA = 0.015*
Control +LA	47.73^{a}	C vs FS = 0.0001***
Flaxseed + LA	42.64^{c}	
SEM	0.27	

$^{a-d}$ Means within the same column with different superscripts differ significantly ($p<0.05$)

The results on the effects of dietary treatment on the deposition of C18:2 in the egg yolk and treatment differences are summarized in Table 4C.2. Incorporation of FS with and without probiotic supplementation significantly ($p<0.05$) increased the amount of C18:2 in the egg yolk by 18%. There was no significant difference between the two probiotics. The pairs of contrasts tested had no significant effect except for the C vs FS diet which was highly significant ($p=0.0001$); the FS diet resulted in a higher C18:2 content than the C diet.

Table 4C.2 Flaxseed and probiotic supplementation effect on yolk C18:2 of SCW Leghorns from 26 to 46 weeks of age.

Diets	C18:2(%)	Contrasts
Control	12.15[b]	C vs C+ F25= 0.7048[ns]
Control + F25	12.02[b]	FS vs FS + F25=0.7326[ns]
Flaxseed	14.51[a]	C vs C +LA= 0.2103[ns]
Flaxseed +F25	14.41[a]	FS vs FS +LA= 0.374[ns]
Control +LA	12.65[b]	C vs FS =0.0001[***]
Flaxseed + LA	14.89[a]	
SEM	0.14	

[a-b]Means in the same column with different superscript letters significantly differ ($p<0.05$).

Incorporation of C18:3 responded significantly to dietary manipulation (Table 4C.3). Incorporating FS in the diet of layers significantly ($p<0.05$) increased the level of C18:3 in the egg yolk by 14 folds. However, even though there was no statistical differences among the C diets, supplementing the C diet with LA was significantly ($p=0.0314$) better than supplementing with F25. When the C was tested against FS the effect was highly significant ($p<0.0001$). These results are consistent with the results reported by Caston and Leeson,(1990), Jiang et al.,(1991), Cherian and Sim, (1991), Scheideler et al.,(1994), Aymond and Van Elswyk, (1995) and Scheideler and Froning, (1996). These results compare well with the results obtained from the analysis done on the commercial ω-3 eggs, although the latter exhibited slightly higher levels of C18:3.

Table 4C.3 Effects of flaxseed and probiotics on egg yolk C18:3 of SCW Leghorns from 26 to 46 weeks of age

Diets	C18:3(%)	Contrasts
Control	0.34[b]	C vs C + F25 =0.9289[ns]
Control + F25	0.34[b]	FS vs FS + F25 = 0.500[ns]
Flaxseed	4.97[a]	C vs C +LA= 0.0314[*]
Flaxseed + F25	5.48[a]	FS vs FS +LA = 0.8036[ns]
Control + LA	0.33[b]	C vs FS =0.0001[***]
Flaxseed + LA	5.06[a]	
SEM	0.29	

[a-b]Means within the same column with different superscript letters differ significantly ($p<0.05$)

Data on the response of C20:4 to dietary treatments is summarized in Table 4C.4. Inclusion of dietary FS significantly ($p<0.05$) reduced the amount of C20:4 in the egg yolk by 40%. Supplementing FS with probiotics did not have significant effect on C20:4 content in egg yolk. For the differences between treatments, only the contrast between C and FS was significantly different ($p=0.0001$).

Table 4C.4 Dietary flaxseed and probiotic supplementation effects on yolk C20:4 of SCW Leghorns from 26 to 46 weeks of age.

