STRATEGIES FOR IMPROVING FATTY ACID PROFILE OF EGGS FOR PRODUCTION OF OMEGA-3 ENRICHED EGGS

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ABSTRACT

Master of Science **Dept. of Animal Science**

Keyvan Amini

STRATEGIES FOR IMPROVING FATTY ACID PROFILE OF EGGS FOR PRODUCTION OF OMEGA-3 ENRICHED EGGS

Two experiments were carried out to evaluate the effects of Pearl Millet in combination with different levels of flaxseed and natural pigment (Oro Glo 15®) on quantity of n-3 fatty acids in eggs, laying performance and yolk pigmentation. In the first experiment, six different diet treatments were used for six weeks, with 24 hens per treatment (three birds per cage, eight cage replicates). Control diet was a corn-soybean meal diet, and diets containing 0, 2, 4, 8 or 12 % ground flaxseed in which all the corn was replaced by pearl millet. In the second experiment, the diet treatments consisted of pearl millet and three inclusion levels of ground flaxseed (4%, 6% and 8%) and two levels (0.1% and 0.2%) of natural pigment in a factorial arrangement. The experiment lasted for twelve weeks, with 18 hens per treatment (three birds per cage, six cage replicates). In each of the experiments, all the diets were formulated to be isocaloric and isonitrogenous and to meet or exceed NRC requirements. Body weight of the birds and feed consumption were recorded at weekly (first experiment) and biweekly (second experiment) intervals. Number of eggs and egg mass produced were measured and recorded on a daily basis. At the end of each of the experiments, all the hens were euthanized to determine liver integrity. In both of the experiments, flock performance parameters were not different among treatments. In regard to egg traits, in the second experiment after 8

week of the start of the experiment, birds fed with diets containing 8% flaxseed produced significantly ($P < 0.05$) smaller eggs compared to hens fed 4% flaxseed. Yolk pigmentation was lower ($P < 0.05$) for the eggs produced by hens fed diets containing pearl millet compared with those produced by feeding corn-based diet. However, 0.1% or 0.2% inclusion of the pigment both proved to be suitable to restore yolk pigmentation to marketable levels. No difference was observed among diets in regard to liver haemorrhage. Evaluation of FA profiles indicated that birds fed a diet containing PM as the sole grain source, and low levels of flaxseed (6%) can produce eggs with more than 350 mg / egg of n-3 FA, which is the lower standard to market eggs as "n-3 FA enriched"..

(Keywords: laying hens, pearl millet, flaxseed, natural pigment, flock performance, eggs, liver haemorrhage)

RESUME

Maitrise en Science **Zootechnie**

Keyvan Amini

STRATEGIES D'AMELIORATION DU PROFIL EN ACIDES GRAS DES ŒUFS POUR LA PRODUCTION D'ŒUFS ENRICHIS EN OMEGA- 3

Deux expériences ont été conduites pour évaluer les effets du millet perle canadien en combinaison avec différentes quantités de graines de lin et de pigments naturels (Oro Glo 15®) sur le profil en acides gras des œufs, sur les performances de ponte, et sur la pigmentation du jaune d'œuf. Dans la première expérience, six régimes différents ont été administrés pendant six semaines, avec 24 poules pondeuses par traitement (trois poules par cage, 8 cages de répétition). Le régime témoin était composé de maïs-soja, tandis que dans les régimes expérimentaux, contenant 0, 2, 4, 8 ou 12% de graines de lin moulue, le maïs a été remplacé par du millet perle. Dans la seconde expérience, les traitements expérimentaux ont consisté en l'introduction de trois quantités différentes de graines de lin moulue (4%, 6% et 8%), et de deux différentes quantités (0.1% et 0.2%) de pigment naturel (Oro Glo 15®), dans un arrangement factoriel. L'expérience a duré douze semaines, avec 18 poules pondeuses par traitements (trois poules par cage, six cages de répétition). Dans chacune des expériences, les régimes ont été établis de façon à être isocaloriques et isoazoté, et conformes aux recommandations NRC. Le poids corporel des oiseaux et leur consommation alimentaire ont été relevés de façon hebdomadaire (première expérience), ou bihebdomadaire (deuxième expérience). Le nombre d'œufs pondus, ainsi que leur poids, ont été relevés de façon journalière. A

la fin de chaque expérience, toutes les poules pondeuses ont été euthanasiées pour déterminer l'état d'intégrité de leur foie. Dans les deux expériences, les paramètres de performances des lots n'ont pas été différents selon les régimes. Concernant les caractéristiques des œufs, dans la seconde expérience, huit semaines après le début de l'expérience, les poules nourries avec un régime contenant 8% de graines de lin ont produit des œufs plus petits ($P < 0.05$) par rapport aux poules nourries avec un régime contenant 4% de graines de lin. La pigmentation du jaune a été plus faible ($P < 0.05$) pour les régimes contenant du millet perle par rapport au régime témoin à base de maïs; cependant, les inclusions de 0.1% ou 0.2% de pigment ont été efficaces pour restaurer une pigmentation de jaune d'œuf à des niveaux commercialisables. Concernant l'hémorragie hépatique, aucune différence n'a été observée entre les différents régimes. L'évaluation du profil en acides gras des œufs a montré qu'un régime à base de millet perle contenant de faibles quantités de graines de lin peut fournir assez d'acides gras n-3 pour la production d' « œufs enrichis en oméga 3 ».

(**Mots clés**: poules pondeuses, millet perle, graines de lin, pigment naturel, performances de lot, œufs, hémorragie hépatique)

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I dedicate this work to the one who is all my reasons, my mother.

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CHAPTER 1: GENERAL INTRODUCTION

INTRODUCTION

The cereal grains form the major components of poultry rations. Thus, it is important to use cereal grains that provide a nutritionally balanced and economically sound diet. Corn is the principal energy source in most poultry diets in the world. In the last decades, there has been an increasing demand for corn as the main ingredient in poultry feed; this has necessitated a search for alternative energy sources for corn in poultry diets. Pearl millet (*Pennisetum glaucum*) is one of the world's most important food crops adapted to very dry and hot climates. It has a long history of use as human food in Africa and Asia and has several agricultural and environmental advantages. It has the potential to be a crop well suited to many parts of North America. Studies have shown that it is a promising grain crop for areas in which drought, soil type, short season or excessive heat reduces the yield potential of corn. New varieties of pearl millet are well adapted to Canadian conditions and are developed to be used in rotational programs by potato producers (Ball-Coelho *et al* .2003); however, the market for the grain is not well developed. A considerable interest has been expressed by the broiler, layer and turkey producers as to the possible value of pearl millet as a feed ingredient for chickens and turkeys. Poultry may prove to be an ideal market for this grain and poultry producers are interested in the value of pearl millet. Pearl millet has proved to be a more economical source of feed compared to sorghum (Evans and Singh, 2005).

Investigations conducted by Kumar *et al.* (1991) , Collins *et al.* (1997), Abd-Elrazig and Elzubeir (1998) and other workers have proved that pearl millet may wholly or partially replace yellow corn in layers' diets. These researchers have

shown that this grain is as acceptable and as readily consumed as corn. It is possible to replace yellow corn in both mash and grain mixtures with millet and yet obtain satisfactory and comparable results (Garcia and Dale, 2006). Moreover, on the basis of nutrient composition and feed trials, the results of the reported work indicate that pearl millet is as good as or better than most of cereal grains commonly used in poultry rations (Collins, *et al.* 1995; Kumar *et al.*, 1991).

Evidence from epidemiological studies and clinical trials has emphasized the effects of omega-3 (n-3) fatty acids on decreasing the risk of heart disease and enhancing immune system of humans. Evidences also suggest that the current Western diet could be deficient in n-3 fatty acid content (Simopoulos, 2000). This situation has stressed the need for alterations in the patterns of consumption of these lipids.

Eggs contain high-quality protein and almost all vitamins and minerals needed for the human diet; however, they have been blamed for an increasing risk of heart disease due to cholesterol content. This has resulted in a great reduction in the per capita consumption of eggs during the last decades (Noble *et al.*, 1990; Chanmugam, *et al*, 1992). Today, the most prevalent causes of mortality in the developed world are those associated with heart diseases. This fact has raised concern about the relationship between health and diet, in general, and the dietary lipid profile, in particular. As there is a limited consumption of n-3 polyunsaturated fatty acids (PUFA) in the human diet, researchers have encouraged the alternative production of foods with high n-3 PUFA content. The fatty acid composition of poultry meat and eggs usually reflects the composition of bird's diet (Cruikshank,

1934; Ajuyah *et al.*, 1992) and fatty acid profile in the eggs can be modified by changes in the hens' diet (Cherian and Sim, 1991; Grobas *et al*, 2001; Cherian, Holsonbake and Goeger, 2002). The inclusion of n-3 fatty acids in poultry products is achievable by feeding n-3 fatty acid rich diets to birds. The aim of increasing the n-3 fatty acid content of poultry products is to augment the n-3 consumption in humans. This has opened the possibility to develop "Omega Eggs" with higher levels of n-3 fatty acids for the health-conscious consumer at premium prices. In "Omega Eggs" the yolks fatty acid profile has been modified by altering the hens' diet. Designer eggs comprise about four percent of the total egg production in Canada. A large (60 g) egg labelled as "Omega Egg" contains at least 350 mg of n-3 fatty acids (Scheideler and Lewis, 1997).

