Effects of Feeding Extruded Flaxseed on Layer Performance

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ABSTRACT

Two experiments were conducted to evaluate the effects of 2 extruded flaxseed meals (EF1 and EF2; flaxseed:alfalfa 4:1) on n-3 fatty acids deposition in eggs, egg production parameters, egg components (i.e. yolk, albumen and eggshell percentages) and cholesterol concentrations, and apparent total tract nutrient digestibility of laying hens. Moreover, in Experiment 2, birds were euthanized for measurements of blood plasma and liver fatty acid concentrations. Seventy two layers were randomly allotted to 1 of 4 dietary treatments (6 cage replicates; 3 hens/cage) and raised over an 8-week experimental period. Dietary treatments included a standard corn-soybean meal diet containing 0%, 7.5%, 15% and 22.5% EF1 for Experiment 1, and 0%, 3%, 6%, and 9% EF2 for Experiment 2.

In Experiment 1, feeding layers with EF1 up to 22.5% of the diet had no effect on feed intake, egg production and feed conversion ratio. Egg yolk and albumen percentages were similar among treatments. However, eggshell percentage increased ($P < 0.05$) with increasing level of dietary EF1. Layers fed EF1 deposited more ($P < 0.05$) total n-3 poly-unsaturated fatty acids than layers fed the control diet. For example, linolenic acid (LNA) increased ($P < 0.05$) by 610% in layers fed 22.5% EF1 compared with those fed the control diet during week 2. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were also increased ($P < 0.05$) as a result of EF1 supplementation. However, dietary EF1 had no effect on egg cholesterol, but significantly reduced ($P < 0.05$) AMEn and apparent total tract digestibility of dry matter, organic matter, crude protein and gross energy.

In Experiment 2, incorporating EF2 up to 9% of the diet had no effect on feed intake, egg production, feed conversion ratio, and egg components. However, dietary EF2 significantly increased ($P < 0.05$) total n-3 poly-unsaturated fatty acids in egg yolk, plasma and liver tissues. For example, when compared to the control diet, hens fed 9% EF2 had higher LNA (551%, 558% and 363%, respectively) and DHA (60%, 46% and 66%, respectively) in yolk, plasma and liver at week 8. Egg cholesterol was not affected by dietary EF2. Similarly, AMEn and apparent total tract nutrient digestibility of dry matter, organic matter, and gross energy were significantly reduced (*P*

< 0.05) among birds fed 9% EF2.

In conclusion, moderate levels of EF1 (i.e. 7.5%) and EF2 (i.e. 6%) are effective dietary strategies for the production of omega-3 enriched eggs (n-3 fatty acids ≥ 300 mg per egg according to regulations of the Canadian Food Inspection Agency), without compromising egg production and nutrient digestibility.

RÉSUMÉ

Deux études ont été effectués pour évaluer l'effet de deux types de moulées à base des graines de lin extrudées (EF1 et EF2; lin:luzerne 4:1) sur le dépôt des acides gras oméga-3 et du cholestérol dans l'oeuf, les paramètres de production, les composants de l'œuf (les pourcentages du jaune, l'albumine et de coquille), et la digestibilité totale apparente des nutriments chez des poules pondeuses. En outre, dans l'étude 2, les poules avaient été euthanasiés pour mesurer les concentrations des acides gras dans le sang et les tissues du foie. Soixante-douze poules pondeuses ont été répartis au hasard à 1 des 4 traitements diététiques (6 cage répliquées; 3 poules/cage) et ont été élevés pendant une période expérimentale de 8 semaines. Les traitements diététiques étaient: une moulée standard à base de maïs-soja contenant 0%, 7.5%, 15% et 22.5% de EF1 dans l'étude 1, et 0%, 3%, 6% et 9% de EF2 dans l'étude 2.

Dans l'étude 1, l'incorporation de EF1 jusqu'à 22.5% de la moulée n'a eu aucun effet sur la consommation, la production des œufs et l'indice de conversion alimentaire. Les pourcentages du jaune d'œuf et de l'albumen étaient similaires entre les traitements. Cependant, le pourcentage de coquille d'œuf avait augmenté ($P < 0.05$) lorsque les moulées contenaient des plus forts niveaux de EF1. En comparaison avec la moulée contrôle, les poules pondeuses nourrit avec EF1 ont déposés plus ($P < 0.05$) d'acides gras oméga-3 polyinsaturés dans l'œuf. Par exemple, pendant la deuxième semaine de l'étude, l'acide linolénique (LNA) avait augmenté ($P < 0.05$) par 610% chez les pondeuses alimentés avec 22.5% de EF1 comparativement à celles nourries avec la moulée contrôle. De plus, les concentrations des acides Eicosapentaénoïque (EPA) et Docosahexaénoïque (DHA), autres types d'acides gras oméga-3, dans l'œuf étaient plus élevés ($P < 0.05$) en raison d'une supplémentation en EF1 dans la moulée. Cependant, l'incorporation de EF1 dans la moulée n'a eu aucun effet sur les niveaux de cholestérol de l'œuf, mais a significativement réduit ($P < 0.05$) AMEn et la digestibilité totale apparente de la matière sèche, la matière organique, la protéine brute et l'énergie brute.

Dans l'étude 2, l'incorporation de EF2 jusqu'à 9% de la moulée n'a eu aucun effet sur la consommation, la production d'œuf, l'indice de conversion alimentaire, et les composants de l'œuf.

Cependant, EF2 a augmenté de façon significative ($P < 0.05$) les concentrations en acides gras oméga-3 polyinsaturés dans le jaune d'œuf, le sang (plasma) et les tissus du foie. Par exemple, en comparaison avec la moulée contrôle, les poules nourries avec 9% de EF2 avaient des concentrations plus élevés en LNA (551%, 558% et 363%, respectivement) et DHA (60%, 46% et 66%, respectivement) dans les jaune d'œufs, le sang (plasma) et les tissus du foie à la huitième semaine de l'étude. Toutefois, EF2 n'avait aucun effet sur le niveau de cholestérol dans l'œuf. Par contre, AMEn et la digestibilité totale apparente des nutriments de matières sèche, la matière organique, et de l'énergie brute ont été significativement réduite ($P < 0.05$) chez les pondeuses nourrit avec 9% d'EF2.

En conclusion, des niveaux modérés d'EF1 (7.5%) et EF2 (6%) pourraient être des régimes alimentaires efficaces pour la production d'œufs enrichis en acides gras oméga-3 (n-3 acides gras ≥ 300 mg par œuf conformément à la réglementation du Canadian Food inspection Agency), sans compromettre la production d'œuf et de la digestibilité des nutriments.

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CHAPTER I.

GENERAL INTRODUCTION

The poultry industry in Canada is highly developed and a major contributor to the Canadian economy. In 2014, Canada had approximately 2,600 chicken producers and 1,000 egg producers, exporting approximately $271 million in chicken and egg products to countries such as the United States, Germany and Russia. Over 30 million worth of processed eggs were exported, 55% of which were produced in Quebec and Ontario. White Leghorn is the most popular poultry breed for egg production in Canada, however Rhode Island Reds are also raised on many layer farms (Agriculture and Agri-Food Canada, 2015).

Flaxseed is an ancient crop that has been cultivated for a long time. It dates back to 7,000 years BC and originates in the Middle East or Indian regions according to historical records. It has been widely grown in many countries throughout the world. Since 1994, Canada has become the world leader of flaxseed production, accounting for nearly 30% of global production. Alberta, Saskatchewan and Manitoba are the main flaxseed producing provinces in Canada (Flax Council, 2015). Because of its high polyunsaturated fatty acid content (73% of total fatty acids), especially linolenic acid (53% of total fatty acids), flaxseed has been greatly valued and widely used in the poultry industry for the development of functional foods. Linolenic acid, a type of omega-3 fatty acid, has been found to be beneficial for human health such as in reducing the risks of cardiovascular disease, inflammation and cancer (Connor, 2000). For this reason, health practitioners recommend daily intake of a proper concentration of omega-3 fatty acids in order to reduce the incidence of multiple illnesses. However, the level of omega-3 fatty acids in Western people's diet is extremely low. From a study conducted by the Canadian Health Measures Survey in 2012, about 97.4% of Canadians are at risk of coronary heart disease. Therefore, there are growing interests for the production and consumption of omega-3 enriched eggs for human health benefits.

The most efficient way to modify the fatty acid compositions of eggs is through dietary manipulations (Grobas et al., 2001). For example, feeding layers up to 15% of flaxseed in diets

significantly increased omega-3 fatty acids in egg yolk (Ansari et al., 2006). It has been demonstrated that the concentration of omega-3 fatty acids increases proportionally with the increasing level of flaxseed in the diet. Although flaxseed has several beneficial nutritional components, it also contains several anti-nutritional factors which limit its potential usage in poultry. Alzueta et al. (2003) reported that the soluble fiber mucilage of flaxseed markedly increased intestinal digesta viscosity which reduced nutrient digestibility. In poultry, it is recommended that flaxseed be processed in order to destroy or deactivate the anti-nutritional factors. The extrusion technology is an efficient detoxification technique for flaxseed which operates under controlled conditions of pressure, temperature, screw speed and feeding rate (Wu et al., 2008). It is well-known that extrusion may eliminate most of undesirable compounds and improve nutrient utilization in poultry. Belisle Solution Nutrition Inc., has developed a new extruded flaxseed product (OmegaPlus) consisting of 80% flaxseed and 20% Alfalfa (absorbent). OmegaPlus is easily distinguished from traditional extruded flaxseed (Linpro) which contains 50% flaxseed and 50% peas (absorbent). Neveu et al., (2014) reported that α-linolenic acid and linoleic acid contents in milk increased by 60% and 29%, respectively when lactating cows were fed extruded flaxseed (OmegaPlus). Until today, this product has not been tested in laying hens. Therefore, the objectives of this study were to evaluate the effects of feeding extruded flaxseed supplemented diet on the performance of laying hens. The specific objectives were to determine the effects of extruded flaxseed on: 1) egg production; 2) egg characteristics; 3) nutrient digestibility; 4) egg fatty acid and cholesterol concentrations; and 5) fatty acid compositions of blood plasma and liver.

CHAPTER II.

LITERATURE REVIEW

2.1 Flaxseed

Flaxseed (*Linum usitatissimum*) is one of the most ancient crop that has been grown for a long time. Historical records show that it dates back to 7,000 BC and originates in the Middle East or Indian regions (Flax Council, 2015). Recently, it has been cultivated in many countries throughout the world most of which are in the northern hemisphere. Canada is the world's largest producer of flaxseed, accounting for nearly one third of total production, and is also the leading exporter since 1994. In Canada, flaxseed is mainly produced from 3 provinces, namely Alberta, Saskatchewan and Manitoba. In the year 2014, the flaxseed production in Canada was 816.2 thousand tons, and China, USA, Europe and Japan were the major exporting countries (Flax Council, 2015).

The initial use of flaxseed was in breads and cereals as food ingredient (Stitt, 1994). However, after the industrial revolution, flaxseed oil became very popular and was mainly used in paints. Today, a large number of products containing flaxseed have been developed for human and pet food market. Flaxseed is a rich source of omega-3 linolenic acid (LNA) and phenolic components such as lignans which have been shown to possess human health benefits (Oomah and Mazza, 2000). Because consumer's interest towards functional food have increased over the years, flaxseed has become a promising resource for the production of healthy and high quality food products.

2.1.1 Chemical composition of flaxseed

The composition of flaxseed is provided in Table 2.1. Flaxseed consists of 38 to 45% fat, which constitutes the largest chemical component, 20% protein, 28% fiber, 7% moisture and 4% ash. Chemical composition of flaxseed may vary according to samples, growing environment, method of measurement and seed processing (Daun et al., 2003).

Table 2.1. Chemical composition of flaxseed

Source	Fat %	Protein %	Total fiber %	Water %	Ash %
Flax Council of Canada	41.0	20.0	28.0	7.0	4.0
USDA	42.16	18.29	27.3	6.96	3.5

USDA: United States Department of Agriculture

Flaxseed contains high fat percentage and a unique mix of fatty acids with greater proportion of polyunsaturated fatty acids, especially the essential omega-3 LNA which accounts for 53% of total fatty acids (Table 2.2).

Table 2.2. Fatty acid composition of flaxseed

Fatty acid (% of total fatty acids)	
C16:0	5-6
C18:0	3-6
C18:1n-9	19-29
C18:2n-6	14-18
C18:3n-3 (LNA)	45-52
Total monounsaturated	18
Total polyunsaturated	73
Total saturated	9

Adapted from Bhatty, 1995 and Cunnane et al., 1993

2.1.2 Anti-nutritional factors of flaxseed

Flaxseed naturally contains several anti-nutritional factors such as cyanogenic glycoside, mucilage, linatine and phytic acid (Ganorkar and Jain, 2013). Cyanogenic glycoside and linatine release hydrogen peroxide when ingested and have adverse effects on growth of birds (Mazza and Oomah, 1995; Mandokhot and Singh, 1983). However, hydrogen peroxide can be denatured by

heat treatment such as that used during oil extraction of flaxseed. In most cases, whole flaxseed fed to chickens without heat treatment has detrimental effects on bird performance. Flaxseed contains a pyridoxine antagonist known as linatine which can cause vitamin B6 deficiency in chicks (Kratzer, 1946). It is therefore recommended that flaxseed diets be supplemented with extra vitamin B6 to offset the negative influence of linatine on this vitamin. Flaxseed mucilage is a water soluble and indigestible fiber which consists of galactose, xylose, rhamnose and galacturonic acid (Fedeniuk and Biliaderis, 1994). It has been shown to increase gut viscosity which decreases nutrient digestibility by increasing feed passage rate and reducing diffusion rates of endogenous enzymes (Classen and Bedford, 1991; Fengler and Marquardt, 1988; Rodriguez et al., 2001). Because of its strong chelating properties, phytic acid has a negative effect on zinc and iron absorption and protein digestibility which ultimately results in poor growth of birds (Thompson, 1989). Therefore, it is important that flaxseed is appropriately processed (e.g. grinding, heat treatment and extrusion) before being fed to chickens in order to destroy or reduce the concentration of anti-nutritional factors that it naturally contains.

2.1.2.1 Feeding ground/milled flaxseed

Previous studies have shown that the form of flaxseed (e.g. whole vs. ground) affects layers performance (Choi et al., 1986). Caston et al. (1994) reported that feeding whole flaxseed up to 20% of the diet had a negative effect on hen weight gain at 52 and 73 wk of age. Similarly, Aymond and Van Elswyk (1995) found that egg production was decreased by feeding 15% of whole flaxseed to young hens over a 5-wk period. However, Jiang et al. (1991) reported that feeding 15% of ground flaxseed to 16-month-old birds had no influence on egg production and egg weight during 4 wk. Scheideler and Froning (1996) also reported that egg weight was not affected by feeding 10% of ground flaxseed, while egg production was increased in birds fed 5 and 10% of ground flaxseed diets than the control diet.

Ground/milled flaxseed also influences the fatty acid compositions of egg yolk. Due to the higher laxation possibility of whole flaxseed for birds, feeding ground/milled flaxseed can be the

most effective way to increase α-linolenic acid (ALA) bioavailability (Tarpila, et al., 2002). Many researchers suggest that deposition of omega-3 fatty acids in egg yolk is more efficient when flaxseed is ground or milled. The study of Van Elswyk (1997) indicated that 16.2 mg ALA/g of yolk was deposited when feeding 10% of milled flaxseed while 10% of whole flaxseed yielded only 13.5 mg ALA/g of yolk. Similar result was reported by Botsoglou et al. (1998), who found that ALA availability for yolk deposition was reduced by feeding layers with 10% of whole flaxseed compared with 10% of ground flaxseed. Aymond and Van Elswyk (1995) observed higher deposition of omega-3 fatty acids in egg yolk when birds were fed 15% of ground flaxseed compared to the same level of whole flaxseed. These differences may be explained by the fact that ground/milled flaxseed is better digested than whole flaxseed and releases more fat to be deposited in egg yolk. In addition, the use of ground/milled flaxseed decreased saturated fatty acids and omega-6 fatty acids, but was not different from whole flaxseed.