Diets	C20:4(%)	Contrasts
Control	2.21[a]	C vs C+ F25= 0.9289[ns]
Control + F25	2.21[a]	FS vs FS + F25=0.2613[ns]
Flaxseed	1.32[b]	C vs C + LA= 0.8998[ns]
Flaxseed + F25	1.27[b]	FS vs FS + LA=0.8036[ns]
Control + LA	2.21[a]	C vs FS = 0.0001[***]
Flaxseed + LA	1.34[b]	
SEM	0.04	

[a-b] Means within the same column with different superscript significantly differ ($p<0.05$)

The mean values for C20:5 are presented in Table 4C.5. Incorporation of FS in the diet of layers significantly ($p<0.05$) increased the level of C20:5 in the egg yolk. Supplementing the FS with probiotics significantly ($p<0.05$) increased the level of C20:5 in the egg yolk. This suggests that supplementing FS with probiotics had an additive effect over and above the effect of FS. The effect of treatment differences between C and FS was highly significant ($p=0.0002$) and FS vs FS + F25 was also significant ($p=0.0115$). This indicates that F25 supplementation increased C20:5 better than supplementing with LA. These results on the effect of incorporating FS on C20:5 are in agreement with the observations made by Caston and Leeson, (1990), Cherian and Sim, (1991), Jiang et al., (1991), Scheideler et al., (1994), Aymond and Van Elswyk, (1995) and Scheideler and Froning, (1996).

Table 4C.5 Effects of Flaxseed and probiotics on egg yolk C20:5 of SCW Leghorns from 26 to 46 weeks of age.

Diets	C 20 :5(%)	Contrasts
Control	0.00^{c}	C vs C +F25= 1.000^{ns}
Control + F25	0.00^{c}	FS vs FS + F25= 0.0115*
Flaxseed	0.05^{b}	C vs C +LA= 1.000^{ns}
Flaxseed + F25	0.08^{a}	FS vs FS + LA= 0.1043^{ns}
Control + LA	0.00^{c}	C vs FS = 0.0002***
Flaxseed + LA	0.07^{ab}	
SEM	0.01	

$^{a-c}$Means in the same column with different superscript letters significantly differ ($p<0.05$)

The experimental diets had significant effect on the incorporation / deposition of C22:6 in the egg yolk (Table 4C.6). Inclusion of FS in the diet significantly ($p<0.05$) increased C22:6 in the egg yolk by 58% compared to the C diet. Supplementing FS with either probiotic had no significant effect over and above the effect of FS. These results were confirmed by the differences between treatments, where there was no significant difference in the contrasts except for the difference between the C and the FS diet which was highly significant ($p<0.0001$). Aymond and Van Elswyk,(1995) and Scheideler and Froning , (1996) also found significant increase in C22:6 when they incorporated FS in the diet.

Table 4C.6 Flaxseed and probiotic supplementation on yolk C22:6 of SWC Leghorns from 26 to 46weeks of age

Diets	C 22:6(%)	Contrasts
Control	0.83[b]	C vs C +F25 =0.9101[ns]
Control + F25	0.83[b]	FS vs FS +F25 = 0.328[ns]
Flaxseed	1.99[a]	C vs C +LA =0.4841[ns]
Flaxseed + F25	1.95[a]	FS vs FS +LA = 0.4532[ns]
Control + LA	0.86[b]	C vs FS = 0.0001[***]
Flaxseed + LA	2.03[a]	
SEM	0.05	

[a-b] Means in the same column with different superscript letters differ significantly

Conclusion

The results of this study demonstrated that incorporating ingredients such as FS in the layer diets with a high content of unsaturated fatty acids can alter the egg yolk fatty acid composition favourably toward increasing polyunsaturated fat content of the egg. This study has also showed that probiotic supplementation except for C18:1, does not have a significant effect on the fatty acid composition of the egg. From this study it can also be concluded that, even though the FS ingredient does not have the long chain metabolites products of linolenic acid (EPA and DHA), and has a very high level of their precursor linolenic acid (56% of the lipid fraction), the laying hen has the somewhat unique ability to efficiently convert linolenic acid into EPA and DHA