Flaxseed, canola and menhaden oil, are common feed ingredients used to increase n-3 fatty acid contents in poultry products. However, menhaden oil supplementation produces off-flavours in the eggs, and its long term supplementation causes hepatic lipidosis in hens. On the other hand, long term supplementation with flaxseed causes reproductive alterations, attributed to phytoestrogenic compounds (Ahn *et al.*, 1995; Van Elswyk *et al.*; 1997a; Aymond *et al.*, 1995), and is also associated with a high incidence of liver haemorrhages in hens (Bean and Leeson, 2003). A fishy or fish-related flavour has also been reported in the eggs from hens fed flaxseed (Jiang *et al.*, 1992; Ahn *et al.*, 1995). Furthermore, feeding high levels of flaxseed has been ascribed to some changes in production parameters (Aymond and Van Elswyk, 1995). Caston *et al.* (1994) reported an increased feed consumption in laying hens, although it is attributable to

differences in metabolizable energy of experimental diets containing 10 or 20% flaxseed.

Pearl millet's nutritional characteristics are attractive for poultry: relatively high protein content (10 to 16%) (Burton *et al.*, 1972), metabolizable energy of about 3000-3300 kcal/kg (Burton *et al.*, 1972; Adeola *et al.*, 1994; Singh *et al.*, 2005), and apparent absence of major anti-nutritional factors. Compared to common cereals, pearl millet is rich in oil, with a typical fat content above 5%. Linolenic acid (C18:3n-3) (LNA) comprises 4% of the total fatty acids in this oil (Rooney, 1978), giving it a higher content of n-3 fatty acids than other cereal grains. Corn is notably deficient in n-3 fatty acids, with LNA comprising only about 0.9% of total fatty acids. Replacing corn with pearl millet in laying hen diets reduces the ratio of n-6 to n-3 fatty acids in eggs, and although production parameters and egg flavour is not affected, it results in reduced yolk pigmentation compared with a corn based feed (Collins *et al.* 1997). Therefore, the potential exists to develop a nutrition program to produce omega enriched eggs using a combination of pearl millet, low levels of flaxseed and a natural pigment, in order to preserve the quality of omega eggs and also the health and well-being of the hens. The objectives of this thesis are: 1) evaluation of the effects of pearl millet (replacing corn) with different inclusion levels of flaxseed on flock performance and egg trait parameters as well as liver integrity of laying hens and also their effect on egg n-3 fatty acid content and n-6/n-3 fatty acid ratio, 2) evaluation of a natural pigment in a pearl millet based diet in combination with low levels of flaxseed in a longer trial for production of "Omega Eggs".

CHAPTER 2: LITERATURE REVIEW

2.1 Pearl Millet

Pearl millet (*Penissetum glaucum*) is the most important millet species among the several small-seeded tropical cereals grown worldwide for human food and animal feed. It is generally agreed that pearl millet was domesticated in Africa, probably on the southern edge of the Sahara, west of the Nile, some 3,000 to 5,000 years ago and subsequently spread to southern Asia (Harlan 1975; Brunken *et al.* 1977). In 1986, pearl millet was grown worldwide on a total of 40 million hectares (ha) according to United Nations Food and Agriculture Organization. Though figures are not available for separate species in all countries, pearl millet is grown on about 26 million hectare in the warm tropics and divided equally between Africa, particularly in the West African Sahel region, and the Indian subcontinent. In these areas, pearl millet is grown almost exclusively as human food, and indeed is the staple cereal of 90 million people who live in agro-climatic zones where there are severe stress limitations to crop production mainly due to heat, low and erratic rainfall, and soil type.

Pearl millet is known as the world's most drought-resistant grain, and maintains its seed production capabilities in climates even drier than those tolerated by sorghum. If enough moisture is present for the seeds to germinate and the shoots to emerge, then it is very rare for the crop to fail totally, even in severe drought (Andrews *et al.* 1996). It is mostly grown for both grain and fodder productions, under hot and dry conditions on uncultivable soils of low water holding capacity, where other crops generally fail to yield grain.

Pearl millet appears to be more tolerant of sandy and acidic soils than other summer grain crops. It is deep-rooted and can use residual nitrogen, phosphorus and potassium and therefore may not need the levels of fertilizer required by other summer grains. These characteristics enhance pearl millet's desirability in lower input, dry land production systems. It is well suited for double-cropping after small grains and vegetables.

In the past decades, breeders have used the large gene pool to innovate strategies to maximize the potential of this crop in grain production. Breeders have produced dwarf strains of pearl millet with heights of about one meter instead of the traditional two meters or more. The short season varieties can be planted as a summer crop, following a winter crop of wheat or canola. In Africa, since fertilizers are mostly not used and cultivation is by hand or animals, actual grain yields are low (0.55 to 0.66 metric tons/hectare) but US trials have averaged 2.3 to 3.5 metric tons per hectare in the Midwest and south. A yield of 5.3 metric tons /hectare has been reported in USA. Average pearl millet yield in USA is still lower than the average corn yield and usually about 85% of the yield of grain sorghum. However, Stegmeier *et al.* (1987) found that on sandy soils pearl millet may out-perform grain sorghum.

2.2 Chemical Composition of Pearl Millet Grain

The chemical composition of pearl millet is comparable to sorghum and corn. Tables 1 and 2, show chemical composition and nutritive values of pearl millet, sorghum and corn, used as fed and also on dry mater basis, respectively.

Table 1: Chemical composition and nutritive value of pearl millet, sorghum, and corn used as fed

Parameter	Pearl Millet	Sorghum	Corn
Dry matter (%)	89	87	89
Metabolizable Energy (kcal/kg)	2675	3288	3350
Crude protein (%)	14	8.8	8.5
Fat (%)	4.3	2.9	3.8
Crude Fibre (%)	3.0	2.3	2.2
Linoleic acid (%)	0.84	1.13	2.20
Calcium (%)	0.05	0.04	0.02
Total phosphorus (%)	0.32	0.30	0.28
Non-Phytin Phosphorus (%)	0.12	0.09	0.08

Source: National Research Council, 1994

Table 2: Chemical composition of pearl millet, sorghum, and corn on dry matter basis

Parameter	Pearl Millet	Sorghum	Corn
Dry matter (%)	88-89.3	88.4-99.2	88.4-89.6
Crude protein (%)	7.1-14.4	10.0-14.1	8.8-10.4
Crude fibre (%)	2.3-3.0	1.5-5.9	2.3-2.8
Fat (%)	4.3-5.1	1.8-5.7	1.8-3.8
Total ash (%)	2.6-6.2	1.77-3.60	1.8-3.2
Nitrogen-Free Extract (%)	-	65.3-84.2	64-90
Calcium (%)	0.03-0.31	0.01-0.36	0.02-0.07
Phosphorus (%)	0.11-0.77	0.13-0.69	0.11-0.45
Available Carbohydrates (%)	60.8	56-63	-

Source: Rajashekher Reddy, A. *et al.* ,2003

Pearl millet, like sorghum, contains high protein levels compared with corn. However, depending on strain, growing conditions and harvest period, pearl millet can vary somewhat in its crude protein (CP) content. Oshodi *et al.* (1999) cited a protein content of 11.4% , Haydon and Hobbs (1991) stated a value of 16.6% , Burton *et al.*(1972) reported it to be 16% and Singh *et al.* (1987) listed strains with protein contents ranging from 9.2% to 21.4% . Abd-Elrazig and Elzubeir (1998) also analyzed three cultivars of pearl millet and reported a range of 9.6% to 12.7% of crude protein content. In an experiment, Ejeta (1987) analysed several varieties between 12% and 14% of protein content and NRC (1994) listed pearl millet with a 14% CP content. The protein content of the grain can be increased from 12% to 13% by application of nitrogen fertilizers (Hoseney and Varriano-Marston, 1980).

Table 3 compares the amino acid composition of corn, sorghum and pearl millet. Pearl millet's higher content of essential amino acids is mainly due to its higher total protein. When expressed as percentage of protein, the essential amino acid content of pearl millet is slightly higher than that of corn, and absolutely higher than that found in low tannin sorghum (Sullivan *et al.* 1990; Ejeta *et al.* 1987; Badi *et al.* 1976). Lysine is the limiting amino acid in pearl millet. The other amino acids are present in adequate amounts, when compared with typical animal requirements. Singh *et al.* (1987) compared several inbred pearl millet genotypes with respect to protein and amino acid content. When expressed as a percentage of grain weight, the high protein genotypes were 60% higher in total crude protein, but only 37% higher in lysine than low protein types.

Table 3: Protein and amino acid composition (% of protein) of pearl millet, sorghum and corn

	Grain		
	Pearl Millet	**Sorghum**	**Corn**
Crude protein (%)	15.7	10.5	9.0
Methionine	0.25	0.17	0.18
Cystine	0.24	0.16	0.19
Lysine	0.45	0.24	0.27
Threonine	0.48	0.31	0.32
Tryptophan	0.08	0.10	0.07
Arginine	0.74	0.37	0.44
Isoleucine	0.37	0.34	0.31
Leucine	1.14	0.99	1.07
Valine	0.49	0.44	0.42
Histidine	0.39	0.20	0.26

Source: National Research Council, 1994 ; Degussa Feed Additives, 2001

As shown in table 4, estimates of apparent metabolizable energy corrected for nitrogen (AME_N) in pearl millet used as poultry feed reported by different workers show a considerable variation. It should also be taken into consideration that the NRC (1994) estimate is about 20% lower than the values from recent reports. Differences may be due to the use of discrete strains of grain or chicken, different harvest periods, or grinding and handling differences.