2.1.3 Performance of laying hens fed flaxseed

Flaxseed is a leading source of omega-3 fatty acids which are essential to human growth. In order to provide healthy products and expand food market, flaxseed has been widely used as an ingredient added to diets for poultry in commercial industry. However, the effects of flaxseed on performance of laying hens may vary according to different strain of hens, age of hens, diet composition and experimental period.

2.1.3.1 Flaxseed and egg production

Data on the effects of feeding flaxseed on egg production are highly inconsistent (Table 2.3). Feeding 5% (Yannakopoulos et al., 1999) and 10% (Yannakopoulos et al., 1999; Novak and Scheideler, 2001; Bean and Leeson, 2003) of flaxseed through the diets had no effect on egg production. No effect of 15% of flaxseed on egg production was also demonstrated by Antruejo et al. (2011). In contrast, a positive effect on egg production was observed by feeding 5 and 10% of ground flaxseed (Scheideler and Froning, 1996). Aymond and Van Elswyk (1995) and Leeson et

al. (2000) reported a reduction in egg production when flaxseed was included at 15 and 20% of the diet, respectively. It has been suggested that flaxseed contains anti-nutritional factors which can impair digestion and absorption of nutrients and finally lead to the decrease in egg production at higher inclusion levels.

Table 2.3. Effects of flaxseed on egg production

Author	Flaxseed inclusion (%)	Effect
Yannakopoulos et al. (1999)	5 and 10	unchanged
Novak and Scheideler (2001)	10	unchanged
Bean and Leeson (2003)	10	unchanged
Antruejo et al. (2011)	15	unchanged
Scheideler and Froning (1996)	5 and 10	10% increase
Leeson et al. (2000)	20	7% decrease

2.1.3.2 Flaxseed and egg weight

The effects of flaxseed inclusion on egg weight vary depending on several factors such as inclusion level and feeding form (Table 2.4). Rizzi et al. (2009) reported that feeding 10% of linseed oil increased egg weight. While AI-Nasser et al. (2011) showed that egg weight was unchanged among hens fed 5, 7.5 and 15% of flaxseed. In agreement with AI-Nasser et al. (2011), Ahmad et al. (2013) reported no effect on egg weight by feeding 5, 10 and 15% of flaxseed. Similarly, Jiang et al. (1991) found that feeding 15% of ground flaxseed had no effect on egg weight. In contrast, Scheideler and Froning (1996) observed that egg weight was reduced when whole and ground flaxseed were fed at 5 and 10% of the diet. Whitehead (1993) reported a relationship between egg weight and regulation of hormone metabolism, especially estrogen activity. The decrease in egg weight is likely due to flaxseed phytoestrogen which may result in a decrease in concentrations of serum estrogen (Novak and Scheideler, 2001).

Table 2.4. Effects of flaxseed on egg weight

Author	Flaxseed inclusion (%)	Effect
Rizzi et al. (2009)	10	14% increase
Jiang et al. (1991)	15	unchanged
AI-Nasser et al. (2011)	5, 7.5 and 10	unchanged
Ahmad et al. (2013)	5, 10 and 15	unchanged
Scheideler and Froning (1996)	5 and 10	2 and 3% decrease

2.1.3.3 Flaxseed and feed intake

Different effects on feed intake as a result of flaxseed feeding have been reported (Table 2.5). Caston et al. (1994) and Leeson et al. (2000) observed that birds fed 20% of ground flaxseed consumed more feed than those fed the control diet or 10% of ground flaxseed. This is likely due to a reduction in apparent metabolizable energy and concomitant compensation on increased feed consumption. In contrast, Pheko et al. (1998) reported that feed intake was not changed when birds were fed 0 and 15% of flaxseed. Similar result was observed by Novak and Scheideler (2001) who found that feeding 10% of flaxseed had no detrimental effects on feed consumption. However, Hayat et al. (2009) reported that feed intake was decreased in hens fed 10% of whole flaxseed diet compared with the control diet. This reduction in feed intake is likely attributed to anti-nutritional factors in flaxseed which have a negative effect on palatability. Hulan et al. (1989) illustrated that feed intake in poultry was affected by palatability problems.

Table 2.5. Effects of flaxseed on feed intake

Author	Flaxseed inclusion (%)	Effect
Caston et al. (1994)	20	5-17% increase
Pheko et al. (1998)	15	unchanged
Novak and Scheideler (2001)	10	unchanged
Hayat et al. (2009)	10	4% decrease

2.1.3.4 Flaxseed and feed conversion ratio

Most studies showed that feed conversion ratio was not affected by flaxseed supplementation (Table 2.6). Basmacioglu et al. (2003) reported that inclusion of 4.3 and 8.6% flaxseed in the diet did not change feed conversion ratio. Similar results were observed by Hayat et al. (2009) and AI-Nasser et al. (2011) who showed that feeding flaxseed at levels of 5, 7.5 and 10% had no influence on feed conversion ratio. However, Sari et al. (2002) reported that feed conversion ratio was lower in layers fed 5, 10 and 15% of flaxseed diets than those fed the control diet. Supplementation of flaxseed increases dietary fat concentration and higher fat is known to lower feed conversion ratio.

Table 2.6. Effects of flaxseed on feed conversion ratio

Author	Flaxseed inclusion (%)	Effect
Basmacioglu et al. (2003)	4.3 and 8.6	unchanged
AI-Nasser et al. (2011)	5, 7.5 and 10	unchanged
Hayat et al. (2009)	10	unchanged
Sari et al. (2002)	5, 10 and 15	4, 7 and 8% decrease

2.1.4 Effects of flaxseed on egg components

Egg component includes three main parts: yolk, albumen and shell. Over 50% of egg protein is deposited in the albumen which provides high-protein and low-fat nutrition for humans. In addition to protein, the yolk also contains cholesterol and minerals. The effects of flaxseed supplementation on egg component are showed in Table 2.7. Several studies indicated that feeding low (i.e. 4.3%, Basmacioglu et al., 2003) or moderate (i.e. 10%, Scheideler and Froning, 1996; Kirubakaran et al., 2011; Hayat et al., 2009) levels of flaxseed had no effects on egg component. Caston et al. (1994) reported that eggs laid by hens fed 10 and 20% of ground flaxseed had lower percentage of yolk. The decrease in yolk percentage was also observed by Scheideler and Froning (1996) when feeding 15% of ground flaxseed. Similar reduction in egg yolk percentage was

reported by Bean and Leeson (2003) when layers were fed 10% of flaxseed. On the contrary, Rizzi et al. (2009) observed no change in yolk percentage by 10% of linseed oil-rich diet while albumen percentage increased and shell percentage decreased. Their findings were supported by Novak and Scheideler (2001) and Aziza et al. (2013), who noted an increase in albumen percentage as a result of feeding 10% of flaxseed. However, the results of Yannakopoulos et al. (2005) showed that feeding diets enriched with flaxseed decreased albumen percentage.

Table 2.7. Effects of flaxseed on egg components

Author	Flaxseed inclusion (%)	Yolk wt (%)	Albumen wt (%)	Shell wt (%)
Caston et al. (1994)	10 and 20	7% decrease	-	-
Bean and Leeson (2003)	10	2% decrease	-	-
Novak and Scheideler (2001)	10	unchanged	1% increase	2% decrease
Aziza et al. (2013)	10	unchanged	3% increase	10% decrease
Scheideler and Froning (1996)	4.3 and 8.6	unchanged	unchanged	unchanged
Basmacioglu et al. (2003)	10	unchanged	unchanged	unchanged
Kirubakaran et al. (2011)	10	unchanged	unchanged	unchanged
Hayat et al. (2009)	10	unchanged	unchanged	unchanged

2.1.5 Effects of flaxseed on nutrient digestibility

The addition of flaxseed in poultry diet has been shown to cause a detrimental effect on nutrient digestibility. The reduction in nutrient digestibility can be attributed to the presence of anti-nutritional factors such as water soluble mucilage, water insoluble non-starch polysaccharides, phytate and trypsin inhibitor in flaxseed (Madhusudhan et al., 1986). Aziza et al. (2013) reported a lower digestibility of crude protein for layers fed 10% of flaxseed compared with those fed the control diet. The negative effect on crude protein digestibility is likely due to the high phytic acid content in flaxseed. Phytic acid can precipitate most proteins at acidic pH by forming strong

electrostatic linkages with residues of lysine, arginine and histidine (Graf and Eaton, 1990). Moreover, it also inhibits the biological functions of proteolytic enzymes (Deshpande and Damodaran, 1989). Alzueta et al. (2003) reported a similar reduction in fatty acid digestibility from 89 to 60% for birds fed 16% of flaxseed diet. The authors attributed the reduction in fatty acid digestibility to the presence of mucilage in flaxseed-containing diets. Fat digestibility reduction could also result from the cell wall polysaccharides which encapsulated oil and/or the presence of other anti-nutritional factors (Slominski et al., 2006). Mucilage increased intestinal viscosity after mixing with water or other fluids (Rodriguez et al., 2001). Furthermore, viscous digesta depressed enzymatic activities and minimized nutrients diffusion capacity which made it difficult for nutrient digestion (Smits and Annison, 1996). Similarly, Scheideler et al., (1998) observed that feeding 11% of flaxseed to birds at age of 9-week and 8% of flaxseed to birds at age of 16-week decreased digestibilities of protein and fat. Flaxseed also contains significant amounts of water insoluble non-starch polysaccharides (dietary fiber) that act as a physical barrier encapsulating intracellular nutrients, suggesting that fiber content of flaxseed impaired the utilization of energy (Jia et al., 2009). Ortiz et al. (2001) reported that increasing dietary flaxseed fiber level in broiler diet from 4 to 24% decreased AMEn from 2,815 to 2,091 kcal/kg.

2.1.6 Effects of flaxseed on egg composition

Several studies have shown that health promoting fatty acids in broiler meat and eggs can be increased through incorporating omega-3 fatty acids content into poultry diets. Flaxseed contains high levels of linolenic acid and can be used to enrich eggs with omega-3 fatty acids. Omega-3 fatty acids such as LNA are essential because they cannot be synthesized by the human body. However, people in western countries do not consume sufficient food rich in omega-3 fatty acids. Daily intake of omega-3 fatty acids has many health benefits such as reducing the risk of heart disease, delaying the loss of immunological functions and providing inhibitory effects on several cancers; the consumption of omega-3 enriched eggs may offer an alternative source of these essential fatty acids to Canadians (Lewis et al., 2000).

2.1.6.1 Flaxseed and egg yolk fatty acid

Generally, most of yolk fatty acids are unsaturated fatty acids, comprising approximately 48% monounsaturated and 18% polyunsaturated fatty acids (Table 2.8). However, fatty acid profile of egg yolk may be affected by several factors such as diet, age and strain (Milinsk et al., 2003). The fact that diet supplemented with flaxseed can decrease saturated fatty acids (SFA) and increase unsaturated fatty acids (USFA), especially n-3 fatty acids, is well documented. Ferrier et al. (1995) reported that concentrations of LNA (101%) and DHA (7%) were markedly increased by feeding layers with 10 and 20% of flaxseed. Similar results were reported by Yalcyn et al. (2007) who observed that feeding 4.3 and 8.6% of flaxseed diets increased the concentration of LNA by 463% and 850%, respectively. The authors also found higher concentration of DHA (162% and 205%) in egg yolk from flaxseed-fed group than control group. This is likely due to high content of LNA in flaxseed. Linoleic acid as the precursor for long chain polyunsaturated fatty acids can be converted to DHA in the liver, which ultimately leads to the increase in DHA concentration.

Processed flaxseed used in other studies has shown similar modifications in egg yolk fatty acid profile. Scheideler and Froning (1996) compared ground flaxseed to the control diet and stated that there was a significant increase in LNA concentration. However, no difference in the concentration of LNA was observed by feeding the same level of whole and ground flaxseed.

Due to the increase in LNA and DHA concentrations, flaxseed supplementation increases total n-3 fatty acids in egg yolk. However, total n-6 fatty acids are reduced at the same time, particularly that of arachidonic acid (AA). Jiang et al. (1991) reported that laying hens fed 10% of ground flaxseed diet had lower level of AA in egg yolk compared with hens fed the control diet. Their results are consistent with Hayat et al. (2009) who observed that concentration of AA was significantly reduced in response to flaxseed supplementation and Sari et al. (2002) who also observed that feeding 5, 10 and 15% of flaxseed diets resulted in a lower yolk concentration of AA (1.65%, 1.57% and 1.32% vs 2.38%). The reduction in AA concentration as a result of flaxseed is likely due to the competition for desaturation and elongation enzymes which regulates the conversion of LNA to DHA and LA to AA (Fraeye et al., 2012). When LNA is present in a large

amount, it is preferred by enzymes over LA (Craig-Schmidt et al., 1987).

Table 2.8. Fatty acid composition of egg yolk

Fatty acid (% of total fatty acids)	
C14:0	0.36
C16:0	25.1
C16:1	3.23
C18:0	8.37
C18:1n-9	46.7
C18:2n-6 (LA)	13.1
C18:3n-3 (LNA)	0.51
C20:4n-6 (AA)	1.83
C22:6n-3 (DHA)	0.85
Total monounsaturated	50.0
Total polyunsaturated	16.3
Total saturated	33.8

Adapted from Samman et al. (2009)

2.1.6.2 Flaxseed and egg cholesterol

Recently, there has been growing interest among researchers, egg producers and consumers in lowering the concentration of cholesterol in egg yolk. This is because cholesterol increases the risk for cardiovascular disease and heart attack, and reduces egg production when eggs contained higher amount of cholesterol (Bartov et al., 1971). Hence, there is an interest in using flaxseed to alter egg yolk cholesterol concentration. Based on the previous studies, the effects of flaxseed on egg cholesterol are very consistent (Table 2.9). No change in egg total cholesterol was reported by Ferrier et al. (1995) when 10 and 20% of flaxseed were fed to laying hens. Caston and Leeson (1990) observed that egg total cholesterol was unaffected in hens fed ground flaxseed at levels of

10, 20 and 30%. Their findings were supported by Botsoglou et al. (1998) and Hayat et al. (2009) who all stated that feeding 10% of whole or ground flaxseed did not affect egg total cholesterol. Similarly, Basmacioglu et al. (2003) who found no difference in egg total cholesterol between 4.3 and 8.6% of flaxseed and control diets (219, 202 and 205 mg, respectively). The lack of cholesterol changes to flaxseed supplementation can be attributed to the fact that sufficient cholesterol in the egg yolk is essential for the development of embryo (Hargis, 1988).

Table 2.9. Effects of flaxseed on egg cholesterol

Author	Flaxseed inclusion %	Effect
Ferrier et al. (1995)	10 and 20	unchanged
Caston and Leeson (1990)	10, 20 and 30	unchanged
Botsoglou et al. (1998)	10	unchanged
Hayat et al. (2009)	10	unchanged
Basmacioglu et al. (2003)	4.3 and 8.6	unchanged

2.1.7 Effects of flaxseed on fatty acid composition of blood plasma and liver

Studies on the effects of flaxseed supplementation on fatty acid composition of plasma and liver are rather limited. However, the results were similar to those findings for egg yolk. Inclusion of flaxseed significantly increases the concentrations of n-3 fatty acids, especially LNA and DHA in blood plasma and liver. Caston et al. (1994) found that feeding 10 and 20% of ground flaxseed increased liver concentration of LNA by 856% and 1,700%, respectively. The authors also observed an approximately 2-fold increase in liver DHA concentration from hens fed 10 and 20% of ground flaxseed diets. Their findings are in accordance with Cherian and Hayat (2009) who observed that there were 800% and 1,700% increase in LNA concentration of plasma and liver respectively by feeding 10% of flaxseed to layers. A significantly increased DHA concentration in plasma and liver was also reported by the authors as a result of flaxseed supplementation. It is suggested that the increase in n-3 fatty acids of plasma and liver may take a similar pattern as egg

yolk. However, the concentration of AA changed differently in plasma and liver. Incorporating flaxseed to laying hens did not affect liver AA concentration while decreased AA concentration in plasma (Caston et al., 1994; Nain et al., 2012; Cherian and Hayat, 2009).