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SECTION V

GENERAL DISCUSSION

The use of probiotic supplementation and feeding flaxseed to laying hens has become an area of interest in research due to their effects on modifying the saturated fatty acid profile in the egg yolk and also influencing plasma and egg yolk cholesterol. One of the objectives of the study presented in section I was to evaluate the combined effects of probiotic supplementation and feeding FS on the general performance of the birds. This study showed that probiotic supplementation improved feed consumption, egg weight and haugh units ($p < 0.05$) while egg production, feed conversion ratio, body weight gain, shell thickness and yolk weight were not significantly affected by probiotic supplementation. These results may be explained by the fact that healthy animals are generally characterized as having a well functioning intestinal tract. This is fundamental for the efficient conversion of feed for maintenance and for growth or production (Jin et al. 1997). The intestinal bacterial flora of domestic animals has an important role in the digestion and absorption of feed ingested by the host animal. It takes part in the metabolism of dietary nutrients such as carbohydrates, proteins, lipids and minerals and also the synthesis of vitamins. The production parameters such as shell thickness, feed conversion ratio, body weight gain and egg production did not respond to probiotic supplementation; this may have been due to the low amount of viable *Lactobacillus* counts because the probiotic culture was mixed with feed by hand which probably did not provide a homogeneous mixture of feed. In the other

studies in which these parameters were improved by probiotics, the probiotic cultures were dissolved directly into drinking water and in that case availability and viability of the *Lactobacillus* count was assured. Feeding FS to laying hens, however had no detrimental effect on the general performance of the birds except that it significantly ($p<0.05$) reduced body weight gain of the layers during the 20wk period which could be attributed to changes in apparent metabolizable energy in the FS diets. Secondly, this could be explained by too much feed loss that was observed in the FS fed groups. Even though there was no significant ($p<0.05$) difference in yolk weight among treatments, the FS fed groups had slightly lower yolk weights compared to C groups. Decline in yolk weight following feeding FS may be explained by the high level of ω-3 PUFA in FS which were hypothesized to be related to the influence of the ω-3 PUFA on hepatic metabolism (Van Elswyk et al., 1997). Research has demonstrated that ω-3 PUFA in mammals reduce hepatic lipid biosynthesis and secretion while promoting peroximal β- oxidation of fat (Harris 1989). The net effect of ω-3 PUFA on mammalian liver therefore is a decrease in circulating lipids. Evidence that ω-3 PUFA may influence avian liver lipid metabolism in a similar way has been reported by Phettelace and Watkins (1990). Another explanation why FS diets produced relatively smaller egg yolk sizes as suggested by Whitehead et al., (1993) could be that, the reduction in egg/yolk weight in response to ω-3 PUFA may be related to a reduction in circulating oestradiol; which is however responsible for maintaining hepatic lipogenesis for yolk formation. As a result, reduced oestradiol levels could reduce hepatic lipid production, thus reducing the availability of lipophilic material for yolk formation. It is however still unclear by what mechanism ω-3 PUFA might reduce serum levels of oestradiol.

The results on the effects of probiotic supplementation and feeding FS on plasma and egg yolk cholesterol showed that, feeding FS significantly ($p<0.05$) increased egg yolk cholesterol. This was attributed to the high levels of unsaturated fatty acid in FS which proved to have a synergistic effect on cholesterol absorption in the ovary and results in increased egg yolk cholesterol (Hargis and VanElswyk, 1990). These results indicate that since the cholesterol deposited in the egg is formed in the liver, the increase in cholesterol synthesis which occurs with feeding of polyunsaturated fats results in increased deposition of cholesterol in the egg. Chen et al.,(1965), and Weiss et al., (1967a) have suggested that transport of liver cholesterol to the ovary also appears to be influenced by the nature of dietary fats. Thus, synthesis of phospholipid, essential components of lipoprotein, is enhanced by unsaturated fats whereas dietary saturated fats are less readily utilized for the synthesis of phospholipid in the hen's liver. Another explanation to increased egg yolk cholesterol by feeding FS was suggested by Vagas and Naber (1984); when body energy stores are substantial, any excess of energy ingested will be reflected as increased body weight and increased cholesterol biosynthesis (with excess cholesterol transferred to the egg yolk). Thus, when the bird is in positive energy balance , the concentration of egg yolk cholesterol increases; while when the hen is losing weight or consuming less than 340 kcal/day, yolk cholesterol is inversely related to the body weight. Although feeding FS increased egg yolk cholesterol relatively higher than the C, when both FS and C were supplemented with probiotics, plasma and egg yolk cholesterol were significantly ($p<0.05$) reduced. The liver and ovary are the primary sites of cholesterol biosynthesis in laying birds. The liver is however, the major source of lipid found in the egg yolk (Weiss et al. 1967a, b; Shivaprasad and Jaap,1977). Although most of the cholesterol found in the egg yolk is