Table 4: Estimates of apparent metabolizable energy, nitrogen-corrected (AME_N) content of pearl millet grain for poultry (as-fed basis)

AME_N, kcal/kg (species, feeding time)	Authors	Publication date
2760 (broilers, 6 weeks)	Sharma *et al.*	1987
3054 (broiler chicks, 10 days)	Fancher *et al.*	1987
3000 (broiler chicks, 3 weeks)	Smith *et al.*	1989
3346 (broilers ,41 days)	Amato and Forrester	1992
3464 (TME, roosters)	Amato and Forrester	1993
2675	NRC	1994
3300 (ducks, 21 days)	Adeola *et al.*	1994
3350 (ducks , mature males)	Ragland *et al.*	1997
3506 (White Leghorn roosters)	Collins *et al.*	1997
2736 (broilers,2-42 days)	Rao *et al.*	2004

There are few reports on the vitamin content of pearl millet in the past years. Choline, niacin, pantothenic acid and riboflavin are high in pearl millet as compared to corn. Pearl millet contains almost double the quantity of thiamine compared to corn (Rajashekher Reddy *et al.* 2003). Morrison (1977) found only 0.5 ppm α-tocopherol, which would suggest low vitamin E level. NRC (1994) has listed no estimate for vitamin E. Simwemba *et al.* (1984) estimated the riboflavin, thiamin and niacin contents of pearl millet at 3.0, 5.4 and 44.2 µg/g, respectively. These values can be compared with the NRC (1994) estimates of 1.6, 6.7 and 53 µg/g, respectively. Simwemba *et al.* (1984) found that niacin content were different

among cultivars, while thiamine level was affected by growing location. Akingbala *et al.* (2002) reported 29.4 and 140 μg/kg of dry weight, as Vitamin A and Vitamin B_2 content of pearl millet, respectively. Table 5, demonstrates NRC (1994) values for vitamin composition of pearl millet, sorghum and corn.

Table 5: Vitamin composition (ppm) of pearl millet, sorghum and corn

Vitamin	Pearl Millet	Sorghum	Corn
Biotin	-	0.26	0.06
Choline	793	668	620
Folacin	-	0.2	0.4
Niacin	53	41	24
Pantothenic acid	7.8	12.4	4.0
Pyridoxine	-	5.2	7.0
Riboflavin	1.6	1.3	1.0
Thiamine	6.7	3.0	3.5
Vitamin E	-	7	22

Source: National Research Council, 1994.

Phytic acid is the main phosphorus store in mature grains. It easily forms complexes with minerals and proteins. Most phytate-metal complexes are insoluble and make several minerals unavailable for animals and birds. The phytate content of pearl millet is similar to that of corn and sorghum and is not a serious problem in diets for chickens. Like most grains, pearl millet seems to be low in calcium and available phosphorus. Smith *et al.* (1989) reported only 0.07% Ca and 0.11% available P. Since nearly 70% of phosphorus in pearl millet is present as phytate, like the other grains, the methods for releasing this phosphorus seem to be important. Mahajan and Chauhan in 1987 found out that a 72 hour *in vitro*

fermentation doubled the free phosphate content, probably due to action of natural phytase in the grain. In 1995, Murry and Lewis showed that microbial phytase added to a pearl millet diet, enhanced P availability for swine. Table 6 compares mineral composition of pearl millet with sorghum and corn.

Table 6: Mineral composition of pearl millet, sorghum and corn.

Mineral	Pearl Millet	Sorghum	Corn
Calcium (%)	0.05	0.04	0.02
Phosphorus (%)	0.32	0.30	0.28
Non-phytin phosphorus (%)	0.12	0.09	0.08
Sodium (%)	0.04	0.01	0.02
Sulfur (%)	0.13	0.06	0.08
Potassium (%)	0.43	0.35	0.30
Chloride (%)	0.14	0.09	0.04
Iron (ppm)	25	45	45
Magnesium (ppm)	0.16	0.15	0.12
Manganese (ppm)	31	15	7
Copper (ppm)	22	10	3
Selenium (ppm)	-	0.2	0.03
Zinc (ppm)	13	15	18

Source: National Research Council, 1994.

Pearl millet has higher quantity of fat than most grains. In 1972, Burton *et al.* estimated an average of 4.5% fat with a range of 3.0 to 6.3%. Other reports of fat content of pearl millet include: 5.5% (Rooney, 1978); 4.1% (Smith *et al.* 1989) and 9% (Eiche, 1994). NRC (1994) estimated an ether extract of 4.3% (table 1). As shown in table 4, the energy content of pearl millet reported by NRC (1994) is low, compared with other estimates. Therefore, this fat estimate might also be somewhat

conservative. It is likely that fat content varies with genotype and growing conditions. Jellum and Powell (1971) measured the effects of seed maturity on fatty acid composition. They found that, the time after pollination affects seed fatty acid composition, but the temperature does not. All mature grains of the same strain had similar composition, regardless of planting season or temperature.

The fatty acid composition of pearl millet probably varies somewhat by strain. Table 7 shows published estimates of fatty acid composition of pearl millet.

Table 7: Estimates of fatty acid compositions of pearl millet seeds (% of total fatty acids)

	Author				
Fatty Acid	**Jellum and Powell, 1971**	**Rooney, 1978 (Av. Several Cultivars)**	**Pansu *et al.* (1981) (Several Cultivars)**	**Osagie and kates, 1984**	**Ibrahima *et al.*,2004 (Several Cultivars)**
16:0	19.9	19.0	19.6-23.4	16.3	18.2-20.1
18:0	3.9	5.0	2.6-5.5	5.4	3.5-5.9
18:1	27.7	25.0	22.1-32.1	26.6	25.7-30.3
18:2	42.2	46.0	38.9-48.4	47.9	40.5-46.5
18:3	5.0	3.2	2.2-4.0	3.0	2.1-2.6
20:0	0.4	0.5	Not Determined	0.7	0.6-0.8

Rooney (1978) measured fatty acid compositions of different strains, grown at different seasons and harvested at different stages of maturity. Jellum and Powell (1971) and Osagie and Kates (1984) both investigated different analytical procedures for determination of fatty acids with a single strain of pearl millet. Rooney's results listed in Table 7 are averages for mature grain. Ground pearl

millet may develop off-flavours in long term storage. These flavours are correlated with hydrolytic and oxidative changes in fat fraction (Lai and Varriano-Marston, 1980). Moreover, micro-organisms often utilise sugars and produce alcohols during long period storage under typical conditions (Hoseney and Varriano-Marston, 1980). Table 8 shows a comparison of fatty acid composition of pearl millet with that of some other feed ingredients. Composition of each feed ingredient may vary considerably by strain and growing conditions, but the general trends in this table can be used to compare their fatty acid estimates. Collins' results (1997) for pearl millet listed here are the averages obtained from four different cultivars.

Table 8: Fatty acid composition of corn, pearl millet, canola oil, menhaden oil and flax oil (% of total fatty acids)

	Corn		Pearl Millet		Canola oil	Menhaden	Flax oil
Source >	Collins,1997	Chow, 1992	Rooney, 1978	Collins,1997	da Silva, 2005	Chow, 1992	Chow, 1992
Fatty Acid ˅							
Saturated	18.3	14.5	25.0	22.0	9.03	37.6	9.5
Monounsaturated	20.0	27.6	26.2	27.8	63.32	34.6	19.9
Linoleic (C18:2n-6)	54.6	57.0	45.1	44.1	14.98	4.4	15.9
α-Linolenic (C18:3n-3)	1.25	0.9	3.7	2.9	12.07	0	52.7
Long chain n-3	0	0	0	0	0	23.5	0
n-6/n-3 ratio	43.6	63.3	12.2	15.21	1.24	0.82	0.30

Because of relatively high total fat content and high percentage of linolenic acid in its fat, pearl millet seems to be the grain highest in linolenic acid. The implication of this fact forms the basis of much of this thesis.

The fibre content of pearl millet is higher than sorghum and corn (Table 1). Pearl millet contains 0.66% water soluble non-starch polysaccharides and 3.88% water insoluble non-starch polysaccharides (Bailey *et al.*, 1979).

Anti-nutritional compounds can be classified broadly as those naturally present in the grains and those occurring due to contamination which may be of fungal origin or may be related to soil and other environmental influences. These factors modify the nutritional value of grains and some of them have serious nutritional and health consequences. Pearl millet has fewer anti-nutritional factors than most grain ingredients. Rye and sorghum grains contain tannins, which limit palatability and inhibit protein digestion, but pearl millet is free of tannins. Pearl millet contains phytic acid, a phosphorus compound which may inhibit mineral absorption, at levels comparable to wheat (Simwemba *et al.* 1984). Some evidences exist for goiterogenic compounds in pearl millet, but problems have only been seen in diets very low in iodine (Osman and fatah, 1981; Klopfenstein *et al.*, 1982). Yellow coloured pearl millet has less goiterogenic effects than brown or grey millet.

Like other cereals, pearl millet is susceptible to fungal growth and mycotoxin production under certain environmental conditions. Wilson *et al.* (1993) found that pearl millet is more resistant to *Aspergillus flavus* infestation. However, it is susceptible to *Fusarium* infection. When harvest was delayed 50 days after seed set, Wilson *et al* (1995) isolated *Fusarium* species in 71.5% of the grain samples. Infestation of pearl millet by parasitic fungi, *Claviceps fusiformis*, causes an outbreak of ergotism (Peraica and Domijan, 2001).

2.3 Pearl Millet as Feed Ingredient for Poultry

With the escalation of poultry feed prices and the real prospect of declining availability of grains for livestock, it is opportune that research efforts be directed to finding alternative feed ingredients. The costs of traditional poultry feeds have continued to rise steadily since the early 1970's. This has stimulated research in developing alternative and cheaper sources for poultry. It is well known that nearly 60% of total poultry production costs are ascribed to feed costs. One of the methods of decreasing this cost is to replace the traditional feed ingredients with equally efficient alternatives.