2.2 Extruded flaxseed

Extrusion is a highly intensive process which forces feed to flow through a die under controlled conditions of temperature and pressure (Anjum, et al., 2013). It combines several steps such as feeding, pinching, heating, and mixing. High temperatures of the extrusion processing leads to a rapid hydrolysis of cyanogenic glycosides and produces hydrocyanic acid (HCN), while HCN can be removed along with the evaporating water during the release of pressure (Feng et al., 2003). Generally, a twin-screw extruder is applied on extrusion of flaxseed (Harper, 1979).

As a detoxification technique, it is often applied to alter the chemical composition of flaxseed. Wu et al. (2009) demonstrated that about 60.3% mucilage could be removed from flaxseed at different extruder die temperatures ranging from 80 to 160 ℃. Similarly, a decrease in content of NDF of flaxseed was reported by Gonthier et al. (2004) as a result of extrusion.

Furthermore, extrusion can lead to a rapid release of intracellular flaxseed oil which results in the loss of fat. Therefore, a suitable absorbent is needed during the extrusion process for flaxseed oil absorption. For the extruded flaxseed product Linpro, peas are used as absorbent in the ratio of 50% flaxseed and 50% peas. Another extruded flaxseed product (OmegaPlus) which was later developed consisting of 75% flaxseed and 25% ground alfalfa (Neveu et al., 2013). However, there are limited studies investigating the effects of extruded flaxseed on the performance of layer chickens.

2.2.1 Performance of laying hens fed extruded flaxseed

The results from a layer study by Nain et al. (2012) showed that feeding 7.5 and 15% of extruded flaxseed (Linpro) did not influence egg weight and feed intake. Similar finding was observed by Imran et al. (2015) who reported no difference in egg weight between extruded

flaxseed diets (i.e. 10 and 20%) and the control diet. However, layers had lower feed consumption when feeding 10 and 20% of extruded flaxseed. In addition, Imran et al. (2015) also found that egg production decreased in response to increasing level of extruded flaxseed in the diet.

2.2.2 Effects of extruded flaxseed on egg composition

Extruded flaxseed increases the deposition of omega-3 fatty acids in eggs. Nain et al. (2012) observed that feeding 7.5 and 15% of extruded flaxseed (Linpro) for 6 days significantly increased egg yolk concentration of LNA by 212% and 348%, respectively. Imran et al. (2015) also reported a 10-fold increase in egg yolk LNA concentration for layers fed 30% of extruded flaxseed. Both of these two studies showed that the concentration of DHA was markedly increased as well. However, Jia et al. (2008) found lower deposition of LNA in egg yolk from layers fed 15% of Linpro diet than those fed 15% of flaxseed diet (305 mg vs 429 mg). The authors attributed the reduction in LNA content to the lower proportion of flaxseed in Linpro diet compared with the flaxseed diet. In addition, Omega-6 fatty acids concentrations were reduced as a result of extruded flaxseed supplementation. A reduction in egg yolk AA concentration from hens fed 7.5 and 15% of Linpro diets was found by Nain et al. (2012). Similarly, feeding 10 and 20% of extruded flaxseed supplemented diets decreased the concentration of AA in egg yolk by 41 and 59% respectively (Imran et al., 2015). Moreover, Imran et al. (2015) observed that egg total cholesterol was not affected by 10, 20 and 30% of extruded flaxseed diets.

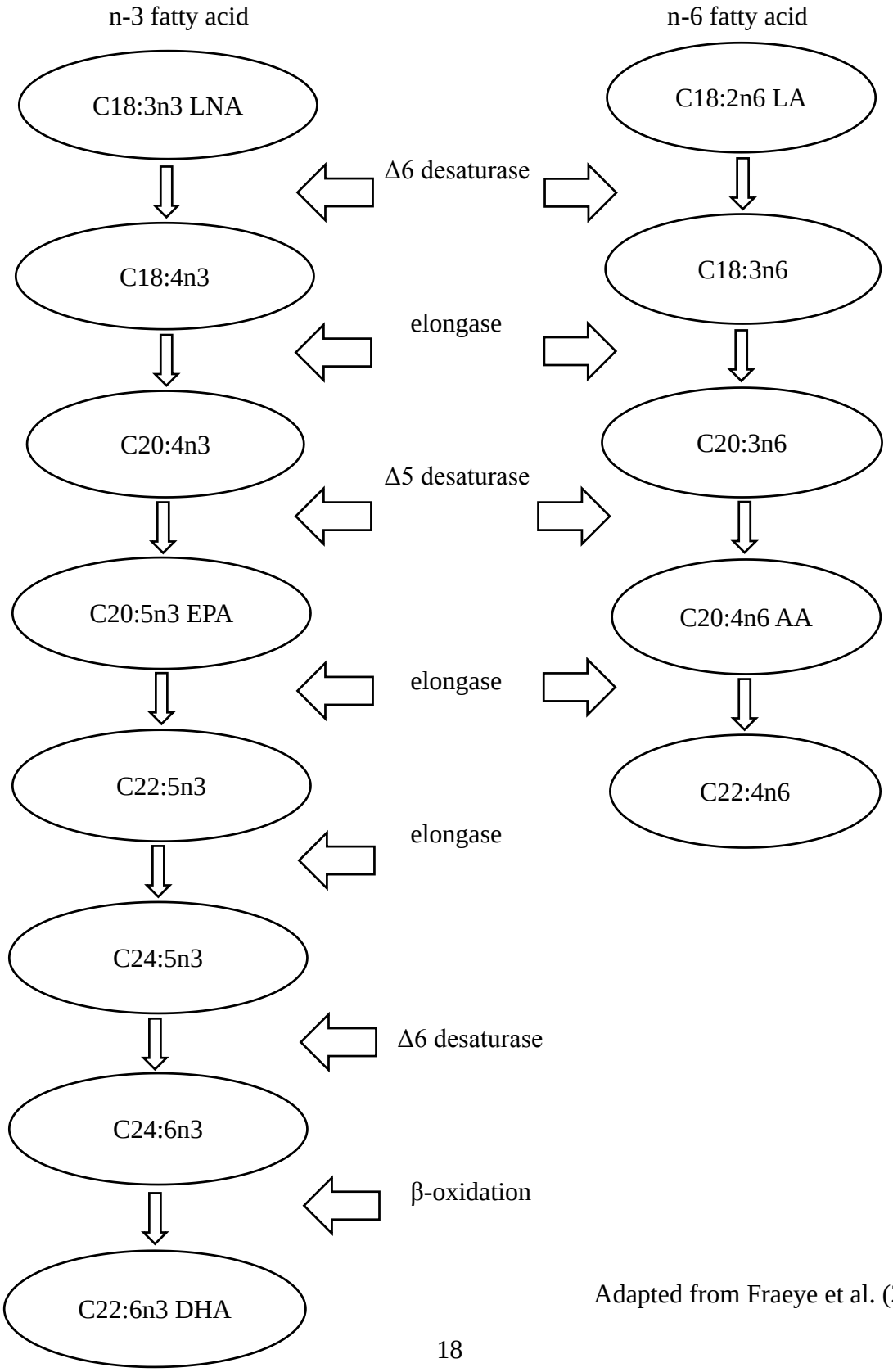
2.3 Metabolism of polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) comprise two families, the n-3 and n-6 PUFA which can be distinguished by the location of double bond on the methyl end. The series of n-3 PUFA contain three major USFA including linolenic acid (C18:3n3, LNA), eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6n3, DHA). Linoleic acid (C18:2n6, LA), arachidonic acid (C20:4n6, AA) and docosatetraenoic acid (C22:4n6) are the major types of n-6 PUFA. Both LNA and LA are categorized as essential fatty acids as they cannot be synthesized by

the human body and are necessary for normal human health (Spector, 1999). These fatty acids also act as substrates for the biosynthesis of longer chain PUFAs (Burdge, 2006). Linolenic acid is the precursor for generating more unsaturated fatty acids such as EPA and DHA after desaturation and elongation reactions (Burdge and Wootton, 2002). However the rate of this conversion in humans is affected by dietary linoleic acid intake (Adam et al., 1986). Initially, LNA is converted in the liver to EPA via the function of desaturase, followed by the elongation by adding two carbons; then EPA continues to be catalyzed through reactions of desaturation, elongation and β-oxidation, and is finally converted to DHA (Fraeye et al., 2012; Fig. 1). Similarly, the same desaturase and elongase transform LA to higher unsaturated derivatives (i.e. AA and docosatetraenoic acid). Although LNA is the preferred substrate, the catalytic enzymes also have an influence on LA (Burdge, 2004). Δ^6 desaturase is regarded as the rate-limiting enzyme (Benatti et al., 2004). The competition of these two fatty acids for the involved enzymes therefore determines which one takes the predominant place.

In addition, dietary manipulation also regulates the activity of desaturase enzyme (Poisson and Cunnane, 1991). Several studies with rats have showed that LNA could significantly suppress the metabolism of LA, such that 10 times more LA was needed to produce the equivalent result in return (Holman, 1998). The consumption of diets enriched with LNA can effectively provide more EPA and DHA to consumers. When people ingest higher amount of LA over LNA, this impedes the health benefits associated with n-3 PUFA leading to numerous health problems; therefore, diets should be balanced for n-3 and n-6 PUFA targeting for low ratio of n-6 to n-3 PUFA (Simopoulos, 2002). Health Canada recommendations for daily intake of LNA are 1.1 and 1.6 g/day for women and men, respectively (Morris, 2003a).

Fig. 1 Metabolism of two families of PUFA



Adapted from Fraeye et al. (2012)

2.4 Physiological role of n-3 PUFA

Over the past years, the biological function of n-6 PUFA is well known by researchers as it relates to the essential fatty acid deficiency diseases such as dermatitis, infertility and growth retardation (Lauritzen et al., 2001). The possible explanations are summarized as: first, linoleic acid as a component of skin ceramide is associated with barrier function (Hansen, 1989); second, arachidonic acid can be converted to eicosanoids which are important for many physiological responses and pathological processes (Harizi et al., 2008); third, metabolites of arachidonic acid probably serve as intracellular second messengers in regulation of signal transduction (Chen et al., 1999). Later, the physiological role of n-3 PUFA was better understood. Except being a substitute of n-6 PUFA under some certain conditions, their unique functions have been investigated and identified. It is well documented that n-3 PUFA are beneficial for the prevention of cardiovascular disease, cancer, diabetes, and inflammatory and autoimmune disorders.

2.4.1 Cardiovascular disease

The effects of n-3 PUFA on cardiovascular disease (CVD) has been recognized since 60 years ago. The British physiologist Hugh Sinclair made his early investigation in Greenland Eskimos exploring the adverse effects of some essential fatty acids deficiency on CVD. Later, his group observed a positive connection between the high intake of diet abundant in EPA and DHA and low death rate as a result of coronary atherosclerotic disease (Dyerberg et al., 1975). Generally, increased consumption of EPA and DHA reduced the concentrations of serum triglyceride and very low density lipoprotein (VLDL) cholesterol (Sanders et al., 1989). Prichard et al. (1995) reported that serum triglyceride was also decreased and high density lipoprotein cholesterol was increased as a result of moderate level of fish oil. In addition, a large dose of n-3 PUFA in human would reduce platelet aggregation and blood viscosity, and this is also helpful to lower the occurrence of CVD. Compared with the Danish population with high incidence of CVD, Greenland Inuits obtained substantial amounts of n-3 PUFA from marine animals, while Danish obtained saturated fatty acids from land animals only. Through analyzing the plasma lipid composition of Eskimos,

less cholesterol and triglycerides were found (Dyerberg et al., 1975). Similarly, Japanese had lower rate of atherosclerosis and acute myocardial infarction than North Americans due to higher intake of aquatic products (Menotti et al., 1999). It seems that n-3 PUFA can lower the risk of CVD by changing blood lipid status and concentration.

2.4.2 Inflammatory and autoimmune disorders

Studies have shown that inflammatory and immune reactions can be modified by supplementing tissues with n-3 PUFA and the derivative leukotriene seems to play an important role in adjusting this process. It can be generated from both n-3 and n-6 PUFA. Arachidonic acid is the substrate for prostaglandins of the 2 series (PGE2) and leukotrienes of the 4 series (LTB4); when n-3 EPA increases, more prostaglandins of the 3 series (PGE3) and leukotrienes of the 5 series (LTB5) are produced. Compared with LTB4, LTB5 can cause less inflammation and immunologic response (Shapiro et al., 1993). In the study of Kremer et al. (1987), it was found that the ingestion of fish oil alleviated active rheumatoid arthritis and decreased the production of neutrophil LTB4 in patients with arthritis. Under conditions of ulcerative colitis, LTB4 acted as the key mediator and was capable of attracting additional neutrophil to mucosa and deteriorating the disease (Stenson et al., 1992). In fact, fish oil achieved this goal by prohibiting the 5-lipoxygenase pathway in neutrophil and monocytes and suppressing the role of LTB4 and increasing the generation of LTB5 (Lee et al., 1985). When changing dietary fat from beef tallow to fish oil, the spontaneous autoimmune renal disease could be avoided in a genetic strain of NZB mice (Prickett et al., 1983). These protective effects of n-3 PUFA are attributed to EPA which inhibits the production of metabolites of AA.

CHAPTER III.

Effects of extruded flaxseed on egg production, egg components, nutrient digestibility, and egg yolk fatty acid and cholesterol compositions of laying hens

3.1 ABSTRACT

A study was conducted to determine the effects of feeding extruded flaxseed (EF1) on egg production, egg components, egg yolk fatty acid and cholesterol concentration, and apparent total tract nutrient digestibility in layer chickens. Seventy two White Leghorn laying hens (58-week-old) were randomly allotted to 4 treatments. Each treatment was replicated six times with three hens per replicate. Hens were fed diets containing EF1 at 0, 7.5, 15 and 22.5% over an 8-week experimental period. Results showed that feeding EF1 had no effect on feed intake, egg production, feed conversion ratio (FCR), and egg weight. Egg components (yolk, albumen and shell percentages) were similar among treatments with the exception of shell percentage which was greater for hens fed 22.5% EF1 than hens fed 7.5 and 15% EF1. Apparent total tract digestibility (ATTD) of DM and OM were highest ($P < 0.05$) for 0 and 7.5% EF1, intermediate ($P < 0.05$) for 15% EF1 and lowest ($P < 0.05$) for 22.5% EF1. Hens fed 15 and 22.5% EF1 had lower ATTD of GE than hens fed 0 or 7.5% EF1. Apparent total tract digestibility of CP and AMEn were lower ($P < 0.05$) for hens fed 22.5% EF1 than those fed 0 and 7.5% EF1. Fatty acid compositions of egg yolk were significantly altered by EF1 supplementation. Feeding EF1 increased ($P = 0.0008$) unsaturated and decreased ($P < 0.0001$) saturated fatty acid concentrations in egg yolks. Relative to the control diet, feeding 7.5, 15 and 22.5% EF1 increased linolenic acid concentrations in eggs by 137, 471 and 610% respectively at week 2. It was concluded that feeding EF1 up to 22.5% had no negative effects on egg production parameters, egg components or egg yolk cholesterol concentration. However, dietary supplementation with EF1 can significantly alter egg fatty acid profile by increasing n-3 PUFA depositions in egg yolks. Feeding moderate to high levels (15 and

22.5%) of EF1 can reduce ATTD and energy utilization. In this study, the production of omega-3 enriched eggs (n-3 fatty acids ≥ 300 mg per egg according to regulations of the Canadian Food Inspection Agency) was achieved by feeding 7.5% EF1 to laying hens over a period of 2 weeks.