synthesized in the hen's liver, transported by the blood in the form of lipoproteins,and deposited in the developing follicles, the concentration of plasma cholesterol is not closely associated with the concentration of egg yolk cholesterol. The mechanism by which probiotics reduce plasma cholesterol was extrapolated from studies in humans, rats and pigs fed fermented milk which suggested that fermented milk contained bacterial metabolites which inhibit cholesterol synthesis by the body. Lactobacillus containing probiotics have proved to have a direct effect on cholesterol levels by assimilation and removal from the growth medium; or its degradation to bile acids followed by deconjugation to prevent resynthesis (Grunewald, 1982). Another mechanism by which probiotics may reduce egg cholesterol is that some organism present in the probiotics preparation could assimilate the cholesterol present in the gastro intestinal tract for their own cellular metabolism, thus reducing the amount of cholesterol absorbed and or synthesized (Gilliland et al. 1985). Supplementing both C and FS diet with LA was significantly better than supplementing with F25 in reducing plasma cholesterol, TG and egg yolk cholesterol. This is probably because F25 was a cocktail of 3 species of Lactobacillus and 2 species of Streptococcus while LA is a pure Lactobacillus *acidophilus* culture. Research on probiotic supplementation has proved that species of Lactobacillus have been proposed as likely dietary adjuncts, and LA is widely regarded as the most suitable candidate organism (Havenaar and Huiit Veld, 1992). These results suggest that LA in the F25 cocktail which represented only 4% of the mixture was probably in too low concentration to show the beneficial effects on plasma, egg yolk cholesterol and TG, as the pure LA culture supplementation did. Nevertheless, the probiotic effect of lowering the plasma and egg yolk cholesterol in layers requires further investigation. It is however, important to note that, the results from plasma, egg cholesterol and

TG showed a similar pattern with respect to the effect of probiotics. The effects of probiotic supplementation in these three analysis were observed after at least 8 weeks of study (2 periods). These observations were in agreement with studies by Mohan et al., (1995a) and Haddadin et al., (1996) who observed the effect of probiotics on laying hen plasma cholesterol and TG after 10 and 40 weeks of supplementation, respectively.

Fatty acid profile in the egg yolk responded significantly to the incorporation of FS in the diet of layers. The magnitude of change in different fatty acids, however, was variable. All diets containing 15% FS reduced C 18:1ω -9 by 12% ($p<0.05$). On the other hand C18:2ω -6 and C18:3 ω-3 contents of yolks from FS treatments increased by 20% and 93% ($p<0.05$), respectively, closely resembling their changes in the experimental diets (24.7% and 92.4% over the C diet, respectively). The longer chain metabolites of C18:3 such as EPA and DHA were increased by FS treatments whereas C20:4, the long chain product of C18:2 was reduced. Consequently, the ratio of ω -6 to ω -3 PUFA in yolk was 37:1 in the C groups; and it was significantly ($p<0.05$) reduced to 3:1 by feeding FS. This ratio from the FS group was in agreement with the ratio we found when we analyzed the purchased market ω -3 chicken eggs of 2:1. There was an important interaction between C18:3 and C20:4 ω -6. Negative relationship ($p<0.05$) between C20:4, and C18:3, and C20:4, and long chain ω -3 fatty acids were observed in the egg yolk. These results compared well with the report by Jiang et al., (1991). This interaction, as explained by Jiang et al., (1991) is perhaps due to the well known fact that the enzymatic pathway for the synthesis of arachidonic acid (AA) from C18:2 is shared by ω -3 fatty acids (Brenner, 1981), and C18:3 inhibits the Δ6 desaturase enzyme and thereby reduces the conversion of C18:2 into AA (Ireitani and Narita, 1984; Garg et al., 1988). The higher