Research on the use of pearl millet in poultry diets has been carried out to ease competition for energy sources and to reduce feed costs. Studies conducted by several research workers have shown that millet compared favourably with corn in poultry diets (Singh and Barsoul, 1976; Sharma *et al.* 1979; Smith *et al.* 1989, Mohan *et al.*, 1991; Collins *et al.* 1997; Abd –Elrazig *et al.* 1998). Singh and Barsoul (1976) found pearl millet could replace corn on an equal weight basis without effecting grain or feed efficiency. When pearl millet was compared to corn on an isocaloric and isonitrogenous basis, it gave comparable gain and efficiency (Sharma *et al.*, 1979). Smith *et al.* (1989) reported that pearl millet could replace corn in the diets of chicks at up to 100% without adversely affecting gain and feed efficiency. Collins *et al.* (1997) and Mohan *et al.* (1991) concluded that pearl millet is suitable as a layer grain and can be included at up to 60%. Results from poultry-feeding experiments in the USA using pearl millet with corn or sorghum indicate

that pearl millet is a suitable feed ingredient. Poultry are probably the major consumers of pearl millet in the United States.

Pearl millet appears to be a satisfactory feed grain for laying hens. Reddy and Reddy (1970) observed comparable performances in regard to food efficiency, egg production and body weight over a 7 x 28 d experimental period when corn was replaced with pearl millet at 320g/kg. Chawla *et al.* (1987) used pearl millet in place of corn (w/w) at lower level of 200 g/kg and found no differences in layer performance over 135 days. Kumar and Reddy (1991) found increased egg size and better feed conversion ratio when pearl millet was substituted for 60% of corn. The high methionine and energy content of pearl millet might explain these results. Abd-Elrazig *et al.* (1998) used three different cultivars of pearl millet and found no significant differences in egg production, feed consumption and feed conversion ratio between experimental diets and a corn-based reference diet.

2.4 Metabolism of Polyunsaturated Fatty Acids

It is believed that there are particular metabolic pathways for discrete groups of fatty acids. This assumption is because of the fact that fatty acids are not used on equal bases for oxidation, which depends on their chain length and degree of unsaturation (Leyton, *et al.*, 1987). Dietary essential fatty acids include two families: n-3 and n-6, designated for the position of the double bond nearest the methyl end of the molecule. The α-linolenic acid (LNA, C18:3 n-3) is the representative of n-3 family, and Linoleic acid (LA, C18:2 n-6) is the most important fatty acid of n-6 series. Longer chain n-3 fatty acids, include

Eicosapentaenoic acid (EPA, C20:5n-3), Docosahexaenoic acid (DHA, C22:6n-3), and long chain n-6 fatty acids include Arachidonic acid (C20:4n-6) (AA). Unlike the other groups of fatty acids, the n-3 and n-6 families follow similar pathways and the same enzymes catalyze the reactions involved in their biosynthesis. Consequently, there is a close interaction between these families which makes the discussion of both appropriate for purposes of this work.

Generally the n-3 and n-6 series follow two pathways: 1) β-oxidation which takes place in the mitochondria and peroxisomes of cells (Coates and Tanaka, 1992) and then desaturation and elongation. N-6 and n-3 fatty acids are desaturated and elongated in mammalian and avian liver. The major end product of the n-6 system is AA, while the n-3 system usually ends in DHA 2) the biosynthesis of the membrane phospholipids, leukotrienes and prostaglandins and thromboxanes. These are produced enzymatically by cyclization and oxidation of the parent long chain fatty acids.

The factors that determine which of these pathways predominate are the degree of unsaturation (Leyton, *et al.* 1987), the presence of other fatty acids that compete for the same enzymes (Opara and Hubbard, 1993), the type of protein in the diet (Ikeda *et al.*, 1994) and the tissue involved in the metabolism of specific fatty acid (Pawlosky *et al.*, 1994). High dietary consumption of short chain n-6 PUFA, supplied by ingredients such as corn oil ,lead to high tissue levels of AA, while diets rich in n-3 PUFA will result in higher tissue levels of DHA. There are scarce quantities of fatty acids longer than 18 carbons in plants, but in animals the 20 and 22 carbon fatty acids have important membrane and intracellular functions.

The elongation and desaturation of LNA depends on the factors such as the consumption of other n-6 fatty acids. LNA competes with LA for Δ-6 desaturase, the enzyme catalyzing desaturation process. Even though the Δ-6 desaturase has a preference for LNA over LA, the latter is generally consumed in much larger quantities and so competes effectively with LNA (Gerster, 1988). Terrestrial plant fats often have high levels of the n-6 family, and corn is especially rich with LA, comprising an average of 48% of total fatty acids (NRC, 1994). However, a few plant oils are rich in the LNA, notably flax, and to a lesser extent, canola. The relative excess of n-6 fatty acid in modern diets not only inhibits the formation of EPA and DHA, but also enhances the synthesis of eicosanoids involved in inflammatory, cardiovascular and immunological diseases (Chan *et al.*, 1993). Given the importance of maintaining a balance of n-3 and n-6, a ratio of n-6/n-3 has been used extensively by researchers. Consideration should be given to the fact that n-6/n-3 ratio varies depending on the source of n-3 fatty acids since these fatty acids are not quantitatively equivalent in all sources (Takita *et al.*, 1994; Woods *et al.*, 1996).

2.5 Beneficial effects of dietary n-3 fatty acids on human health

There is presently a great interest in n-3 polyunsaturated fatty acids (n-3 PUFA) in regard to its beneficial effects on human health. It is well known that n-3 fatty acids potentially prevent cardiovascular diseases, diabetes, several auto-immune disorders and some types of cancer. They also play a significant role in neonatal growth (Simopoulos, 2000).

The usually low incidence of vascular diseases among Inuits has caught the attention of scientists worldwide (Kroman and Green, 1980; Middaugh, 1990). Epidemiological evidence in these populations suggest their diets may reduce the incidence of cardiovascular diseases (Hirai *et al.* 1989;Kromhout , 1989) .The dietary habits of Inuits include a high consumption of fat, cholesterol and protein that is considered to predispose to heart diseases. However, the fat consumed came from marine sources rich in n-3 fatty acids (Nobmann *et al.*, 1992; Newman *et al.*, 1993).

Research on other human populations has found a high correlation between fish consumption and a low incidence of vascular diseases leading to the conclusion that high intake of fish may account for such benefits (Kagawa *et al.*, 1982; Kromhout *et al.*, 1985; Yamori *et al.*, 1985; Daviglus *et al.*, 1997). These findings were reviewed by Caggiula and Mustad (1997) and Simopoulos *et al.* (2003), leading to renewed interest in elucidating the complex role of n-3 fatty acid on human health. Nettleton (1991) suggested that the high n-6: n-3 ratio typical in American diets might inhibit the conversion of linolenic acid to EPA due to substrate competition. Neuringer (1986) has suggested that the n-6: n-3 ratio in the diet should be lower than 10:1, and a total of about two grams/day of n-3 PUFA would be desirable. In a study on consumer acceptability by Marshall *et al.* (1994), they found that 71% of consumers would be willing to pay a premium price for shell eggs which are enriched with n-3 fatty acids at levels comparable to those in fish.

2.6 Dietary Manipulation of fatty Acid composition of Eggs

The fatty acid content of the yolk is greatly influenced by the fatty acid profile of the bird's diet. Many studies have confirmed this fact over the decades, with early studies usually comparing oilseeds and animal fat sources (Cruikshank, 1941; Sell *et al.* 1968; Naber, 1978), and later studies incorporating fish oils, newer oilseeds such as canola, and novel marine algae (Nitsan *et al.* 1999).

Usually, egg composition would be altered within 1-2 weeks of dietary changes (Collins, 1997). According to Van Elswyk (1997a) n-3 fatty acid enriched eggs can be obtained by enriching layer feeds with flaxseed and/or canola, as these easily promote the incorporation of n-3 fatty acids in the egg yolk. The addition of these n-3 fatty acid-rich fat sources in layer feeds aims to change the lipid profile of the eggs, which represents an excellent marketing strategy to provide healthy eggs for health-conscious consumers.

In general, the two most important ingredients that are used in layers' diets for the purpose of incorporation of n-3 fatty acids in the eggs are:

2.6.1 Flax

Flaxseed is a rich source of protein (22.4%) and lipids (37.4%) for poultry (Lee *et al.*, 1995). Gonzalez-Esquerra and Leeson (2000 b) found out that the metabolizable energy of flaxseed differs significantly by age of the birds (3560, 2055 and 2118 kcal/ kg for 72 wk, 6 wk and 9 d old birds, respectively) which is attributable to greater tolerance of the older birds to laxative effects of flaxseed.

Moreover, dietary flaxseed is a rich source of linolenic acid that has been shown to dramatically increase the n-3 fatty acid content of eggs (Caston and Leeson, 1990; Jiang *et al*, 1991, 1992). Flaxseed is one of the most concentrated sources of linolenic acid available in natural plant feedstuffs for poultry. Flaxseed contains as much as 35% ether extract, of which about 50% is linolenic acid (Panford and deMan, 1990). Caston and Leeson (1990) observed a highly significant increase from 0.38 to 8.9% linolenic acid in the eggs, by inclusion of 20% flaxseed in the diet. Jiang *et al.*(1992) found comparable increases in linolenic acid content of the eggs after feeding 15% flaxseed, but the sensory evaluation of eggs indicated a high incidence (36% of respondents) of off-flavours in hard-cooked eggs produced. Several groups interested in marketing a product which grows well in far northern climates, have investigated the effects of feeding flax to the chickens. The most obvious effect was a very large increase in linolenic acid, and proportionally much smaller, but still significant increase in long chain n-3 PUFA's. Aymond *et al.* (1994) increased egg linolenic acid from 0.95 mg/g yolk to 16.08 mg/g by feeding 15% flaxseed, while long chain n-3 PUFA increased from 4 mg/g to 8.3 mg/g. Using six different strains of layers, Ahn *et al.* (1995) found egg linolenic acid increased from about 0.5% to about 5% and long chain n-3 PUFA increased from about 1% to 3% of total fatty acids when a proprietary blend of "high linolenic acid ingredients" was fed at 17% of the diet.