Key words: laying hen, extruded flaxseed, n-3 fatty acids, cholesterol

3.2 INTRODUCTION

Flaxseed is an excellent source of linolenic acid (LNA), an omega-3 polyunsaturated fatty acid (PUFA), which makes up about 53% of total fatty acids in flaxseed (Bernacchia et al., 2014). Linolenic acid cannot be synthesized by the human body, and therefore should be obtained from dietary sources. It is also a precursor for synthesis of another two long chain omega-3 PUFA, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Over the past years, numerous researches have shown that LNA is an essential fatty acid that has beneficial effects to human health, especially in prevention and treatment of cardiovascular disease, development disorders, hypertension, inflammation and cancer (Simopoulos, 2000). However, the use of flaxseed in the diets of animals is somewhat limited because flaxseed naturally contains several anti-nutritional factors such as cyanogenic glycosides, mucilage and phytic acid (Dale and Batal, 2008). These compounds negatively affect enzyme secretions from the pancreas leading to significant reductions in feed intake of animals (Klosterman et al., 1967) and increase intestinal digesta viscosity which reduce nutrient digestibility (Rodriguez et al., 2001). Extrusion process is the most effective and economical means to deactivate these anti-nutritional factors contained in flaxseed. In addition to eliminating most anti-nutritional factors under high pressure and temperature treatments, the extrusion process releases intercellular oil from flaxseed grains, making the oil more absorbable and available to the animals.

Recent studies have used flaxseed to increase n-3 PUFA depositions in poultry eggs and meat (Ajuyah et al. 1991). Birds are able to absorb the nutrients from flaxseed-supplemented diets and further convert linolenic acid into EPA and DHA for deposition in eggs and meat as lipids. This results in changes in both the compositions and nutritional values of eggs. Scheideler and

Froning (1996) reported that LNA and EPA contents of eggs were significantly increased when layers fed a 10% flaxseed diet compared to the control diet. However, addition of flaxseed into layer diets could decrease the weight and percentage of egg yolk, and this might be related to changes in the cholesterol concentrations of eggs (Scheideler and Froning, 1996). Based on the past research, the effects of flaxseed on performance of laying hens are inconsistent. Moreover, the effects of EF on nutrient digestibility and egg yolk cholesterol have not been investigated yet.

Therefore, the first objective of this study was to determine the effects of diets containing different concentrations of EF1 (OmegaPlus, Belisle Solution Nutrition Inc., Quebec, Canada) on egg production, egg components, and total tract nutrient digestibility in laying hens. The second objective was to evaluate the effects of EF1 supplemented diets on fatty acid and cholesterol compositions of egg yolk.

3.3 MATERIALS AND METHODS

3.3.1 Birds and Housing

A total of seventy two 58-week-old White Leghorn laying hens were randomly assigned to one of four dietary treatments for 8 weeks. Birds were housed in 24 cages, with 6 cage replicates and 3 hens per cage. All birds received equal time illumination per day and were raised at constant temperature throughout the experiment. Birds also had free access to experimental diets and water. All animal procedures were approved by McGill University Animal Care Committee.

3.3.2 Dietary Treatments

Four experimental diets were evaluated, including a control corn-soybean meal diet (0% extruded flaxseed, EF1), 7.5% EF1, 15% EF1, and 22.5% EF1. The EF1 product (OmegaPlus, Belisle Solution Nutrition Inc., Quebec, Canada) contained 80% flaxseed and 20% ground alfalfa meal (Table 3.1). Extrusion was carried out using a modified Insta-Pro 2000RC extruder (model 2000RC, Insta-Pro International, Des Moines, IA) outfitted with an 8100RC volumetric feeder and extrusion temperature was maintained around 142 ℃. All experimental diets were formulated to

be isonitrogenous, and to meet or exceed NRC (1994) nutrient requirements (Table 3.2). Chromic oxide (0.3%) was incorporated in all diets as an indigestible marker to determine apparent total tract digestibility.

3.3.3 Sample Collection

Egg production and egg weight were recorded daily while feed intake and body weight were determined biweekly. Feed conversion ratio was calculated as feed intake divided by egg mass (daily egg production × daily egg weight). For egg component measurements, two eggs per replicate were selected randomly at two-week interval and were broken to separate the yolk and the albumen. Yolk and shell were weighed and albumen weight was calculated by subtracting yolk and shell weight from total egg weight. The proportion of each component was calculated relative to the egg weight.

3.3.4 Nutrient Digestibility

Fecal samples were collected over 48 h at the end of week 4 by placing aluminum foil trays under each cage. Feathers were removed to avoid contamination. Fecal samples were stored in plastic containers, freeze dried and ground using a coffee grinder.

Nitrogen corrected apparent metabolizable energy (AMEn) was calculated as follows:

$$\text{AMEn} = \frac{[\text{GE intake} - \text{GE in the excreta} - 8.73 \times (\text{dietary N intake} - \text{N output from excreta})]}{\text{Feed intake}}$$

N correction factor of 8.73 was obtained from research of Titus (1956).

Apparent total tract digestibility (ATTD) was calculated by using the following equation:

$$\text{ATTD (\%)} = \{100 - 100 \times [(Cr_2O_3 \text{ in feed\%} \div Cr_2O_3 \text{ in feces\%}) \times (\text{nutrient in feces\%} \div \text{nutrient in feed\%})]\}.$$

3.3.5 Chemical Analysis

Standard procedures (AOAC, 1990) were used to determine dry matter and ash contents of

EF1, experimental diets and fecal samples. Neutral detergent fiber (NDF) of EF1 and feed was determined using an Ankom fiber Analyzer (Ankom Technology, Macedon, NY). Heat stable α-amylase was used in NDF analysis (Van Soest et al., 1991). A Leco Nitrogen Analyzer (FP-428 Nitrogen Determinator, Leco Corp., St. Joseph, MI) was used for determination of crude protein (N × 6.25) of EF1, feed and fecal samples. Chromic oxide of feed and fecal samples was determined according to the method described by Fenton and Fenton (1979). Gross energy was determined by an adiabatic bomb calorimeter.

3.3.6 Fatty Acid Analysis

Two eggs from each cage were collected randomly at week 2, 4, 6, and 8, cracked, and then yolks were separated from whites. Yolk samples were freeze dried and finely ground prior to fatty acid analysis. Methyl esters of EF1, feed and egg yolks were directly determined according to O'fallon et al. (2007). Tridecanoic acid (C13:0) was used as internal standard. The fatty acid methyl esters were analyzed by gas chromatography (Varian 3900 equipped with flame ionization detector at 260 ℃ and 117 auto-injector) on a 100 m × 0.25 mm fused silica capillary column (CP74489, Varian, CA). The carrier gas was H_2 and the flow rate was 0.8 ml/min. Both temperature of injector and detector was 260 ℃ and the split ratio was 50:1. The initial column temperature was set at 70 ℃ for 4 min, then increased by 4 ℃/min to 175 ℃ and maintained for 27 min. It was then increased to 214 ℃ by 4 ℃/min and held for 11 min, final temperature increased to 225 ℃ at the rate of 4 ℃/min, held for 5.5 min. Fatty acids were identified by comparing their retention times with standard fatty acid methyl esters (NuCheck, Elysian, MN, USA).

3.3.7 Cholesterol Analysis

To determine cholesterol of egg yolk, two eggs were collected at the end of the experiment from each cage, and yolk cholesterol analysis was determined according to Fenton and Sim (1991). Freeze dried yolk (0.2 g) was directly saponified with 10 mL of alcoholic KOH and heated in water bath at 60°C for 1 h with 1 mL of internal standard (containing 2 mg of 5-α-cholestane in hexane,

Sigma, St. Louis, MO, USA). The mixture was then cooled to room temperature, and 5 mL of water and 10 mL of hexane were added, and cholesterol was extracted by centrifuging the tube at 1,500 g for 5 min. After phase separation, the upper hexane layer was diluted to a concentration of 1 mg/mL and injected into a gas chromatography (Varian model 3900 equipped with flame ionization detector at 300 ℃) on a 30 m × 0.25 mm capillary column (DB-1MS UI, Agilent J&W, USA) with film thickness of 0.25 μm. Column temperature was initially set at 70 ℃ for 0.1 min, and then increased by 40 ℃/min to 300 ℃ and held for 5 min. Injector and detector temperature was programmed at 300 ℃ with split ratio of 20:1. The carrier gas was H_2 and the flow rate was 1.5 ml/min. Nitrogen was the make-up gas at a flow rate of 29 ml/min.

3.3.8 Statistical Analysis

Data for total tract nutrient digestibility and yolk cholesterol were analyzed using the PROC MIXED procedure of SAS (2014) with the following model:

$$Y_{ijk} = \mu + T_i + C_{ij} + e_{ijk}$$

Where

Y_{ijk} = observation,

μ = overall mean,

T_i = fixed effect of i^{th} treatment (i = 1, 2, 3 or 4),

C_{ij} = random effect of j^{th} cage within i^{th} treatment (j = 1, 2, 3, 4, 5 or 6),

e_{ijk} = residual error (k = 1 or 2), $e_{ijk} \sim N(0, \sigma^2_e)$.

Data for performance parameters, egg components and fatty acid composition of yolk were analyzed using the PROC MIXED procedure of SAS (2014) with the following model:

$$Y_{ijk} = \mu + T_i + C_{ij} + W_k + T_i \times W_k + e_{ijk}$$

Where

Y_{ijk} = observation,

μ = overall mean,

T_i = fixed effect of i^{th} treatment (i = 1, 2, 3 or 4),

C_{ij} = random effect of j^{th} cage within i^{th} treatment (j = 1, 2, 3, 4, 5 or 6),

W_k = fixed effect of k^{th} week (k = 1, 2, 3 or 4),

$T_i \times W_k$ = fixed effect of interaction of i^{th} treatment and k^{th} week,

e_{ijk} = residual error (k = 1 or 2), $e_{ijk} \sim N(0, \sigma^2_e)$.

Least squares means method was used to identify differences between treatment means, statistical significance was declared at $P < 0.05$ level.

3.4 RESULTS

3.4.1 Layer Performance

The effects of treatment and duration of feeding on performance parameters are presented in Table 3.3. EF1 supplementation had no effect on feed intake (average 119.83 g), egg production (average 92.90%), FCR (average 2.04) and egg weight (average 63.86 g). However, layers fed 22.5% EF1 lost more weight than those fed other treatments. Significant differences were observed for the duration of feeding in egg production, feed intake and FCR. Egg production was highest ($P = 0.0199$) during week 4 while feed intake was greatest ($P < 0.0001$) during week 2 than other weeks. FCR was lowest ($P = 0.0008$) during week 8 relative to other feeding time.

3.4.2 Egg Components

Percentages of yolk and albumen were not influenced by EF1 supplementation or duration of feeding (Table 3.4). However, shell percentage was greater for hens fed 22.5% EF1 than hens fed other treatments. Shell percentage was also influenced by duration of feeding with a reduction in shell percentage during week 2 compared with week 4, 6, and 8.

3.4.3 Nutrient Digestibility

Feeding EF1 up to 7.5% of the diet had no adverse effect on ATTD and AMEn (Table 3.5).

However, ATTD of DM, OM and GE was lower ($P < 0.0001$) for hens fed 15% EF1 than hens fed 0% and 7.5% EF1. Feeding EF1 at 22.5% of the diet reduced ATTD of CP ($P = 0.0058$) and AMEn ($P = 0.0006$) compared with other treatments.

3.4.4 Egg Fatty Acid and Cholesterol Concentration

Table 3.6 shows fatty acid composition of egg yolk of laying hens fed EF1. Concentrations of LNA (C18:3n3) and DHA (C22:6n3) in egg yolk increased with EF1 supplementation. Highest concentration of LNA was achieved when diet contained 22.5% EF1. There was a dietary treatment by feeding time interaction on the concentrations of saturated fatty acids (SFA) and unsaturated fatty acids (USFA). The concentrations of SFA were lower ($P < 0.0001$) in egg yolks from EF1 diets than that from the control diet. However, USFA levels were higher ($P = 0.0008$) in egg yolks from EF1-containing group than the control group. Palmitic acid (C16:0) and linoleic acid (C18:2n6) were the major saturated and unsaturated fatty acids in the egg yolk, and both were lower ($P < 0.0001$) for layers fed EF1 diets than the control diet. Treatment by feeding time interaction for total n-3 and n-6 polyunsaturated fatty acids (PUFA) were also observed. Dietary supplementation of EF1 significantly increased ($P < 0.0001$) egg yolk concentrations of n-3 PUFA and this increase occurred at the beginning of week 4. However, inclusion of EF1 in the diets significantly decreased ($P < 0.0001$) total n-6 PUFA in egg yolk throughout the experiment. Consequently, the ratio of n-6 to n-3 PUFA in eggs from birds fed EF1 diets was reduced ($P < 0.0001$) compared to those fed the control diet since week 2.

A difference ($P = 0.0402$) in egg yolk cholesterol concentration was observed between 15 and 22.5% EF1 diets (Table 3.7). However, total cholesterol level of eggs (average 262.8 mg) was not different between layers fed the control and EF1 diets.

3.5 DISCUSSION

Published data on the effects of flaxseed on feed intake of laying hens are inconsistent. The present study showed that feeding EF1 up to 22.5% had no effect on feed intake or FCR. In

agreement with our findings, Nain et al. (2012) reported that feeding EF (Linpro, flaxseed:peas 1:1) up to 15% of the diet had no adverse effects on feed intake or FCR. Similar results on feed intake and feed efficiency were reported by Eder et al. (1998) and Sujatha (2002). In contrast, flaxseed supplementation has also been reported to increase (Leeson et al., 2000; Jia et al., 2008) or decrease (Bean and Leeson, 2003) feed intake in layers. Our findings show that egg weight was not affected by EF1 supplementation, although there was an increase in egg weight during week 4. These results are in agreement with those of Novak and Scheideler (2001) who reported no effect of whole flaxseed on egg weight. In contrast, Scheideler and Froning (1996) reported a reduction in egg weight as a result of whole flaxseed inclusion. The authors attributed the decrease in egg weight to estrogen metabolism which was regulated by dietary fatty acids.

In the current study, EF1 diets had no impact on egg production. This result substantiate the findings of others who showed that feeding 15% (Antruejo et al., 2011; Nain et al., 2012) or 20% (Caston et al., 1994) of flaxseed diets had no adverse effects on egg production. In contrast, other researchers showed that feeding 15% of flaxseed improved (Scheideler and Froning, 1996) or depressed (Aymond and Van Elswyk, 1995) egg production. Differences in egg production are likely due to age of the layers, level and form of flaxseed in the diet and other experimental conditions. The performance of body weight change in layers was negatively affected by extruded flaxseed dietary treatments relative to the control diet. Our results are in agreement with the findings of Novak and Scheideler (2001), who found that hen weight was decreased by feeding 10% of flaxseed diet. Jia et al. (2008) also observed that 15% of flaxseed reduced body weight gain in laying hens. A reduction in dietary metabolizable energy may lead to the decrease in body weight of hens fed extrduded flaxseed diets.

Apparent total tract digestibility of nutrients was adversely affected by EF1 supplementation. A reduction in ATTD of DM, OM, and GE was observed with 15 and 22.5% EF1, while a reduction in ATTD of CP and AMEn occurred with 22.5% EF1. Data regarding the effects of flaxseed on ATTD of layers are limited. However, our data are consistent with the results of Rodriguez et al. (2001) who reported a linear decline in ATTD of CP, amino acids and fatty acids

as the level of ground flaxseed in diets increased to 16%. The reduction in ATTD is most likely due to high dietary fiber contents which result from the utilization of ground alfalfa as an oil absorbent during the EF1 manufature. But, it is also feasible to associate the lower ATTD with anti-nutritional factors of flaxseed that might be present in EF1. For example, mucilage which is a water soluble and indigestible fiber has been shown to increase the viscosity of intestinal digesta in chickens causing a significant reduction in nutrient digestion and absorption (Rodriguez et al., 2001; Alzueta et al., 2003).