contents of longer chain ω -3 fatty acids such as EPA and DHA might also hinder the incorporation of AA in the egg yolk, thus resulting in reduced AA content in the egg yolk. However, it was noted that probiotic supplementation had no effect on fatty acid profile. The incorporation of different fatty acid in the yolk was observed after the first period (28 days) of the study. Continuing feeding FS over the subsequent periods did not have any significant effect on changing the fatty acid profile. Thus the results on fatty acid profile were stable throughout the five periods. Van Elswyk, (1997) also reported that C18:3, EPA DHA contents of the egg yolk stabilize after a few weeks of feeding FS. As suggested by Van Elswyk, (1997), given the fact that egg yolk formation only requires nine days, a four week requirement to stabilize yolk ω -3 PUFA deposition in the egg yolk seems surprising. Although studies proved that marked increases in yolk ω -3 PUFA are observed after just one week, yolk fatty acid stabilization appears to require more time. This is perhaps because the hepatic lipid enzyme systems require more than nine days to respond to supplemented ω -3 PUFA. Biologically, C18:3 is a precursor for EPA and DHA. The efficiency of this conversion process is however, debatable. Numerous studies indicated that yolk DHA is not provided directly by dietary FS; rather the production of yolk DHA from dietary C18:3 relies upon in vivo production from elongation and desaturation of C18:3. Hence the mobilization of the enzyme required by the liver to complete these reactions may require several weeks to stabilize.

GENERAL CONCLUSION

In recent years recommendations have been made to reduce cholesterol intake and increase ω - 3 fatty acids consumption by modifying the composition of foods to increase their content of these healthful nutrients. Although the saturated fatty acid profile in the egg seems to be detrimental to human health, this study has demonstrated that altering saturated fatty acid profile in the egg yolk is easier than influencing cholesterol level. Hence feeding layers FS increases the unsaturated fatty acid content of the egg , subsequently decreasing the ratio of ω -6 to ω -3 fatty acids. Flaxseed has proved to have no detrimental effect on production parameters. However, it has the undesirable effect of elevating egg yolk cholesterol. Fortunately ,this effect can be overcome by the supplementation of the FS containing diet with LA. On the other hand probiotics supplementation in this study has clearly demonstrated their effect in lowering plasma and egg yolk cholesterol. However, there is need for a special attention to ensure that probiotic preparations are of adequate concentration and sufficiently stable in storage and during administration to the birds. Their effectiveness has also been shown to depend on the duration of supplementation. Hence there is need to ensure that effective methods of probiotic administration are used. If linolenic acid or the ratio of ω - 6 to ω -3 fatty acids is proved to be important for human health then, manipulation of egg composition seems a viable practice aimed at meeting future consumer demands. Similarly, if ω -3 fatty acid proves to be important for prevention and management of cardiovascular disease (CVD), then, agricultural commodities enriched with high levels of the healthful fatty acids and low in cholesterol may increase the

nutritional value of the foods that form the foundation of our basic diet. They may also find a market niche especially for those individuals who are at risk from CVD and to the consumer who is conscious about CVD and knowledgeable about the benefits associated with ω -3 PUFA. It is however, important to note that the controversy about fishy smell imparted by flaxseed in the egg yolk and meat needs further investigation. The successful incorporation of long chain ω-3 fatty acids and lowering cholesterol demonstrated by feeding FS and probiotic supplementation may in the long run be the most appropriate route of research in an attempt to produce the so called "healthy eggs".

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