There are conflicting reports in terms of effect of flaxseed on egg flavour. Jiang *et al.* (1992) fed 15% flaxseed and found that 36% of taste panellists detected fishy flavours in eggs. These investigators suggested this might have occurred due to

the presence of lipid oxidation or direct transfer of the flavouring characteristics of flaxseed into yolk. However, panellists detected no such flavours in eggs from hens fed sunflower seeds or tallow.

However, Mazalli *et al.* (2004 b) reported no influence of flaxseed on the flavour of hard-cooked eggs, probably because the level of inclusion of flaxseed (9%) was lower than that used by Jiang *et al.* Ahn *et al.* (1995) found that flax tended to produce poorer egg flavour scores, but the effect was significant for only two of the six strains of hens studied.

Aymond and Van-Elswyk (1995) reported that the use of 15% whole flaxseed did not increase the quantity of oxidation of unsaturated fatty acids. However, the experimental diets they used were stored under refrigeration. Scheideler *et al.* (1994) found that a brown variety of flax fed at 15% of diet gave poorer egg flavour scores, but golden flax gave scores which did not differ from the corn-soy controls. Caston *et al.* (1994) fed 10% and 20% flax, and found conflicting evaluations of egg flavours. Increasing the level of dietary flax had a significant and detrimental effect on flavour evaluations, but individual panellists were highly inconsistent in their responses.

According to Scheideler *et al.* (1997), other factors must be considered, such as the level of inclusion of flaxseed, form, physical condition of the flaxseed (whole or ground), temperature of storage of diet and egg, and the amount of antioxidants added in the diet for oxidative stabilization of fresh stored eggs.

Moreover, some anti-nutritional factors are reported to exist in flaxseed. Linatine, which is a trypsin inhibitor (Klosterman, *et al.* 1967), found in mucilage,

may decrease the productivity of the birds by decreasing the amount of endogenous enzymes released from the pancreas, thus decreasing digestion of feed particles. According to Kennedy *et al.*, (1994) and Aymond *et al.*, (1994) flaxseed contains certain diphenolic compounds which can be converted by bacteria to lignans, which are known to be phytochemicals (phyto-estrogens). They may act as steroid hormones and compete with estrogen, thus decreasing its level in the blood and potentially interfering with productivity of laying hens.

2.6.2 Canola

Canola (the low glucosinolate, low erucic acid form of rapeseed) is a trademarked cultivar of the rapeseed plant. The word "canola" is derived from "**Can**adian **o**il, **l**ow **a**cid". Either the oil or the full-fat seed may be fed to poultry.

Full-fat canola seed contains 41-43% oil and 20 to 25% protein (Lee *et al.*, 1991) and therefore is a valuable source of energy and protein (Leeson *et al.* 1978; Shen *et al.* 1983; Salmon *et al.* 1988). Canola has a rather high level of linolenic acid, typically 9-10% of total fatty acids, and a low level of less than 10% saturated fatty acids (Ajuyah *et al.*, 1991). Compared with flax, canola gives smaller increases in linolenic acid, but similar increases of long chain n-3 PUFA.

In a study in 2005 da Silva Filardi and co-workers found out that inclusion of canola oil to the feed of commercial layers does not cause any significant effect on performance parameters. This was in agreement with the results reported by Baucells *et al.* (2000) and Mazalli *et al.* (2004a) , reporting that addition of flax oil or canola oil did not cause any change in hens' performance . However, addition of

canola oil to the feed decreases the concentration of linoleic acid and at the same time, increases linolenic acid and DHA in the yolk (da Silva Filardi *et al.*, 2005). These reports confirm that canola results in acceptable n-6/n-3 fatty acid ratio in eggs in terms of human nutrition. Moreover, Nwokolo and Sim (1989) fed full-fat canola seed to layers at 10% of diet, and increased egg linolenic acid by 50% and DHA by 26%, compared with control. Cherian and Sim (1991) fed 16% canola seed to laying hens and egg linolenic acid increased from 0.6% to 2.4% of total fatty acids, and long chain n-3 PUFA increased from 1% to 1.7%. In all cases, increases in n-3 PUFA percentage were accompanied by corresponding decreases in saturated fatty acid content.

Therefore, because of favourable changes in fatty acid profiles of the eggs as a result of feeding canola, there has been interest in feeding canola to laying hens in industrial scale. Moreover, erucic acid levels in canola are negligible and glucosinolate levels are as low as 20 μg/g. These levels are low enough to be of little or no concern for poultry (Leeson and Summers, 2001).

2.7 Yolk Pigmentation

Egg yolk color is an important characteristic when estimating the quality of egg. Besides the standards of measurement such as egg weight, shape, shell thickness and shell weight, the color of the yolk is a decisive criterion in the evaluation and standardization of egg quality (Vuilleumier, 1968).

Carotenoids have been used for many years in the poultry industry as a means for pigmenting eggs and meat (Leeson and Summers, 1997). These pigments

control the color of the egg yolk, as well as the shanks and beaks of layers, and also the skin color that may be important in meat birds. The xanthophylls are the most important carotenoids in poultry nutrition, and natural ingredients rich in these compounds are alfalfa meal, corn gluten meal and marigold petal. In general, carotenoid pigments are mostly two types of xanthophylls: Lutein and Zeaxanthin. Brown in 1938 and William *et al.* in 1962 demonstrated that in general, laying hens will transfer to the egg yolk at least part of the carotenoids consumed, and various feed ingredients were found to affect the color of the yolk.

For the laying hens, yolk color is a consequence of pigments consumed in the layer feed, and also the transfer of pigments accumulated in the skin and shanks when bird was immature. This transfer of pigments to the ovary occurs regardless of diet pigments, and is responsible for the "bleaching" effect of the shanks and beak of yellow-skinned birds over time.

Yellow corn is the only grain that contributes sufficient xanthophyll that gives enough color to the skin of broilers and egg yolk of layers. Corn contains much more xanthophylls than do other cereals, although very high levels of pigmentation can be achieved by including other products such as alfalfa and corn gluten meal. Petals of marigold (*Tagetes erecta*) are very effective as yolk pigmentation agents (Madiedo and Sunde, 1964). Table 9 illustrates the xanthophyll levels of some feed ingredients. Since pearl millet in the diets has virtually not enough pigment to impart, in diets in which pearl millet replaces corn, the use of a natural pigment seems to be inevitable.

Table 9: Xanthophyll content of selected ingredients (mg/kg)

Ingredient	Xanthophyll
Corn	20
Wheat	4
Milo	1
Alfalfa meal	175
Corn gluten meal	275
Marigold petal	7000

Source: Leeson and Summers(2005)

In addition to pigmenting the yolk for marketing needs, there is growing evidence that lutein and zeaxanthin may be important nutrients for humans (Leeson and Caston, 2004). These pigments concentrate in the macular region of the eye (Landrum *et al.*, 1997), and are thought to help prevent macular degeneration (Rapp *et al.*, 2000), which together with cataract, are leading causes of blindness in developing countries. By the way, about 20% of North Americans over the age of 65 have some degree of macular degeneration. It seems that diets rich in lutein and zeaxanthin increase the level of these pigments in the macula which act as antioxidants and/or filters against damaging blue light and protect the sensitive area of the inner eye surface (Leeson and Caston, 2004).

Prologue to Chapter 3

Chapter 3 consists of a manuscript, authored by Keyvan Amini and edited by Dr. Ciro Ruiz-Feria, which has been submitted for publication in *British Poultry Science* journal. All the references mentioned in this chapter are listed under the "References" section at the end of this thesis. The tables and figures included in this manuscript are illustrated at the end of this chapter.

Chapter 3 explains the first experiment of this study, performed to evaluate pearl millet as a replacement for corn in laying hens' diets on flock performance, egg trait parameters and n-3 fatty acid content of the eggs as well as n-6/n-3 fatty acid ratio in the eggs.