Results of the current study indicated that birds consuming EF1 diets deposited less SFA and more n-3 PUFA in their eggs than hens fed the control diet. The reduction in SFA was mainly due to the decline in C16:0 fatty acid concentration while the increase in n-3 PUFA were likely due to elevated concentration of C18:3n-3 and C22:6n-3. Similar to our findings, Jia et al. (2008) reported that feeding 15% of Linpro to layers increased n-3 PUFA concentrations in eggs by 106%. A greater increase in n-3 PUFA concentration of egg yolk was also observed by Jiang et al. (1991) by feeding 15% of ground flaxseed. The amount of egg n-3 PUFA in layers fed the control diet was less than 300 mg. However, hens fed diets with EF1 produced eggs with more than 300 mg of n-3 PUFA. According to the Canadian Food Inspection Agency, egg should contain at least 300 mg of n-3 fatty acids per reference amount in order to be labelled as omega-3 enriched egg (CFIA, 2015). The longer chain metabolite of linolenic acid (i.e. C22:6n-3) increased while the metabolite of linoleic acid (i.e. C20:4n-6) decreased as a result of EF1 supplementation. Incorporation of EF1 to the diet of laying hens increased the dietary contents of n-3 PUFA, which were then deposited into egg yolk after ingestion by birds. In the present study, the amount of LNA increased proportionally to EF1 supplementation. A significant increase in DHA concentration was also observed. A concomitant reduction in egg linoleic acid (C18:2n-6, LA) and arachidonic acid (C20:4n-6, AA) concentrations were also achieved as a result of EF1 supplementation. The deposition of DHA in egg yolk was due to conversion of LNA which it served as a precursor through different process of desaturation and elongation in the liver (Brenner, 1971). Meanwhile, the same pathway was also applied to the conversion of LA to AA. However, the formation of

DHA from LNA is rather limited. This is likely due to competition for the involved enzymes (Burdge, 2004). Both LNA and LA compete for Δ^6 and Δ^5 desaturase in the first conversion step to synthesize longer chain PUFA (Holman, 1998). Whereas, when they are in equal amounts, LNA is more easily metabolized by those enzymes over LA (Craig-Schmidt et al., 1987). The decrease in AA concentration observed in egg yolks of birds fed EF diets compared with those from the control diet showed that the synthesis of this fatty acid can be inhibited when birds receive diets enriched with LNA. Similar modifications in egg yolk fatty acids as a result of flaxseed supplementation have been reported by other researchers (Jia et al., 2008; Nain et al., 2012).

Since greater dietary LNA concentration increased the amount of n-3 PUFA and suppressed the synthesis of n-6 PUFA, the ratio of n-6 to n-3 PUFA decreased by 68% for hens fed 22.5% EF1 at week 2. This is in line with the findings of other researchers who reported reduction in n-6 to n-3 ratio as a result of flaxseed supplementation (Jiang et al., 1991; Jia et al., 2008). Nain et al. (2012) also observed 46 and 59% reduction in n-6 to n-3 PUFA ratio in egg yolk from layers fed moderate (7.5%) and high (15%) Linpro diets, respectively, at 18 d. Although both LNA and LA are all essential fatty acids that have physiological functions to human health, a balanced n-6 to n-3 ratio is associated with health benefits including improving cardiovascular system, anti-inflammatory effects and reducing atherosclerosis. An ideal n-6 to n-3 ratio for the human diet is recommended between 4:1 to 1:1 (Simopoulos, 2004). In general, results from our study illustrated that feeding EF1 diets to laying hens not only increased the amount of n-3 PUFA in egg yolk but also lowered ratio of n-6 to n-3 PUFA, which could improve the health benefits of flaxseed-supplemented egg.

Cholesterol concentration in egg yolk was not influenced by EF1 supplementation. In agreement with our results, Hayat et al. (2009), Ferrier et al. (1995), and Caston and Leeson (1990) found no effect of flaxseed supplementation on egg yolk cholesterol concentration. However other researchers reported a decrease (Lin and Pratt, 1992) or an increase (Naber, 1983) in egg yolk cholesterol concentration as the level of omega-3 fatty acid increased.

3.6 CONCLUSIONS

The results of this study showed that feeding EF1 to layers up to 22.5% of the diet had no adverse effects on egg performance parameters, egg component and egg yolk cholesterol concentration. Feeding layers with EF1-supplemented diets may be an effective strategy to enrich eggs with n-3 PUFA, and reduce saturated fatty acids and n-6 PUFA concentrations in eggs, particularly at 22.5% of the diet. However, moderate to high levels (i.e. 15 and 22.5%) of EF1 reduced ATTD and energy utilization. More research are warranted to investigate the effects of lower levels of extruded flaxseed on layer performance.

Table 3.1. Chemical composition of extruded flaxseed (EF1)[1]

Item	% DM
Ash %	12.19
CP %	20.89
Fat %	20.01
NDF %	26.23
Energy Cal/g	4805.93
Fatty acids, % of total fatty acids	
C14:0	0.07
C16:0	7.84
C16:1	0.08
C18:0	3.52
C18:1n9c	21.75
C18:2n6c	22.28
C18:3n3	48.26

[1]OmegaPlus: manufactured by Belisle Solution Nutrition Inc., Quebec, Canada.

Table 3.2. Ingredients and chemical composition of dietary treatments

	Extruded Flaxseed inclusion %			
Ingredients %	0	7.5	15	22.5
Corn	52.57	50.06	47.46	44.84
Soybean	30.54	26.57	22.62	18.66
OmegaPlus[1]	-	7.50	15.00	22.50
Soybean oil	3.91	2.94	1.97	1.00
Salt	0.31	0.32	0.33	0.34
Chromic oxide	0.30	0.30	0.30	0.30
Premix[2]	0.50	0.50	0.50	0.50
Calcium	10.33	10.22	10.10	9.99
Phosphorus	1.22	1.26	1.30	1.34
Methionine	0.12	0.12	0.13	0.13
Choline chloride	0.10	0.10	0.10	0.10
Sodium carbonate	0.10	0.10	0.10	0.10
Threonine	-	0.01	0.03	0.05
Lysine-HCL	-	-	0.07	0.15
Calculated analysis				
ME, kcal/kg	2870.16	2724.24	2576.03	2427.33
CP, %	19.00	19.00	19.00	19.00
Fiber, %	2.35	2.81	3.28	3.74
Total methionine, %	0.43	0.43	0.43	0.43
Total lysine, %	1.08	1.01	1.00	1.00
FA, % of total FA				
C16:0	14.02	12.43	11.16	10.21
C18:0	2.20	2.30	2.57	2.71
C18:1	22.44	22.29	21.52	21.08

C18:2n6c	54.17	48.48	41.58	35.94
C18:3n3	4.31	11.88	20.78	27.84

[1] Manufactured by Belisle Solution Nutrition Inc., Quebec, Canada.

[2] Composition of premix: Vitamin A 11,530 KIU/kg; Vitamin D 2,400 KIU/kg; Vitamin E 74.168 IU/kg; Copper 24mg/kg; Iron 200mg/kg; Magnesium 122mg/kg; Selenium 0.38mg/kg; Zinc 131mg/kg; Cobalt 0.46mg/kg; Fluorine 19mg/kg; Iodine 0.80mg/kg.

Table 3.3. Effects of extruded flaxseed on egg production parameters and change in BW

Treatment	Week	Egg wt (g)	Egg production (%)	Feed intake (g)	FCR	Change in BW (g)
0% EF		63.38	92.76	118.94	2.05	33.78[a]
7.5% EF		64.65	88.39	121.55	2.15	- 25.94[ab]
15% EF		63.69	95.49	119.49	1.97	- 22.25[ab]
22.5% EF		63.70	94.94	119.32	1.98	- 36.17[b]
SEM		0.86	2.04	2.39	0.07	14.81
	2	63.66	91.52[ab]	123.67[a]	2.14[a]	- 20.57
	4	63.62	95.19[a]	119.31[b]	1.98[b]	- 19.87
	6	63.92	91.27[b]	119.25[b]	2.06[ab]	- 7.68
	8	64.22	93.60[ab]	117.06[b]	1.97[b]	- 2.46
SEM		0.46	1.49	1.33	0.05	9.04
P-value						
Treatment		0.7472	0.0891	0.8676	0.2808	0.0141
Week		0.1090	0.0199	<0.0001	0.0008	0.1975

[a-b]Means within treatment and week with no common superscript differ significantly ($P < 0.05$).

Table 3.4. Effects of extruded flaxseed on egg components

Treatment	Week	Yolk wt (%)	Albumen wt (%)	Eggshell wt (%)
0% EF		27.77	59.11	13.12[ab]
7.5% EF		27.15	60.17	12.68[b]
15% EF		27.35	60.07	12.58[b]
22.5% EF		26.88	59.67	13.45[a]
SEM		0.25	0.37	0.18
	2	27.52	60.13	12.35[b]
	4	27.09	59.99	12.92[a]
	6	27.32	59.56	13.12[a]
	8	27.22	59.34	13.44[a]
SEM		0.24	0.31	0.16
P-value				
Treatment		0.1221	0.1998	0.0101
Week		0.6281	0.2148	<0.0001

[a-b]Means within treatment and week with no common superscript differ significantly ($P < 0.05$).

Table 3.5. Effects of extruded flaxseed on total tract nutrient digestibility for a period of 4 weeks

	Extruded Flaxseed inclusion %					
Total tract digestibility %	0	7.5	15	22.5	SEM	*P*-value
DM	71.12[a]	69.90[a]	65.54[b]	60.55[c]	0.86	<0.0001
OM	78.50[a]	76.47[a]	71.45[b]	67.49[c]	0.79	<0.0001
CP	44.59[a]	41.52[a]	34.60[ab]	25.76[b]	3.39	0.0058
GE	81.03[a]	78.28[a]	73.97[b]	70.64[b]	0.94	<0.0001
AMEn	2941.77[a]	2882.07[a]	2787.40[ab]	2679.52[b]	38.60	0.0006

[a-c]Means within treatment with no common superscript differ significantly ($P < 0.05$).

Table 3.6a. Effects of interaction of dietary treatment and feeding time on fatty acid composition of eggs (mg/60g of egg) of laying hens

	Week 2				Week 4			
Fatty acid	0%	7.5%	15%	22.5%	0%	7.5%	15%	22.5%
C14:0	24.6	23.7	21.9	23.3	25.5	23.2	22.3	22.3
C14:1	4.0	3.9	3.1	3.6	3.8	3.4	3.2	3.2
C15:0	4.3	3.6	4.9	4.8	4.8	4.4	4.3	4.3
C16:0	2410.0^{bc}	2328.6^{cd}	2322.2^{cd}	2383.5^{c}	2686.7^{a}	2509.8^{abc}	2463.4^{abc}	2394.4^{bc}
C16:1	215.8	220.6	199.9	226.1	221.3	217.8	213.2	206.1
C18:0	807.1	802.0	711.0	780.7	876.8	845.0	856.8	867.7
C18:1	3664.1	3567.1	3733.7	3745.8	3897.1	3818.9	3879.3	3798.9
C18:2n6c	1778.3^{abc}	1563.0^{c}	1824.4^{abc}	1742.0^{abc}	2104.0^{a}	1861.4^{abc}	1829.7^{abc}	1782.0^{abc}
C18:3n3	78.6^{g}	186.5^{fg}	448.9^{bc}	557.8^{b}	80.2^{g}	212.1^{fg}	371.6^{cde}	482.7^{bc}
C20:1	17.0	15.2	18.4	17.0	17.2	14.7	14.8	15.5
C20:2	13.0	10.1	11.9	10.2	15.3	11.5	11.1	10.9
C20:3n6	14.6	14.4	15.0	15.0	15.8	14.8	14.0	14.5
C20:4n6	180.8	139.2	127.3	113.6	193.3	145.3	121.3	108.3
C22:6n3	167.0^{a}	154.8^{a}	164.6^{a}	161.6^{a}	107.4^{bc}	139.7^{abc}	151.1^{a}	146.4^{ab}
Total SFA[1]	3260.3^{bc}	3170.4^{cd}	3075.7^{cd}	3207.3^{bcd}	3610.2^{a}	3397.5^{abc}	3362.2^{abc}	3303.5^{abc}
Total USFA[2]	6141.1^{ab}	5882.3^{b}	6557.1^{a}	6602.0^{a}	6664.1^{a}	6448.4^{ab}	6618.0^{a}	6577.0^{a}

Total n-3 PUFA[3]	245.5^{fg}	341.3^{ef}	613.5^{bc}	719.4^{b}	187.6^{g}	351.8^{ef}	522.8^{cd}	629.0^{bc}
Total n-6 PUFA[4]	1973.6^{abcde}	1716.6^{e}	1966.7^{abcde}	1870.6^{cde}	2313.0^{a}	2021.5^{abcde}	1965.0^{abcde}	1904.8^{bcde}
Ratio n-6/n-3[5]	8.1^{b}	5.1^{cd}	3.2^{e}	2.6^{e}	12.4^{a}	5.8^{c}	3.8^{de}	3.0^{e}

[a-g]Means within the interaction of treatment and week across the rows with no common superscript differ significantly ($P < 0.05$).

[1]Total SFA was calculated as 14:0 + 15:0+ 16:0 + 18:0.

[2]Total USFA was calculated as 14:1 + 16:1 + 18:1 + 18:2n6c + 18:3n3 + 20:1 + 20:2 + 20:3n6 + 20:4n6 + 22:6n3.

[3]Total n-3 PUFA was calculated as 18:3n3 + 22:6n3.

[4]Total n-6 PUFA was calculated as 18:2n6c + 20:3n6 + 20:4n6.

[5]Ratio n-6/n-3 was calculated as total n-6 PUFA/total n-3 PUFA.

Table 3.6b. Effects of interaction of dietary treatment and feeding time on fatty acid composition of eggs (mg/60g of egg) of laying hens

	Week 6				Week 8					*P*-value
Fatty acid	0%	7.5%	15%	22.5%	0%	7.5%	15%	22.5%	SEM	EF x Time
C14:0	25.9	23.9	20.1	21.6	21.9	21.3	21.8	17.3	0.77	<0.0001
C14:1	3.9	3.4	3.1	3.2	2.8	2.8	3.2	1.7	0.17	<0.0001
C15:0	4.0	4.0	4.3	3.9	4.4	4.5	3.8	5.0	0.15	<0.0001
C16:0	2655.7^{ab}	2555.2^{abc}	2327.8^{cd}	2353.5^{c}	2488.7^{abc}	2415.6^{bc}	2413.7^{bc}	2085.0^{d}	34.90	<0.0001
C16:1	232.9	219.2	221.6	220.0	185.5	189.6	218.6	145.0	8.08	<0.0001
C18:0	835.4	826.0	759.2	822.8	781.7	752.0	828.1	747.1	25.26	0.0010
C18:1	3880.8	3872.1	3608.6	3784.8	3672.8	3604.2	3961.2	3411.1	76.06	<0.0001
C18:2n6c	1975.8^{ab}	1847.4^{abc}	1875.8^{abc}	1674.6^{bc}	2063.0^{a}	2070.2^{a}	1668.4^{bc}	2029.4^{a}	49.31	<0.0001
C18:3n3	75.1^{g}	238.6^{ef}	549.5^{b}	555.3^{b}	77.4^{g}	309.0^{def}	378.2^{cd}	747.5^{a}	18.12	<0.0001
C20:1	17.5	16.2	14.6	15.4	17.4	16.5	16.4	14.6	0.61	<0.0001
C20:2	12.9	10.9	9.3	9.1	14.6	13.1	9.2	11.2	0.47	<0.0001
C20:3n6	14.0	13.8	13.4	12.8	15.0	14.3	13.2	14.6	0.39	0.0366
C20:4n6	183.5	134.0	104.6	93.7	181.9	133.3	109.5	100.9	3.26	0.0035
C22:6n3	101.0^{c}	140.2^{abc}	139.1^{abc}	146.4^{ab}	102.5^{c}	130.3^{abc}	146.5^{ab}	128.9^{abc}	5.53	<0.0001
Total SFA[1]	3535.3^{ab}	3422.8^{abc}	3125.4^{cd}	3214.4^{bc}	3312.9^{abc}	3208.8^{bcd}	3280.8^{abc}	2871.0^{d}	47.55	<0.0001

Total USFA[2]	6505.3[a]	6503.9[ab]	6548.3[a]	6523.3[a]	6341.5[ab]	6492.0[ab]	6532.8[a]	6613.9[a]	85.29	0.0008
Total n-3 PUFA[3]	176.1[g]	378.8[ef]	688.6[b]	701.6[b]	179.9[g]	439.3[de]	524.7[cd]	876.4[a]	18.74	<0.0001
Total n-6 PUFA[4]	2173.2[abc]	1995.2[abcde]	1993.8[abcde]	1781.0[e]	2259.9[ab]	2217.8[abc]	1791.0[de]	2144.8[abcd]	50.20	<0.0001
Ratio n-6 /n-3[5]	12.4[a]	5.3[c]	3.0[e]	2.6[e]	12.6[a]	5.1[cd]	3.4[e]	2.5[e]	0.18	<0.0001

[a-g]Means within the interaction of treatment and week across the rows with no common superscript differ significantly ($P < 0.05$).