CHAPTER 3

Evaluation of Pearl Millet and Flaxseed for Egg Production and their effect on egg n-3 Fatty Acid Content[1]

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ABSTRACT

A six week trial was conducted to evaluate the effects of Pearl Millet (PM), as a replacement for corn, and in combination with flaxseed (FS), on hens' productivity, egg trait parameters and egg n-3 (Omega-3) fatty acid (FA) content. Six diet treatments were used: a control diet (CTL, corn-soybean meal based diet), and diets containing 0, 2, 4, 8 or 12 % ground FS in which all the corn was replaced by pearl millet (PM-0, PM-2, PM-4, PM-8, and PM-12), respectively. All diets were formulated to be isocaloric and isonitrogenous and to meet the NRC (1994) requirements. White Leghorn hens of the Shaver strain were used (eight cage replicates per treatment, 3 hens per cage). At the end of each week, three eggs were randomly collected from each cage to measure egg trait parameters including yolk pigmentation score and then yolks were separated, pooled and lyophilized for fatty acid determination by Gas Chromatography (GC). Body weights (BW) and feed consumption were recorded weekly, whereas egg production (number of eggs and egg mass produced) were recorded daily. At the end of the experiment, all the hens were euthanized to determine liver haemorrhage score. Egg traits and flock performance parameters were not statistically different among treatments ($P < 0.05$) except at week four, where birds fed treatment diet PM-12 produced smaller eggs than hens fed diet treatments PM-0 and PM-2. Yolk pigmentation scores were lower ($P < 0.05$) for the PM-0 and PM-2 diets (1.60 ± 0.24 and 1.80 ± 0.20 respectively), increased with higher levels of FS (2.75 ± 0.47 for PM-12 diet) but did not reach the pigmentation levels of the control diet (6.00 ± 0.01). Liver haemorrhage scores were not affected by dietary treatment ($P < 0.05$). Hens fed the PM-8 and PM-12

diets produced eggs with n-3 FA content higher than required to be considered as n-3 FA enriched egg, and had a lower n-6/n-3 FA acid ratio compared to eggs of hens consuming CTL or the PM based diets with lower FS supplementation. These results suggest that PM can be used to substitute corn in the diets of laying hens, and may reduce the amount of FS needed to obtain n-3 FA enriched eggs.

(Keywords: laying hens, pearl millet, flaxseed, flock performance, eggs, liver haemorrhage)

INTRODUCTION

Pearl millet (*Pennisetum glaucum*) is a promising grain crop for areas in which drought, soil type, short season or excessive heat reduces the yield potential of corn. Recent investigations have focused on developing new varieties of PM adapted to Canadian conditions and used in rotational programs with potato (Ball-Coelho *et al.*, 2003). However, the Canadian market for the grain is not well developed. A search for new and improved feed ingredients to provide more certainty, flexibility and cost-effectiveness in poultry diets has led to the investigation of PM as poultry feed. Studies on formulation of poultry feeds using PM have been carried out to ease the competition for energy sources and to reduce feed costs (Evans and Singh, 2005).

Pearl millet's nutritional profile makes it a suitable feed ingredient for poultry. It has relatively high protein content (10-13.7%) and its metabolizable energy of 3000-3300 Kcal/Kg is comparable to that of corn (Burton *et al.*, 1972; Singh *et al.*, 2005). Pearl millet has fewer anti-nutritional factors than most grain crops. In contrast to sorghum and rye, pearl millet is low in tannins, which are

known to inhibit protein digestion and limit palatability. Pearl millet has an excellent amino acid profile: 40% richer in lysine and methionine, and 30% richer in threonine compared to corn (Rajashekher *et al.*, 2003). Therefore, substitution of PM for corn reduces the need for high protein feed ingredients and supplemental amino acids.

On the other hand, consumption of Omega-3 Fatty Acids (n-3 FA) brings positive effects on health and has potential in prevention and treatment of cardiovascular diseases, diabetes, arthritis and certain types of cancer. They cause a reduction in the concentration of triglycerides in blood; improve immune response, brain and eye function, and infant development (Kinsella *et al.*, 1990; Sanders, 1993; Uauy *et al*, 1999; Simopoulos, 2000).

The content and profile of FA in the eggs can be modified by changes in the diet (Cherian and Sim, 1991; Grobas *et al.*, 2001; Cherian *et al.*, 2002). This has opened the possibility to develop designer eggs with higher levels of n-3 FA (omega-3 enriched eggs) for the health-conscious consumer at premium prices. Designer eggs comprise about four percent of the total egg production in Canada. A large egg (60 g) must contain 350 mg of n-3 FA in order to be labelled as "Omega-3 enriched Egg" (Scheideler and Lewis, 1997). The production of eggs with higher content of n-3 FA is possible by supplementing the diets with feed ingredients rich in n-3 FA, such as menhaden oil (Hargis *et al.*, 1991; Cherian *et al.*, 2002) and flaxseed (Cherian and Sim, 1991). However, menhaden oil supplementation produces off-flavours in the eggs, and its long term supplementation causes hepatic lipidosis in hens. On the other hand, long term

supplementation with FS causes reproductive alterations, attributed to phytoestrogenic compounds (Van Elswyk *et al.*, 1994; Aymond *et al.*,1994; Ahn *et al.*, 1995), and is also associated with a high incidence of liver haemorrhages in long term use (Bean and Leeson , 2003). Furthermore, a fishy or fish-related flavour has been reported in the eggs from hens fed a diet with 15% inclusion of FS (Jiang *et al.*, 1992).

Pearl millet is high in oil content in comparison with other cereals, with an average fat content of more than 5%. According to Rooney (1978) linolenic acid (C18:3; n-3) comprises 4% of the total fatty acids in its oil, giving it a higher content of n-3 fatty acids than other cereal grains. In corn only 0.9% of the total fatty acids consist of n-3 fatty acids. Linoleic acid and linolenic acid which are respectively, the main n-6 FA and n-3 FA's compete for enzyme sites of Δ-6 desaturase, a catalyzing enzyme in the fatty acid elongation process (Adam *et al.* 1986). Therefore, the ratio of n-6 to n-3 fatty acids in the diet has been reported to be more important than the absolute amount of n-3 FA consumed (Boudreau *et al.*, 1991). This suggests that n-3 fatty acids in the diet should be increased, and the ratio of n-6: n-3 fatty acids should be decreased (Kinsella *et al.*, 1990).

The ratio of n-6:n-3 fatty acids in the eggs produced by hens fed a corn based diet, corn plus PM, or PM alone was 13.1 , 10.1 and 8.3, respectively, and although production parameters and egg flavour were not affected, feeding PM resulted in reduced yolk pigmentation compared with the corn based feed (Collins *et al.* 1997). The n-6: n-3 FA ratio in eggs from hens consuming diets containing menhaden oil

and FS was 3.92 and 2.75, respectively (Huang *et al.*, 1990; Aymond and Van Elswyk, 1995).

Considering that PM supplies more n-3 FA for deposition into eggs than corn, but it is apparently not enough to produce "Omega Eggs", the potential exists to develop a nutrition program to produce n-3 FA enriched eggs using a combination of PM and low levels of FS, in order to reduce off-flavours in the eggs produced and to preserve the health and wellbeing of the hens. The objectives of this study were to evaluate the effects of PM as replacement for corn with added low levels of FS on flock performance, egg trait parameters, liver integrity of laying hens, and the effect on egg n-3 FA content and n-6/n-3 FA ratio.

MATERIALS AND METHODS

Birds, Housing and Animal Care

White Leghorn hens (62 wk old) of the Shaver White strain (ISA Poultry) in their first cycle of production were used. The birds were housed in commercial type wire laying cages in a room with automatic light and ventilation control. Each cage accommodated three hens, allowing for a space of 400 square cm per hen. Hens were subjected to 16h light: 8h dark throughout the experiment. Feed and water were provided *ad libitum*. All experimental procedures involving animals were conducted according to a protocol reviewed and approved by McGill University Institutional Animal Care and Use Committee.

Dietary Treatments

Six different dietary treatments were used: a control diet (CTL, corn-soybean meal based), and diets containing 0, 2, 4, 8 or 12 % ground FS in which all the corn was replaced by pearl millet (PM-0, PM-2, PM-4, PM-8, and PM-12), respectively (Table 1) . Eight cage replicates were randomly assigned to each treatment with three birds per cage for a total of 144 hens. All diets contained a fixed level of 4.1% canola oil. The diets were formulated to be isocaloric and isonitrogenous and to meet NRC (1994) requirements. The pearl millet grain (PM) used in the experiment was developed by AERC[2] (Canadian Grain Pearl Millet Hybrid, CGPMH 1) and was milled by a hammer mill through a 1.5 mm sieve.

Flock Performance Parameters

Feed intake and BW were recorded on a weekly basis, whereas egg production (number of eggs and egg mass produced) was recorded daily. The flock performance parameters determined included hen-day egg production, egg mass produced per bird per day, feed consumption per bird per day and feed conversion ratio. At the end of the experiment, all the hens were euthanized to determine liver haemorrhage score. Livers were scored according to the method described by Schumann *et al.* (2000) based on a 1 to 5 scale, where a score of 1 indicated no haemorrhaging, score 2 denoted sparse small patches of hemorrhagic lesions, score 3 indicated large hemorrhagic patches on some lobes of liver, score 4 signified large haemorrhages patches on all lobes of liver and a score of 5 denoted excessive haemorrhaging all over liver.

[2] Agriculture Environmental Renewal Canada Inc.; www.aerc.ca

Egg Quality Parameters

Eggs were collected for two consecutive days at the end of each week, and three eggs per each cage replicate were randomly selected for measuring egg weight, yolk weight and shell weight and thickness. Albumen weight was calculated accordingly. Yolk pigmentation was determined using the Roche® color fan on a 1-15 scale.

Fatty Acid Analysis

Three yolks from each cage replicate were pooled at the end of each week, and lyophilized. Yolk fat was extracted according to the methods described by Folch *et al.* (1957) and converted to methyl esters using the Meth-Prep II[3] methylation kit. Approximately 150 mg of the lyophilized egg yolk sample was weighed into a 50-ml centrifuge tube, mixed with 8 ml of Folch solution (2:1 chloroform- methanol mix), and homogenized with a Polytron[4] for two minutes on ice. 200 µl recovery standard (C17:0; 5 mg/ml) was added to each tube, and the extraction mixture was sonicated on ice water for 30 min., vortexing occasionally. The mixture was allowed to stand for 30 min on ice, and then was filtered through a funnel with a bed of glass wool and sodium sulphate, and the tube and funnel were rinsed twice, using 4 and 3 ml of Folch solution, respectively. Four ml of 0.73% NaCl solution was added to each tube and vortexed, and then the tube was centrifuged at ~800 *g* for 5 min. Then 1.2 ml of the lower layer extracted solution was transferred to the GC auto sampler vials and 0.2 ml of Meth-Prep II was added and allowed to stand for an hour at room temperature for the methylation reaction to

[3] Alltech Associates, Inc. Deerfield ,IL, USA
[4] Brinkmann Instruments , Rexdale ,Ontario, Canada

take place. 70 µl of internal standard (C19:0 fatty acid methyl ester, 20 mg/ml) was added to the vial shortly before injection. Reaction mixture was then injected into the Gas Chromatograph (GC). The FA compositions of the six diet treatments were also determined by the same method (Table 2).