[1]Total SFA was calculated as 14:0 + 15:0+ 16:0 + 18:0.

[2]Total USFA was calculated as 14:1 + 16:1 + 18:1 + 18:2n6c + 18:3n3 + 20:1 + 20:2 + 20:3n6 + 20:4n6 + 22:6n3.

[3]Total n-3 PUFA was calculated as 18:3n3 + 22:6n3.

[4]Total n-6 PUFA was calculated as 18:2n6c + 20:3n6 + 20:4n6.

[5]Ratio n-6/n-3 was calculated as total n-6 PUFA/total n-3 PUFA.

Table 3.7. Effects of extruded flaxseed on egg cholesterol

	Extruded Flaxseed inclusion %					
Item	0	7.5	15	22.5	SEM	*P*-value
Cholesterol Concentration in egg yolk (mg/g)	14.72[ab]	14.84[ab]	14.62[b]	15.24[a]	0.15	0.0402
Total cholesterol per egg (mg)	264.11	267.71	261.54	257.65	3.61	0.2529

[a-b]Means within treatment with no common superscript differ significantly ($P < 0.05$).

CHAPTER IV.

In Experiment 1, we evaluated the effects of graded levels (0, 7.5, 15 and 22.5%) of a locally produced extruded flaxseed (EF1) in the diets of layer chickens on egg production, egg components, apparent total tract nutrient digestibility (ATTD), and fatty acid and cholesterol compositions of egg yolk. Our findings show that dietary supplementation with EF1 significantly increased n-3 PUFA depositions, especially linolenic acid (C18:3n3; LNA), in egg yolks. In fact, the production of omega-3 labelled eggs (n-3 fatty acids ≥ 300 mg per egg according to regulations of the Canadian Food Inspection Agency) was possible after 2 weeks of feeding with the 7.5% EF1-supplemented diet. Egg production parameters (egg production, feed intake and feed conversion ratio), egg components (i.e. yolk and albumen percentages) and egg yolk total cholesterol were not affected by any dietary level of EF1. However, energy utilization (AMEn) and ATTD of dry matter, organic matter, crude protein and gross energy were significantly reduced when diets contained 15 and 22.5% EF1.

Therefore, based on findings of this study, an improved version of the EF product (EF2) was developed by Belisle Solution Nutrition Inc. Indeed, the improved EF product contained higher concentrations of total fat (31% vs 20% on DM basis) and linolenic acid (60% vs 48% on DM basis). In the next study, we were interested to know whether omega-3 labelled eggs could be produced with lower dietary levels of the new EF and without any detrimental effects on energy utilization and nutrient digestibility.

CHAPTER V.

Effects of an improved extruded flaxseed on egg production, egg components, nutrient digestibility, egg cholesterol and fatty acid compositions of yolk, plasma and liver of laying hens

5.1 ABSTRACT

The objective of the present study was to determine the effects of graded levels of an improved extruded flaxseed (EF2) in layer diets on egg production, egg components, apparent total tract nutrient digestibility, and fatty acid concentrations in egg yolk, blood plasma, and liver. Cholesterol concentration of egg yolk was also determined. Seventy two layers (58-week-old) were randomly assigned to 4 groups (6 cage replicates; 3 hens/cage). Dietary treatments included: 0, 3, 6, and 9% of EF2. Results showed that feed intake, egg production, feed conversion ratio (FCR), and egg weight were not affected by dietary EF2. Apparent total tract digestibility (ATTD) of dry matter and gross energy was lower ($P < 0.05$) for birds fed 9% EF2 than those fed the control diet, while apparent organic matter digestibility and AMEn were lower ($P < 0.05$) for birds fed 6 and 9% EF2 compared with those fed the control diet. Dietary EF2 increased ($P < 0.05$) n-3 fatty acids in egg yolk, plasma and liver. Birds fed 6% EF2 produced eggs with over 300 mg of n-3 fatty acids after week 2, the highest of n-3 fatty acid concentrations were achieved for birds fed 9% EF2. Cholesterol concentration in egg yolk was greater ($P < 0.05$) for layers fed 6 and 9% EF2 than those fed the control diet. However, total cholesterol per egg was not influenced by any treatment. It was concluded that feeding EF2 up to 9% of the diet had no adverse effects on layer performance and total cholesterol contents of eggs. However, moderate to high levels of EF2 (6 and 9% of the diet) reduced nutrient ATTD and AMEn. Therefore, omega-3 enriched eggs can be achieved by feeding layers EF2 at levels not exceeding 6% of the diet.

Key words: laying hen, extruded flaxseed, n-3 fatty acids, blood plasma, liver

5.2 INTRODUCTION

Flaxseed (*Linum usitatissimum*) is a rich source of poly-unsaturated fatty acids particularly omega-3 fatty acids, constituting 53% of total fatty acids (Gonthier et al., 2004). Dietary manipulation by incorporating flaxseed in layer diets is an effective method of increasing the n-3 poly-unsaturated fatty acids (n-3 PUFA) concentration of chicken eggs (Novak and Scheideler, 2001). Omega-3 fatty acids include linolenic (LNA, C18:3n-3), eicosapentaenoic (EPA, C20:5n-3), docosapentaenoic (DPA, C22:5n-3), and docosahexaenoic (DHA, C22:6n-3) acid. These fatty acids have received considerable attention for their health promoting effects (Lewis et al., 2000). The biological functions of n-3 PUFA in human and animal health have been studied extensively. It is well documented that omega-3 have therapeutic and beneficial effects to prevent coronary heart disease, arthritis and brain disorders (Simopoulos, 1991; Bazinet and Laye, 2014). As essential fatty acids to humans, they have to be obtained from dietary sources. During the deposition of n-3 fatty acids in egg yolk, a high turnover of lipids and lipoproteins occurs in the liver of avian species where de novo fatty acids are synthesized (Hermier, 1997).

Several studies have shown that n-3 PUFA of egg yolk could be increased by feeding dietary n-3 fatty acid sources such as flaxseed up to 10% of the diet (Bean and Leeson, 2003; Kirubakaran et al., 2011) However, adverse effects on layer performance and egg production were reported when layers were fed diets containing 15 (Jia et al., 2008) or 20% of flaxseed (Leeson et al., 2000). This can mainly be attributed to anti-nutritional factors in flaxseed such as mucilage and non-starch polysaccharides. Anti-nutritional factors may interfere with nutrients of flaxseed diets and therefore decreasing the energy utilization (Ortiz et al., 2001). Physical treatments (e.g. seed rupture and heat treatment) can significantly improve nutrient digestibility and dietary apparent metabolizable energy utilization (Shen et al., 2004). Additional benefits of heat treatment can be achieved by inactivating heat-labile anti-nutritional factors (e.g. antitrypsin factors). Extrusion is an effective process which can be used to improve the feeding value of flaxseed. Layers fed canola seed or extruded flaxseed (Linpro, flaxseed:peas 1:1) had greater egg production and better feed conversion ratio than those fed flaxseed (Jia et al., 2008). During the extrusion

process of oilseed, the rapid release of intracellular oil may lead to considerable oil losses. Therefore, the addition of a binder may help to reduce oil losses during extrusion of oilseed (Akraim et al., 2007). OmegaPlus was successfully used to increase omega-3 fatty acids in milk of dairy cows (Neveu et al., 2014). Recently, an improved extruded flaxseed product (EF2; OmegaPlus, Belisle Solution Nutrition Inc., Quebec, Canada) was developed to contain higher concentrations of total fat (31% vs 20% on DM basis) and linolenic acid (60% vs 48% on DM basis) when compared to the extruded flaxseed product (EF1) used in the first study. Until today, no study has evaluated the effects of EF2 on layer performance. Moreover, the effects of extruded flaxseed on fatty acid profile of blood plasma and liver are still unknown. Therefore, the objectives of this study were to determine the effects of dietary EF2 on layer performance, egg components, egg cholesterol, nutrient digestibility, and fatty acid compositions of yolk, plasma and liver.

5.3 MATERIALS AND METHODS

5.3.1 Birds and Housing

Seventy two 58-week-old White Leghorn laying hens were randomly assigned to 4 experimental groups (6 cages with 3 birds per cage). Three hens were housed in one cage and six adjacent cages were considered as replicates for each experimental diet. Birds were subjected to the equal time illumination per day and were raised at constant temperature. All animal procedures were approved by the Animal Care Committee of McGill University.

5.3.2 Dietary Treatments

Four experimental diets were formulated. These included a control corn-soybean meal diet (0%), 3%, 6%, and 9% EF2. The EF2 product (OmegaPlus, Belisle Solution Nutrition Inc., Quebec, Canada) contained 80% flaxseed and 20% ground alfalfa meal (Table 5.1). Extrusion was carried out using a modified Insta-Pro 2000RC extruder (model 2000RC, Insta-Pro International, Des Moines, IA) outfitted with an 8100RC volumetric feeder and its temperature was maintained around 142 ℃. All the diets were formulated to be isonitrogenous, and to meet or exceed NRC

(1994) requirements of poultry for macro- and micronutrients (Table 5.2). Chromic oxide (0.3%) was incorporated in all diets as an indigestible marker to determine apparent total tract digestibility. Feed and water were provided ad libitum during an 8-week experimental period.

5.3.3 Sample Collection

Egg production and egg weight were recorded daily while feed intake and body weight were determined biweekly. Feed conversion ratio was calculated as feed intake divided by egg mass (daily egg production × daily egg weight). For egg component measurements, two eggs per replicate were selected randomly at two-week interval and were broken to separate the yolk and the albumen. Yolk and shell were weighed and albumen weight was calculated by subtracting yolk and shell weight from total egg weight. The proportion of each component was calculated relative to the egg weight. Two eggs from each replicate were collected randomly at week 2, 4, 6, and 8, cracked, and then yolks were separated from whites. Yolk samples were freeze dried and finely ground prior to fatty acid and cholesterol analyses.

At the end of experiment, blood samples from two birds in each cage were collected using heparin-treated syringes and stored in heparinized tubes. Plasma was obtained by centrifuging blood samples at 2,000 g for 10 min at 4 ℃ and kept at -80 ℃. Following blood collection, birds were killed and livers were removed from carcass. Liver samples were also freeze dried and finely ground for later analysis.

5.3.4 Nutrient Digestibility

Fecal samples were collected over 48 h at the end of week 4 by placing aluminum foil trays under each cage. Feathers were removed to avoid contamination. Fecal samples were stored in plastic containers, freeze dried and ground using a coffee grinder.

Nitrogen corrected apparent metabolizable energy (AMEn) was calculated as follows:

$$AMEn = \frac{[GE\ intake - GE\ in\ the\ excreta - 8.73 \times (dietary\ N\ intake - N\ output\ from\ excreta)]}{Feed\ intake}$$

N correction factor of 8.73 was obtained from research of Titus (1956).

Apparent total tract digestibility (ATTD) was calculated by using the following equation:

ATTD (%) = {100 − 100 × [(Cr_2O_3 in feed% ÷ Cr_2O_3 in feces%) × (nutrient in feces% ÷ nutrient in feed%)]}.

5.3.5 Chemical Analysis

Standard procedures (AOAC, 1990) were used to determine dry matter and ash contents of EF2, experimental diets and fecal samples. Neutral detergent fiber (NDF) of EF2 and feed was determined using an Ankom fiber Analyzer (Ankom Technology, Macedon, NY). Heat stable α-amylase was used in NDF analysis (Van Soest et al., 1991). A Leco Nitrogen Analyzer (FP-428 Nitrogen Determinator, Leco Corp., St. Joseph, MI) was used for determination of crude protein (N × 6.25) of EF2, feed and fecal samples. Chromic oxide of feed and fecal samples was determined according to the method described by Fenton and Fenton (1979). Gross energy was determined by an adiabatic bomb calorimeter.

5.3.6 Fatty Acid Analysis

Methyl esters of EF2, feed, egg yolks, plasma and liver samples were directly determined according to O'fallon et al. (2007). Tridecanoic acid (C13:0) was used as internal standard. The fatty acid methyl esters were analyzed by gas chromatography (Varian 3900 equipped with flame ionization detector at 260 ℃ and 117 auto-injector) on a 100 m × 0.25 mm fused silica capillary column (CP74489, Varian, CA). The carrier gas was H_2 and the flow rate was 0.8 ml/min. Both temperature of injector and detector was 260 ℃ and the split ratio was 50:1. The initial column temperature was set at 70 ℃ for 4 min, then increased by 4 ℃/min to 175 ℃ and maintained for 27 min. It was then increased to 214 ℃ by 4 ℃/min and held for 11 min, final temperature increased to 225 ℃ at the rate of 4 ℃/min, held for 5.5 min. Fatty acids were identified by comparing their retention times with standard fatty acid methyl esters (NuCheck, Elysian, MN, USA).

5.3.7 Cholesterol Analysis

Yolk cholesterol analysis was determined according to Fenton and Sim (1991). Freeze dried yolk (0.2 g) was directly saponified with 10 mL of alcoholic KOH and heated in water bath at 60°C for 1 h with 1 mL of internal standard (containing 2 mg of 5-α-cholestane in hexane, Sigma, St. Louis, MO, USA). The mixture was then cooled to room temperature, and 5 mL of water and 10 mL of hexane were added, and cholesterol was extracted by centrifuging the tube at 1500 g for 5 min. After phase separation, the upper hexane layer was diluted to a concentration of 1 mg/mL and injected into a gas chromatography (Varian model 3900 equipped with flame ionization detector at 300 ℃) on a 30 m × 0.25 mm capillary column (DB-1MS UI, Agilent J&W, USA) with film thickness of 0.25 μm. Column temperature was initially set at 70 ℃ for 0.1 min, and then increased by 40 ℃/min to 300 ℃ and held for 5 min. Injector and detector temperature was programmed at 300 ℃ with split ratio of 20:1. Hydrogen was used as the carrier gas and the flow rate was 1.5 ml/min. Nitrogen was the make-up gas at a flow rate of 29 ml/min.

5.3.8 Statistical Analysis

Data for total tract nutrient digestibility, yolk cholesterol and fatty acid compositions of plasma and liver samples were analyzed using the PROC MIXED procedure of SAS (2014) with the following model:

$$Y_{ijk} = \mu + T_i + C_{ij} + e_{ijk}$$

Where

Y_{ijk} = observation,

μ = overall mean,

T_i = fixed effect of i^{th} treatment (i = 1, 2, 3 or 4),

C_{ij} = random effect of j^{th} cage within i^{th} treatment (j = 1, 2, 3, 4, 5 or 6),

e_{ijk} = residual error (k = 1 or 2), $e_{ijk} \sim N(0, \sigma^2_e)$.

Data for performance parameters, egg components and fatty acid composition of yolk

were analyzed using the PROC MIXED procedure of SAS (2014) with the following model:

$$Y_{ijk} = \mu + T_i + C_{ij} + W_k + T_i \times W_k + e_{ijk}$$

Where

Y_{ijk} = observation,

μ = overall mean,

T_i = fixed effect of i^{th} treatment (i = 1, 2, 3 or 4),

C_{ij} = random effect of j^{th} cage within i^{th} treatment (j = 1, 2, 3, 4, 5 or 6),

W_k = fixed effect of k^{th} week (k = 1, 2, 3 or 4),

$T_i \times W_k$ = fixed effect of interaction of i^{th} treatment and k^{th} week,

e_{ijk} = residual error (k = 1 or 2), $e_{ijk} \sim N(0, \sigma^2_e)$.

Least squares means method was used to identify differences between treatment means, statistical significance was declared at $P < 0.05$ level.

5.4 RESULTS

5.4.1 Layer Performance

Extruded flaxseed supplementation and duration of feeding had no effect on egg weight (average 63.40 g) and egg production (average 93. 36%, Table 5.3). Similar treatment effects were also observed for feed intake (average 126.28 g) and FCR (average 2.15). However feed intake and FCR were lower ($P < 0.0001$) during week 8 than other weeks.

5.4.2 Egg Components

Egg yolk, albumen, and shell percentages were similar among treatments and averaged 27.45, 60.36, and 12.19%, respectively (Table 5.4). Egg components were also not influenced by duration of feeding with the exception of eggshell percentage which was greater ($P < 0.0001$) during week 2 and week 4 than week 6 and week 8.

5.4.3 Nutrient Digestibility

Effects of EF2 supplementation on ATTD are depicted in Table 5.5. Relative to other treatment, feeding 9% EF2 reduced ($P < 0.05$) ATTD of DM, OM and GE and AMEn. Similar negative effects on ATTD of OM and AMEn were observed for layers fed 6% EF2 than layers fed the control diet. However, inclusion of EF2 up to 3% had no effects on ATTD or AMEn. Similar observation was noted for ATTD of DM and GE for layers fed 6% EF2.

5.4.4 Fatty Acid Composition of Blood Plasma and Liver

Extruded flaxseed supplementation significantly altered plasma and liver PUFA concentrations (Table 5.6 and 5.7). Layers fed EF2 had greater ($P < 0.0001$) liver and plasma concentrations of LNA (C18:3n3), DHA (C22:6n3), and total n-3 PUFA compared with layers fed the control diet. Liver concentration of EPA (C20:5n3) was also greater ($P < 0.0001$) for layers fed EF2 supplemented diets than the control diet. Plasma total n-6 PUFA was lower ($P = 0.0054$) for birds fed 9% EF2 compared with other treatments. However, liver total n-6 PUFA concentrations were not altered by EF2 supplementation. Relative to the control diet, EF2 supplementation reduced ($P < 0.0001$) ratio of n-6/n-3 in plasma and liver.

5.4.5 Egg Fatty Acid and Cholesterol Concentration

Feeding EF2 up to 9% of the diet significantly altered the concentrations of saturated (SFA) and unsaturated (USFA) fatty acids in egg yolk (Table 5.8). Layers fed diets supplemented with EF2 deposited more ($P < 0.05$) USFA, but less ($P < 0.05$) SFA. The reduction in SFA was mainly due to a decline in C16:0 as a result of EF supplementation. Treatment by duration interaction was observed for total n-3 and n-6 fatty acids. Total n-3 PUFA increased ($P < 0.0001$) as a result of EF2 with the highest concentration obtained at the level of 9%. However total n-6 PUFA decreased ($P = 0.0356$) in response to the level of EF2 during the course of experiment. Deposition of C18:3n3 in egg yolk increased ($P < 0.0001$) with increasing level of EF2 and the highest deposition was achieved by feeding 9% EF2 at week 8. Similar effects of EF2 supplementation were observed

for EPA. However, egg yolk concentration of DHA was not affected by treatment and duration interaction effect.

Cholesterol concentration in egg yolk was greater (P = 0.0402) for layers fed 6 and 9% EF2 than the control diet (Table 5.9). However, total cholesterol per egg was not influenced by EF2 supplementation, and averaged 292.5 mg.

5.5 DISCUSSION

In the current study, feeding EF2 up to 9% of the diet had no impact on the feed intake, FCR or egg production. These results agree with the earlier research, which showed that feeding 7.5 and 15% EF (i.e. Linpro) had no negative effects on layers performance (Nain et al., 2012). Similar results on layers performance were also observed when 10% of whole flaxseed was fed to layers (Novak and Scheideler, 2001). In contrast, layers fed 15% of Linpro or canola seed had higher egg production and lower feed intake which therefore showed better FCR (Jia et al., 2008).

Egg weight and egg components (i.e. yolk, albumen and shell percentages) were not influenced by either dietary treatments or duration of feeding. Nain et al (2012) reported similar effects of feeding extruded flaxseed-based diets on egg and yolk weight. No effects of whole flaxseed on egg weight, albumen weight and shell weight were also observed by Hayat et al. (2009). Shell weight percentage was significantly redued with feeding time. A 7.46% reduction was noted during week 6 and it continued to decrease to 11.55% at week 8. This is in accordance with Basmacioglu et al. (2003) who found that shell weight percentage was significantly lower in phase 2 (56 d) than in phase 1 (28 d).

Inclusion of EF2 in layer diets had a detrimental effect on nutrient digestibility. This was more pronounced at 6 and 9% EF2. In agreement with our findings, Aziza et al. (2013) reported a reduction in CP digestibility and AMEn for layers fed 10% of whole flaxseed compared with the control diet. The lower nutrient ATTD of EF2-based diets may be related to high fiber contents as a result of ground alfalfa used as an oil absorbent during the manufacture of the EF2 product. In addition, ATTD may have been compromised due to the presence of anti-nutritional factors of

flaxseed such as mucilage, cyanogenic glycosides, or trypsin inhibitors (Bhatty, 1993) in EF2. Mucilage, a water-soluble non-starch polysaccharide, can interfere with nutrient absorption by increasing intestinal viscosity, which has been shown to reduce DM, starch, CP, amino acids, fat, and ME digestibility and utilization (Almirall et al., 1995; Choct et al., 1995; Langhout et al., 1999; Mathlouthi et al., 2002).

Birds fed EF2 supplemented diets deposited greater linolenic acid (LNA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in their eggs, leading to an increase in total n-3 PUFA. In contrast, feeding EF2 diets significantly reduced the concentrations of linoleic acid (C18:2n6c, LA) and arachidonic acid (C20:4n6, AA). LNA as a precursor, can be converted into EPA and DHA in the liver under the action of desaturase and elongase enzymes. However, the conversion of LA to AA competes for the same Δ^6 desaturase enzyme (Holman, 1998). Normally, desaturase enzymes have a superior preference of LNA over LA (Hague and Christoffersen, 1984). In this study, a higher amount of LNA increased the biosynthesis of EPA and DHA but also decreased the conversion of LA to its metabolite PUFA. The reduction in AA concentration in egg yolk of layers fed EF2 diets support the competitive inhibition between n-3 and n-6 fatty acids.

The inclusion of flaxseed to enrich egg with n-3 PUFA is well documented. The increase in n-3 PUFA was mainly due to greater deposition of LNA as a result of EF2 supplementation. Our findings agree with Nain et al. (2012) who reported 96% and 154% increase in total n-3 PUFA concentrations in egg yolk of layers fed 7.5 and 15% of Linpro diets at day 18 compared with those fed the control diet. Scheideler and Froning (1996) reported a linear increase in egg yolk LNA concentration when layers were fed 5, 10 and 15% of flaxseed diets, but DHA concentration had nonlinear response to flaxseed supplementation. Birds fed 10% of flaxseed over 23 weeks deposited more LNA (696%), DHA (57%), and n-3 PUFA (316%) concentrations into their eggs relative to those fed the control diet (Bean and Leeson, 2003). These findings are consistent with previous studies indicating that the increase in LNA is proportional to the flaxseed inclusion (Nain et al., 2012).

Layers fed the control diet produced eggs with less than 300 mg of total n-3 PUFA. However, when feeding layers with 6% (536.2 mg/egg after 2 weeks) and 9% (640.2 mg/egg after 2 weeks) EF2, n-3 PUFA depositions in eggs exceeded by far the 300 mg per reference amount of egg required for certification of omega-3 enriched egg (CFIA, 2015). Scheideler and Froning (1996) produced omega-3 enriched eggs (350 mg/egg of n-3 PUFA compared with 60 mg/egg in control eggs) by feeding 15% of flaxseed. Similarly, Jia et al. (2008) reported that hens fed 15% of flaxseed had the greatest n-3 PUFA amount (562 mg/egg) compared with those fed canola seed (207 mg/egg) and Linpro (427 mg/egg).

Plasma total n-3 PUFA concentration increased by 137 and 322% for layers fed 6 and 9% EF2, respectively. The corresponding increase in liver total n-3 PUFA concentration were 115 and 155%, respectively. Higher LNA, DHA and total n-3 PUFA depositions in liver and plasma occurred when hens were fed flaxseed (Cherian and Hayat, 2009). Nain et al. (2012) suggested that a similar pattern was followed when transferring n-3 PUFA from diet to plasma.

The ratio of n-6 to n-3 PUFA decreased as a result of EF2 supplementation. Similar reductions were also observed for blood plasma and liver. The decline of n-6 to n-3 PUFA ratio in egg yolk, plasma and liver resulted primarily from an increase in LNA and a decrease in AA. Our findings are in line with those of AI-Nasser et al. (2011) who observed 77% reduction in ratio of n-6 to n-3 PUFA in eggs from hens fed 10% of flaxseed for 8 weeks.

There was no effect of EF2 on total amount of cholesterol in egg yolk from current study. This was likely due to the essentiality of keeping sufficient cholesterol for the development of chick embryo in egg. Our findings are consistent with those of Ansari et al. (2006) who observed that egg cholesterol was not affected by feeding 5% and 10% of flaxseed diets. Similar effects were reported for layers fed 10% of flaxseed diet (Botsoglou et al., 1998).

5.6 CONCLUSIONS

Results of this study indicated that feeding EF2 up to 9% of the diet had no negative impact on layer performance and egg components. Apparent total tract nutrient digestibility was not affected by 3% of dietary EF2, but was reduced with moderate to high levels (6 and 9%) of EF2. Supplementation of EF2 significantly increased n-3 PUFA concentrations in egg yolk, blood plasma and liver tissues with the highest concentration achieved at 9% EF2. However, total egg cholesterol was not influenced by EF2 supplementation.

Table 5.1. Chemical composition of improved extruded flaxseed (EF2)[1]

Item	% DM
Ash %	7.19
CP %	20.11
Fat %	30.83
NDF %	26.88
Energy Cal/g	5812.15
Fatty acids, % of total fatty acids	
C14:0	0.06
C16:0	6.23
C16:1	0.06
C18:0	2.45
C18:1n9c	13.86
C18:2n6c	17.14
C18:3n3	60.10

[1]OmegaPlus: manufactured by Belisle Solution Nutrition Inc., Quebec, Canada.

Table 5.2. Ingredients and chemical composition of dietary treatments

	Extruded Flaxseed inclusion %			
Ingredients %	0	3	6	9
Corn	54.56	53.77	52.97	52.17
Soybean	30.18	28.56	26.93	25.31
OmegaPlus[1]	-	3.00	6.00	9.00
Soybean oil	2.28	1.72	1.16	0.59
Salt	0.31	0.31	0.32	0.32
Chromic oxide	0.30	0.30	0.30	0.30
Premix[2]	0.50	0.50	0.50	0.50
Calcium	10.34	10.29	10.25	10.20
Phosphorus	1.21	1.23	1.24	1.26
Methionine	0.12	0.12	0.12	0.12
Choline chloride	0.10	0.10	0.10	0.10
Sodium carbonate	0.10	0.10	0.10	0.10
Threonine	-	0.04	0.01	0.02
Lysine-HCL	-	-	-	0.01
Calculated analysis				
ME, kcal/kg	2781.74	2713.95	2646.07	2577.82
CP, %	19.00	19.00	19.00	19.00
Fiber, %	2.38	2.57	2.76	2.95
Total methionine, %	0.43	0.43	0.43	0.43
Total lysine, %	1.07	1.04	1.02	1.00
FA, % of total FA				
C16:0	14.29	13.44	12.62	11.04
C18:0	2.23	2.34	2.29	2.32
C18:1	22.39	21.11	19.52	17.87

C18:2n6c	56.63	50.78	45.44	38.56
C18:3n3	4.18	12.05	19.84	29.96

[1] Manufactured by Belisle Solution Nutrition Inc., Quebec, Canada.

[2] Composition of premix: Vitamin A 11,530 KIU/kg; Vitamin D 2,400 KIU/kg; Vitamin E 74.168 IU/kg; Copper 24mg/kg; Iron 200mg/kg; Magnesium 122mg/kg; Selenium 0.38mg/kg; Zinc 131mg/kg; Cobalt 0.46mg/kg; Fluorine 19mg/kg; Iodine 0.80mg/kg.

Table 5.3. Effects of extruded flaxseed on egg production parameters and change in BW

Treatment	Week	Egg wt (g)	Egg production (%)	Feed intake (g)	FCR	Change in BW (g)
0% EF		64.06	92.99	127.87	2.16	-53.47[b]
3% EF		63.51	93.51	127.90	2.16	67.86[a]
6% EF		63.43	91.14	122.93	2.14	18.38[a]
9% EF		62.59	95.80	126.43	2.12	71.69[a]
SEM		0.88	1.98	2.41	0.06	30.76
	2	63.34	95.64	131.93[a]	2.20[a]	-7.07[c]
	4	63.48	94.40	124.34[b]	2.08[b]	19.35[b]
	6	63.55	91.93	129.97[a]	2.24[a]	29.54[b]
	8	63.22	91.47	118.90[c]	2.06[b]	62.64[a]
SEM		0.47	1.39	1.45	0.04	16.09
P-value						
Treatment		0.7050	0.4400	0.4394	0.9597	0.0316
Week		0.5781	0.1021	<0.0001	<0.0001	<0.0001

[a-c]Means within treatment and week with no common superscript differ significantly ($P < 0.05$).

Table 5.4. Effects of extruded flaxseed on egg components

Treatment	Week	Yolk wt (%)	Albumen wt (%)	Eggshell wt (%)
0% EF		27.14	60.49	12.37
3% EF		27.59	60.15	12.26
6% EF		27.88	60.25	11.87
9% EF		27.17	60.55	12.28
SEM		0.39	0.49	0.16
	2	27.12	60.15	12.73[a]
	4	27.19	60.08	12.73[a]
	6	27.55	60.67	11.78[b]
	8	27.92	60.53	11.55[b]
SEM		0.31	0.33	0.13
P-value				
Treatment		0.5029	0.9332	0.1612
Week		0.2519	0.3786	<0.0001

[a-b]Means within treatment and week with no common superscript differ significantly ($P < 0.05$).

Table 5.5. Effects of extruded flaxseed on total tract nutrient digestibility for a period of 4 weeks

	Extruded Flaxseed inclusion %					
Total tract digestibility %	0	3	6	9	SEM	*P*-value
DM	69.19[a]	66.74[ab]	65.40[ab]	62.81[b]	0.96	0.0014
OM	75.27[a]	72.82[ab]	71.53[b]	69.76[b]	0.76	0.0005
GE	76.24[a]	75.53[ab]	73.02[ab]	72.15[b]	0.92	0.0141
AMEn	2861.92[a]	2760.48[ab]	2691.23[b]	2640.28[b]	37.72	0.0031

[a-b]Means within treatment with no common superscript differ significantly ($P < 0.05$).