Gas Chromatography: A fused silica capillary column (100 m x 0.25 mm i.d.) with 0.25 µm film thickness on a Varian 3400 CX gas chromatograph[5], equipped with an auto sampler and a flame ionization detector (FID) was used to separate and quantify the fatty acid methyl esters. The injector and detector temperatures were set at 250°C and 275°C, respectively. Helium was used as the carrier gas at a flow rate of 2.0 ml / min. The initial column temperature was set at 80 °C, held for 1 min, and then increased by 30 °C /min to 180 °C. Then it was increased to 195 °C at the rate of 1 °C / min, and finally increased by 20 °C to a final temperature of 230 °C, held for 19 min. Fatty acid methyl esters were identified by comparison with retention times of authentic standards[6] .Peak areas and percentages were calculated using the Saturn GC/MS Workstation Software (Version 5.52)

Statistical Analysis

For the flock performance parameters and egg fatty acid analysis results, cage served as experimental and sampling unit and PROC-GLM procedure of SAS (2003) was used to process the data.

Statistical model used:

$Y_{ij} = \mu + TRT_i + e_{ij}$

Where:

5 Varian 3400,Varian Canada Inc., Mississauga ,ON, Canada
6 Nuchek, Elysian, MN 56028,USA

TRT_i : i = 1,2,3,4,5,6

e_{ij} : j = 1,2,3,4,5,6,7,8

Fixed effect parameters of the model:

a) μ is the overall mean

b) TRT_i is the fixed effect of i^{th} dietary treatment

Random effect parameter of the model:

σ^2_e is the random residual variation of the model

For egg trait results, egg served as sampling unit and cage was the experimental unit. Data were analysed using PROC-MIXED procedure of SAS (2003).

Statistical model used:

$Y_{ijk} = \mu + TRT_i + Cage_{ij} + e_{ijk}$

Where:

TRT_i : i = 1,2,3,4,5,6

$Cage_{ij}$: j = 1,2,3,4,5,6,7,8

e_{ijk} : k: 1,2,3

Fixed effect parameters of the model:

a) μ is the overall mean

b) TRT_i is the fixed effect of i^{th} dietary treatment

Random effect parameters of the model:

a) $Cage_{ij}$ is the random effect of cage

b) σ^2_e is the random residual variation of the model

Treatment means were separated using the least square means function of SAS. Significant differences among treatment means were separated using the Scheffe's multiple comparison test. Statistical differences were declared at $P < 0.05$.

RESULTS

Flock Performance and Egg Parameters

No differences were observed among diet treatments in regard to BW and flock performance parameters, including hen-day egg production, egg mass produced per bird per day, feed consumption and feed conversion ratio (FCR). Yolk weight, albumen weight, shell weight and shell thickness were not significantly affected by diet treatments. Birds fed diet treatment PM-12 produced smaller eggs than hens fed the PM-0 and PM-2, but only at wk four (Table 3). Yolk pigmentation scores were lower for the PM and PM-2 dietary treatments, increased with 12% inclusion level of FS, but did not reach the pigmentation levels of the control diet (table 4). Liver haemorrhage scores were not different among treatments at the end of the trial.

Fatty Acids

Changes in egg n-3 FA content were observed after one week of feeding the experimental diets. In week 1, 2, 4, 5 and 6 of the experiment, hens fed PM based diet deposited more n-3 FA into eggs compared to corn based diet (CTL).

Birds fed the PM-8 and PM-12 diets deposited higher amounts of n-3 FA's into their eggs compared to birds fed the CTL diet and diets with lower levels of FS.

PM-8 and PM-12 diets consistently deposited n-3 FA's into eggs with quantities higher than 350 mg/egg. This quantity of n-3 FA deposition could not be consistently met by feeding the PM-4, PM-2, PM-0 and CTL diet (Figure 1).

In week 1, 2, 4, 5, and 6 of the experiment, hens fed corn based diet produced eggs with highest n-6/n-3 ratio (Figure 2). By the way, PM-0 fed hens produced eggs with higher n-6/n-3 FA ratio compared to diets containing FS. This effect was observed in wk 1, 2, 3, 4 and 6.

DISCUSSION

The effects of PM (Kumar *et al.*, 1991; Collins *et al.*, 1997; Abd-Elrazig and Elzubeir, 1998) and FS (Cherian and Sim, 1991; Jiang *et al.*, 1991; Novak and Scheideler, 2001) on flock performance and on egg FA composition have been previously reported. However, there are no reports on the effects of the concurrent usage of PM and FS on n-3 FA enriched eggs. Our objective was to develop a nutritional program for production of n-3 FA enriched eggs using PM and low levels of FS.

This experiment lasted for six weeks (the experimental period which is also used by other researcher working on modification of FA composition of the eggs (Aymond and Van Elswyk, 1995; Collins *et al.*, 1997; Scheideler *et al*, 1997). Findings of previous workers who used high levels of n-3 FA in the diets indicate incremental trends in the n-3 FA content of the eggs in the first two weeks of treatment, after which the n-3 FA content reaches a plateau (Lin *et al.*, 1995; Herber and Van Elswyk, 1996; Van Elswyk, 1997a). The main objective of this trial was to

investigate the feasibility of our hypothesis, which was reducing the inclusion level of FS in a PM-based diet for production of omega eggs, and meanwhile to investigate the consistency of our results with the results of previous researches in regard to flock performance, egg trait parameters and liver integrity of laying hens.

In this study we did not find dietary effects on BW, feed consumption, feed efficiency and egg production, except in wk 4, when birds fed the PM-12 diet produced smaller eggs than birds fed the PM-0 and PM-2 diets. Reddy and Reddy (1970) observed comparable performances in feed efficiency and egg production when corn was replaced completely with PM. Chawla *et al.* (1987) found no differences in laying performance when corn was replaced by PM over a 20 wk study. Rama Rao *et al.* (2000) reported that broiler breeders fed PM as the principal energy source had similar egg production to that of birds fed corn based diets. Furthermore, Collins *et al.* (1997) found out that PM as a replacement for corn in hens' diets has no effect on feed intake and egg production. Our results agree with the previous results, and further indicate that PM is a safe alternative for replacing corn in the diets of laying hens.

In this study we did not find significant effects on egg weight when corn was replaced by PM. Rama Rao *et al.*, (2000) reported that egg weight was reduced in birds fed PM compared to birds fed a corn based diet. The content of linoleic acid in the diet is one of the most important factors determining egg size (Shultz *et al.*, 1962; Whitehead, 1981), and corn contains higher amounts of linoleic acid than PM. However, when birds consume adequate amounts of energy, the response to extra dietary linoleic acid is minimal (Grobas *et al.* 1999). In the above-mentioned report

by Rama Rao *et al.* (2000) the dietary treatments had low and unequal ME contents (2350 and 2100 kcal/kg for corn- and PM-based diets, respectively), whereas we used isoenergetic diets. A potential disadvantage of PM could be its lower ME content. According to Singh *et al.* (2005) different varieties of PM have different ME contents. The analysis of PM used in this study indicated an ME content of 3000 kcal/kg. We used canola oil in the dietary treatments, in order to meet the energy requirements of the hens. The advantage of canola oil is that it has lower n-6/n-3 FA ratio compared to other vegetable oils (NRC, 1994).

Feeding FS to laying hens has been associated with reduction in BW (Caston *et al.*, 1994;Schumann *et al.*, 2000; Novak and Scheideler, 2001) and this has been attributed to anti-nutritional factors that may reduce the digestion and absorption of feedstuffs providing energy (Gonzalez-Esquerra and Leeson, 2000 a ; Ortiz *et al.*, 2001; Rodriguez *et al.*, 2001). We did not find differences in BW in hens fed the different diets. This may be due to the fact that mature poultry seem to be less susceptible than younger birds to the anti-nutritional factors found in FS (Klosterman, 1974; Jiang *et al.*, 1991; Scheideler and Froning, 1996). The birds in our experiment were at the end of the first cycle of production. Possible differences in the chemical composition of FS, especially in the concentration of some anti-nutritional compounds, could also be taken into consideration as a probable reason (Lee *et al.*, 1995).

Egg shell quality (shell weight and shell thickness) was not affected by the dietary treatments, which is in agreement with previous reports (Novak and Scheideler, 2001; Bean and Leeson, 2003). In the same way, we found that

replacement of corn by PM did not affect yolk weight. This is also in agreement with the results obtained by Collins *et al.* (1997) who reported no change in yolk weight as a result of total or partial replacement of corn by PM.

It has been well documented that replacing corn with PM in the diet of laying hens results in reduced yolk pigmentation (Collins *et al.*, 1997; Abd-Elrazig *et al.*, 1998). Pearl millet does not have enough xanthophylls, the pigment that imparts a golden yellow color in yolks, which is present in high quantities in yellow corn. This may represent another disadvantage of PM compared with corn, especially in markets demanding a well pigmented yolk. We found that higher levels of FS in combination with PM slightly improved yolk pigmentation, although it remained lower than the pigmentation obtained with a corn based diet. Therefore, the use of PM must be concurrent with the use of natural or synthetic pigments to restore egg yolk presentation and market acceptability.