Table 5.6. Effects of extruded flaxseed on fatty acid composition of blood plasma of laying hens

	Extruded Flaxseed inclusion %					
Fatty Acid %	0	3	6	9	SEM	*P*-value
C14:0	0.39	0.40	0.37	0.39	0.02	0.7686
C16:0	30.24	30.09	29.51	29.60	0.33	0.3343
C16:1	3.21	3.41	3.23	3.47	0.18	0.6967
C18:0	8.48	8.24	8.33	8.38	0.21	0.8647
C18:1n9c	37.90	38.32	38.02	36.59	0.49	0.0884
C18:2n6c	16.66[a]	15.48[ab]	15.27[ab]	14.41[b]	0.50	0.0384
C18:3n3	0.80[d]	1.91[c]	3.07[b]	5.26[a]	0.22	<0.0001
C20:4n6	1.64[a]	1.27[b]	1.17[b]	0.88[c]	0.03	<0.0001
C22:6n3	0.70[b]	0.91[a]	1.03[a]	1.02[a]	0.05	0.0001
Total SFA[1]	39.10	38.72	38.21	38.37	0.34	0.2910
Total USFA[2]	60.90	61.28	61.79	61.63	0.34	0.2910
Total n-3 PUFA[3]	1.49[d]	2.81[c]	4.11[b]	6.29[a]	0.24	<0.0001
Total n-6 PUFA[4]	18.30[a]	16.74[ab]	16.44[ab]	15.29[b]	0.52	0.0054
Ratio n-6/n-3[5]	12.32[a]	6.02[b]	4.10[c]	2.48[d]	0.18	<0.0001

[a-d]Means within treatment with no common superscript differ significantly ($P < 0.05$).

[1]Total SFA was calculated as 14:0 + 16:0 + 18:0.

[2]Total USFA was calculated as 16:1 + 18:1n9c + 18:2n6c + 18:3n3 + 20:4n6 + 22:6n3.

[3]Total n-3 PUFA was calculated as 18:3n3 + 22:6n3.

[4]Total n-6 PUFA was calculated as 18:2n6c + 20:4n6.

[5]Ratio n-6/n-3 was calculated as total n-6 PUFA/total n-3 PUFA.

Table 5.7. Effects of extruded flaxseed on fatty acid composition of liver of laying hens

Fatty Acid %	Extruded Flaxseed inclusion %					
	0	3	6	9	SEM	*P*-value
C14:0	0.26[ab]	0.29[a]	0.23[b]	0.23[b]	0.01	0.0008
C15:0	0.05	0.05	0.05	0.05	0.002	0.2059
C16:0	24.74[ab]	25.00[a]	23.42[bc]	23.05[c]	0.37	0.0005
C16:1	1.99[ab]	2.27[a]	1.81[ab]	1.75[b]	0.13	0.0264
C18:0	13.05[ab]	11.98[b]	14.49[a]	14.12[a]	0.41	0.0002
C18:1n9c	34.59[ab]	37.68[a]	31.94[b]	32.86[b]	1.14	0.0042
C18:2n6c	17.61	15.69	17.34	17.34	0.71	0.2214
C18:3n3	0.64[c]	1.13[c]	1.83[b]	2.96[a]	0.15	<0.0001
C20:4n6	5.20[a]	3.78[b]	5.35[a]	4.22[ab]	0.35	0.0046
C20:5n3	0.03[c]	0.07[c]	0.22[b]	0.36[a]	0.02	<0.0001
C22:6n3	1.84[b]	2.05[b]	3.31[a]	3.06[a]	0.16	<0.0001
Total SFA[1]	38.11	37.32	38.20	37.45	0.28	0.0672
Total USFA[2]	61.89	62.68	61.80	62.55	0.28	0.0672
Total n-3 PUFA[3]	2.50[d]	3.25[c]	5.37[b]	6.38[a]	0.23	<0.0001
Total n-6 PUFA[4]	22.81	19.47	22.69	21.57	0.99	0.0763
Ratio n-6/n-3[5]	9.25[a]	6.16[b]	4.26[c]	3.41[d]	0.18	<0.0001

[a-d]Means within treatment with no common superscript differ significantly ($P < 0.05$).

[1]Total SFA was calculated as 14:0 + 15:0+ 16:0 + 18:0.

[2]Total USFA was calculated as 16:1 + 18:1n9c + 18:2n6c + 18:3n3 + 20:4n6 + 20:5n3 + 22:6n3.

[3]Total n-3 PUFA was calculated as 18:3n3 + 20:5n3 + 22:6n3.

[4]Total n-6 PUFA was calculated as 18:2n6c + 20:4n6.

[5]Ratio n-6/n-3 was calculated as total n-6 PUFA/total n-3 PUFA.

Table 5.8a. Effects of interaction of dietary treatment and feeding time on fatty acid composition of eggs (mg/60g of egg) of laying hens

	Week 2				Week 4			
Fatty acid	0%	3%	6%	9%	0%	3%	6%	9%
C14:0	25.2	27.6	27.2	27.1	24.8	27.3	27.2	24.4
C15:0	4.1	3.6	4.8	4.4	4.0	3.8	4.5	4.2
C16:0	2516.7	2545.3	2614.1	2564.6	2514.2	2576.8	2640.1	2434.0
C16:1	233.3	281.5	350.8	326.3	258.0	305.5	361.1	283.9
C18:0	826.0	773.2	776.9	796.0	812.7	806.4	804.0	833.3
C18:1	3850.1	4037.3	3839.4	3816.9	3493.5	3644.6	3778.5	3751.8
C18:2n6c	1548.0^{ab}	1336.7^{b}	1535.6^{ab}	1342.5^{ab}	1583.2^{ab}	1426.2^{ab}	1487.2^{ab}	1351.0^{ab}
C18:3n3	62.1^{e}	121.0^{de}	370.3^{b}	471.2^{ab}	76.7^{de}	184.0^{cd}	365.4^{b}	513.4^{a}
C20:3n6	12.8	9.3	10.7	8.3	12.6	9.1	10.2	8.5
C22:0	16.8	15.4	18.1	15.2	17.1	15.4	18.1	14.5
C20:4n6	182.8	153.3	124.5	105.9	176.9	137.1	118.5	95.3
C20:5n3	1.4^{h}	3.4^{gh}	9.8^{cde}	14.6^{a}	1.8^{h}	5.3^{fgh}	10.4^{bcd}	13.4^{abc}
C22:6n3	95.4	136.0	156.0	154.5	95.8	132.4	149.4	151.8
Total SFA[1]	3388.7^{ab}	3365.1^{ab}	3441.1^{ab}	3407.3^{ab}	3372.7^{ab}	3429.7^{ab}	3493.9^{a}	3310.5^{ab}
Total MUFA[2]	4083.4	4318.8	4190.3	4143.2	3751.4	3950.1	4139.6	4035.7
Total USFA[3]	5985.9	6078.3	6397.2	6240.1	5698.3	5844.2	6280.8	6169.1

Total n-3 PUFA[4]	158.9^{g}	260.3^{efg}	536.2^{b}	640.2^{a}	174.3^{fg}	321.7^{cde}	525.2^{b}	678.5^{a}
Total n-6 PUFA[5]	1743.6^{abc}	1499.2^{bcd}	1670.8^{abcd}	1456.7^{bcd}	1772.6^{ab}	1572.4^{abcd}	1615.9^{abcd}	1454.8^{cd}
Ratio n-6/n-3[6]	11.0^{a}	5.8^{b}	3.1^{e}	2.3^{e}	10.3^{a}	4.9^{cd}	3.1^{e}	2.2^{e}

[a-h]Means within the interaction of treatment and week across the rows with no common superscript differ significantly ($P < 0.05$).

[1]Total SFA was calculated as 14:0 + 15:0+ 16:0 + 18:0 + 22:0.

[2]Total MUFA was calculated as 16:1 + 18:1.

[3]Total USFA was calculated as 16:1 + 18:1 + 18:2n6c + 18:3n3 + 20:3n6 + 20:4n6 + 20:5n3 + 22:6n3.

[4]Total n-3 PUFA was calculated as 18:3n3 + 20:5n3 + 22:6n3

[5]Total n-6 PUFA was calculated as 18:2n6c + 20:3n6 + 20:4n6.

[6]Ratio n-6/n-3 was calculated as total n-6 PUFA/total n-3 PUFA.

Table 5.8b. Effects of interaction of dietary treatment and feeding time on fatty acid composition of eggs (mg/60g of egg) of laying hens

	Week 6				Week 8					*P*-value
Fatty acid	0%	3%	6%	9%	0%	3%	6%	9%	SEM	EF x Time
C14:0	28.6	27.5	26.6	25.7	24.8	25.9	27.5	25.6	0.92	0.0036
C15:0	3.6	3.3	3.8	4.1	4.2	3.8	3.9	4.2	0.15	0.0002
C16:0	2598.8	2564.8	2584.9	2457.1	2560.2	2573.4	2603.5	2395.7	31.65	0.0034
C16:1	304.0	300.5	329.3	296.5	279.9	295.9	317.2	297.2	15.05	0.0010
C18:0	783.5	814.0	817.7	814.1	761.8	779.8	802.6	782.3	19.05	0.3364
C18:1	3550.2	3695.4	3743.6	3475.8	3505.0	3851.5	3776.3	3483.5	89.65	0.1154
C18:2n6c	1402.2^{ab}	1316.6^{b}	1284.3^{b}	1308.4^{b}	1646.5^{a}	1440.6^{ab}	1412.5^{ab}	1359.6^{ab}	42.68	0.0211
C18:3n3	57.1^{e}	154.8^{cde}	257.3^{c}	483.8^{a}	77.0^{de}	171.0^{cde}	265.2^{c}	501.4^{a}	15.49	<0.0001
C20:3n6	10.8	9.0	9.5	8.9	12.5	9.1	9.5	8.5	0.53	0.1395
C22:0	16.3	15.8	16.5	15.0	16.0	14.8	15.4	15.0	0.56	0.0710
C20:4n6	172.2	137.3	122.2	91.4	170.3	146.3	123.5	90.6	3.99	0.2065
C20:5n3	1.5^{h}	5.1^{fgh}	8.7^{def}	14.2^{ab}	1.6^{h}	4.5^{gh}	6.9^{efg}	13.6^{abc}	0.54	0.0002
C22:6n3	87.5	128.6	149.7	151.9	93.6	134.4	157.4	149.9	4.29	0.5217
Total SFA[1]	3430.8^{ab}	3425.2^{ab}	3448.4^{ab}	3316.0^{ab}	3367.1^{ab}	3397.6^{ab}	3452.7^{ab}	3222.9^{b}	34.81	0.0475
Total MUFA[2]	3854.1	3995.9	4069.3	3772.3	3784.9	4147.4	4093.6	3780.6	87.77	0.1586

Total USFA[3]		5585.4	5747.2	5906.1	5830.9	5786.3	6053.2	6068.6	5904.3	90.87	0.0654
Total PUFA[4]	n-3	146.1^{g}	288.4^{ef}	415.8^{cd}	649.9^{a}	172.1^{fg}	309.8^{de}	429.6^{c}	665.0^{a}	15.85	<0.0001
Total PUFA[5]	n-6	1585.2^{abcd}	1462.9^{bcd}	1416.4^{cd}	1408.7^{d}	1829.3^{a}	1596.0^{abcd}	1545.5^{abcd}	1458.7^{bcd}	44.03	0.0356
Ratio /n-3[6]	n-6	11.0^{a}	5.1^{bcd}	3.4^{de}	2.2^{e}	10.7^{a}	5.2^{bc}	3.6^{cde}	2.2^{e}	0.23	0.0009

[a-h]Means within the interaction of treatment and week across the rows with no common superscript differ significantly ($P < 0.05$).

[1]Total SFA was calculated as 14:0 + 15:0+ 16:0 + 18:0 + 22:0.

[2]Total MUFA was calculated as 16:1 + 18:1.

[3]Total USFA was calculated as 16:1 + 18:1 + 18:2n6c + 18:3n3 + 20:3n6 + 20:4n6 + 20:5n3 + 22:6n3.

[4]Total n-3 PUFA was calculated as 18:3n3 + 20:5n3 + 22:6n3

[5]Total n-6 PUFA was calculated as 18:2n6c + 20:3n6 + 20:4n6.

[6]Ratio n-6/n-3 was calculated as total n-6 PUFA/total n-3 PUFA.

Table 5.9. Effects of extruded flaxseed on egg cholesterol

	Extruded Flaxseed inclusion %					
Item	0	3	6	9	SEM	*P*-value
Cholesterol Concentration in egg yolk (mg/g)	15.58[b]	15.95[ab]	16.35[a]	16.22[a]	0.13	0.0402
Total cholesterol per egg (mg)	289.19	296.54	296.33	287.89	3.53	0.2233

[a-b]Means within treatment with no common superscript differ significantly ($P < 0.05$).

CHAPTER VI.

GENERAL DISCUSSION AND CONCLUSION

The objective of this study was to evaluate the performance of laying hens fed two different extruded flaxseed products (OmegaPlus, Belisle Solution Nutrition Inc., Quebec, Canada). The first study determined the effects of extruded flaxseed (EF1, 19% ether extract) fed at 0, 7.5, 15 and 22.5% of the diet, while in the second study EF2 (29% ether extract) was fed at 0, 3, 6 and 9% of the diet.

Results from both studies showed that no adverse effects of extruded flaxseed on egg weight, egg production, feed intake and feed conversion ratio. Similarly, egg components including yolk, albumen and shell percentages were similar among dietary treatments. Apparent total tract nutrient digestibility in both studies was significantly reduced by extruded flaxseed supplementation, especially at moderate to high levels (i.e. 15 and 22.5% EF1; 6 and 9% EF2). In the first study, feeding moderate to high levels of EF1 (i.e. 15 and 22.5%) reduced apparent total tract nutrient digestibility of DM, OM and GE, while feeding high level of EF1 (i.e. 22.5%) reduced apparent total tract digestibility of CP and AMEn. In the second study, apparent total tract digestibility of OM and AMEn were lower in birds fed 6 and 9% EF2 compared with other treatments. However feeding 9% EF2 reduced apparent total tract digestibility of DM and GE. The negative effect of extruded flaxseed on apparent total tract nutrient digestibility is most likely due to greater NDF content of extruded flaxseed diet.

The results of 2 studies also indicated that omega-3 labelled eggs (i.e. $\geq$ 300 mg n-3 PUFA per egg) were successfully produced with 7.5% EF1 (Experiment 1) and 6% EF2 (Experiment 2) after 2 weeks of feeding. Therefore, dietary supplementation with extruded flaxseed is an efficient means of transferring and modifying fatty acids profile of eggs for the production of omega-3 enriched eggs. On the other hand, extruded flaxseed supplementation significantly reduced the concentrations of n-6 PUFA in eggs. In Experiment 2, dietary EF2 increased n-3 polyunsaturated fatty acids concentrations in blood plasma and liver tissues. The elevated concentrations of LNA and DHA contributed to the increase in n-3 polyunsaturated fatty acids. However, n-6

polyunsaturated fatty acids decreased with the increasing level of EF1 and EF2 supplementations. This is mainly because linolenic acid and linoleic acid compete for the same metabolic enzymes for the conversion to longer chain polyunsaturated fatty acids. Therefore, the ratio of n-6 to n-3 polyunsaturated fatty acids in egg yolk was reduced. Effects of EF2 supplementation on blood plasma and liver fatty acid composition were similar to those observed for egg yolk suggesting consistent pattern of nutritional changes. Overall, results of both studies indicated that feeding high levels of EF1 (i.e. 22.5%) and EF2 (i.e. 9%) had no adverse effects on layer performance and total egg cholesterol. Increasing dietary levels of EF1 and EF2 significantly increased the depositions of n-3 PUFA in eggs. However, the fact that lower level of EF2 was required for successful production of omega-3 labelled eggs makes it a more efficient extruded flaxseed product than EF1, and this was due to higher contents of ether extract and linolenic acid. Therefore, it is recommended that EF2 be incorporated into layer diets at levels below 6% for successful production of omega-3 labelled eggs and without any detrimental effects on nutrient digestibility.

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