Liver haemorrhage scores were not affected by the inclusion of FS in the feed. This could be due to the short term of FS supplementation. Schumann *et al.* (2000) reported that FS at inclusion levels of 10% and 40% and for a period of 4 weeks did not increase liver haemorrhage, whereas Bean and Lesson (2003) reported that in a long-term trial (10 periods of 28 d), using 4-10% of dietary FS, liver haemorrhage score was increased. Further research is needed to verify the effect of long term FS supplementation in combination with PM on liver integrity.

Pearl millet is richer in fat content than most grains and the fat from PM has a higher percentage of linolenic acid (Burton *et al.* 1972; Rooney, 1978; Smith *et*

al., 1989; Eiche, 1992). The n-3 FA content of the eggs collected during the six wk period of this trial was strongly influenced by diet (Figure 1).

Hens fed a diet in which PM was the sole grain and without FS supplementation (PM-0) produced eggs with a higher content of n-3 FA, and a lower n-6/n-3 FA ratio compared to eggs produced by hens fed the CTL diet (Figure 2). However, these results were not consistent. For instance, in wk 3, n-3 FA levels in the eggs of birds fed the CTL diet and the PM-0 diet were similar, whereas the n-6/n-3 FA ratio in wk 3 was also similar between the two treatments. Furthermore, the increase in n-3 FA content associated with the PM-0 diet was not high enough to consider the egg as an "omega-3 enriched egg". We found that the levels of n-3 FA in the eggs had a tendency to decrease in weeks 3, 4, 5, and 6 in all treatments. This reduction in FA content could be attributed to reduction in feed intake due to the high ambient temperature recorded in those days.

As expected, the addition of FS in the diets increased the amount of n-3 FA in the eggs and reduced the n-6/n-3 FA ratio. However, 2% FS supplementation had a very limited effect in this regard, and it was necessary to use supplementation levels above 4% to obtain eggs with a higher n-3 FA level and a lower n-6/n-3 FA ratio compared with eggs obtained using CTL and PM-0 diets. However, with 4% FS supplementation and PM it was still not possible to consistently obtain the minimum quantity of 350 mg of n-3 FA per egg required for the production of an "omega-3 enriched egg". Hens fed a diet based on PM and 8% FS supplementation (PM-8) produced eggs with an n-3 FA content higher than that required for marketing eggs as "omega-3 enriched eggs". The n-6/n-3 FA ratio was also reduced

consistently at this level of FS supplementation. Higher levels of FS supplementation (PM-12) increased the levels of n-3 FA in the egg, although the increase was minor and inconsistent as compared with the PM-8 treatment. The n-6/n-3 FA ratio was not further improved by increasing the FS supplementation from 8 to 12%. These results indicate that the use of PM reduces the requirements of FS to produce n-3 FA enriched eggs. Using a corn based diet, it is necessary to use inclusions of 10-15% of FS to obtain an n-3 FA enriched egg (Scheideler and Froning, 1996). As previously discussed, the use of high levels of FS inclusion is associated with egg off-flavours and liver haemorrhage in hens. Thus by reducing the supplementation level of FS with a PM based diet, we expect to reduce, or eliminate those problems. As a matter of fact, we did not find adverse effects on liver integrity in our experiments, although longer term of use is necessary to see pathological lesions in the liver of hens fed FS.

In summary, we found that PM can totally replace the use of corn in the diet of laying hens without adverse effects on feed consumption, egg production, or egg quality, except yolk pigmentation score. Furthermore, since PM contains a higher amount of n-3 FA than corn, the dietary FS inclusion level to produce n-3 FA enriched eggs can be reduced. Lower inclusions of FS may reduce or prevent the adverse effects of long term FS supplementation, such as liver haemorrhage and the development of off-flavours in the eggs. Further research is needed to assess the effects of long term PM and low levels of FS supplementation on laying hen health and production, and to evaluate the use of natural pigments to restore yolk pigmentation.

ACKNOWLEDGMENTS

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TABLES

Table 1: Composition (%) and calculated analyses of the control, corn based diet (CTL), and dietary treatments in which corn was replaced by pearl millet (PM) with different levels of flaxseed (0, 2, 4, 8, and 12%)

	Dietary Treatments					
Ingredients	CTL	PM-0	PM-2	PM-4	PM-8	PM-12
Corn	53.2	0	0	0	0	0
Pearl Millet	0	61.7	59.5	57.3	52.9	48.6
Soybean-Meal 48	23.8	21.0	20.6	20.2	19.4	18.6
Flaxseed	0	0	2	4	8	12
Canola oil	4.1	4.1	4.1	4.1	4.1	4.1
Limestone	6.4	6.5	6.5	6.5	6.5	6.5
Dicalcium Phosphate	2	1.7	1.7	1.7	1.7	1.7
DL-Methionine	0.2	0.2	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2	0.2	0.2
Premix[1]	4.4	4.4	4.4	4.4	4.4	4.4
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Inert filler (sand)	5.5	0	0.6	1.2	2.4	3.5
ME (Kcal/Kg)	2750	2753	2752	2751	2750	2751
CP (%)	16.01	16.01	16.01	16.01	16.02	16.04
Ca (%)	4.24	4.22	4.22	4.23	4.23	4.24
P (%)	0.84	0.84	0.84	0.84	0.84	0.84
Mathionine (%)	0.47	0.49	0.49	0.48	0.48	0.48
Lysine (%)	1.00	1.02	1.02	1.01	1.01	1.01

[1] Contained: Calcium 31.6%; Phosphorus 2.8%; Sodium 1.8%; Cobalt 4 mg/kg ; Copper 100 mg/kg, Fe 2215 mg/kg ; Iodine 10 mg/kg ; Manganese 930 mg/kg ; Zinc 730 mg/kg ; Selenium 3 mg/kg ; Vit. A 100000 IU/kg; Vit. D 25000 IU/kg; Vit. E 400 IU/kg

Table 2: Fatty acid composition of layer diets (% of total fatty acids) for control diet (CTL) and pearl millet based diets containing 0, 2, 4, 8 or 12 % flaxseed (PM-0, PM-2, PM-4, PM-8, and PM-12, respectively)

	Dietary Treatments					
	CTL	PM-0	PM-2	PM-4	PM-8	PM-12
Fatty acid						
C16:0	9.89 ± 0.56	11.99 ± 0.91	11.46 ± 0.83	10.80 ± 0.25	9.82 ± 0.13	8.59 ± 0.33
C16:1	0.09 ± 0.01	0.26 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
C18:0	3.35 ± 0.12	3.67 ± 0.28	3.51 ± 0.04	3.25 ± 0.30	3.06 ± 0.26	2.89 ± 0.04
C18:1	30.18 ± 0.80	41.39 ± 2.31	39.46 ± 1.79	36.30 ± 1.41	36.03 ± 1.53	33.67 ± 2.40
C18:2/n-6	50.36 ± 1.34	35.99 ± 1.30	35.08 ± 1.87	33.76 ± 1.49	32.44 ± 1.56	30.13 ± 1.40
C18:3/n-3	4.14 ± 0.22	5.89 ± 0.12	7.33 ± 0.60	8.41 ± 0.36	12.01 ± 0.48	15.51 ± 1.09

Table 3: Weekly average egg weights (g) for control diet (CTL) and pearl millet based diets containing 0, 2, 4, 8 or 12% flaxseed (PM-0, PM-2, PM-4, PM-8, and PM-12, respectively)

	WEEKS					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Treatments						
CTL	62.61	64.30	65.07	64.22 [ab]	64.14	64.17
PM-0	64.79	66.00	64.99	65.76 [a]	65.09	65.65
PM-2	64.79	66.23	66.41	65.92 [a]	64.89	65.88
PM-4	63.29	64.43	65.14	64.65 [ab]	64.95	65.67
PM-8	64.18	65.16	64.26	65.22 [ab]	64.98	65.72
PM-12	62.69	66.41	66.50	62.48 [b]	64.76	65.15
SEM	2.23	2.77	2.28	1.77	1.20	1.32

a,b Values in the same column with different superscripts are significantly different

Table 4: Roche® Pigmentation Scores for control diet (CTL) and pearl millet based diets containing 0, 2, 4, 8 or 12 % flaxseed (PM-0, PM-2, PM-4, PM-8, and PM-12, respectively)

	WEEKS					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Treatments						
CTL	5.80 [a]	5.82 [a]	6.60 [a]	6.60 [a]	6.60 [a]	6.00 [a]
PM-0	1.60 [c]	2.00 [b]	2.00 [c]	1.40 [c]	1.80 [c]	1.60 [c]
PM-2	1.75 [c]	1.40 [b]	2.20 [bc]	1.60 [bc]	2.00 [bc]	1.80 [bc]
PM-4	2.00 [c]	2.00 [b]	2.00 [c]	2.20 [bc]	2.00 [bc]	2.40 [bc]
PM-8	2.20 [c]	2.00 [b]	2.80 [b]	2.80 [b]	2.60 [bc]	2.40 [bc]
PM-12	3.00 [b]	2.20 [b]	2.60 [bc]	2.20 [bc]	2.80 [b]	2.75 [b]
SEM	0.17	0.20	0.18	0.32	0.22	0.25

a,b,c Values in the same column with different superscripts are significantly different

FIGURES

Figure 1: Total n-3 fatty acid content in the eggs of hens fed a corn-soybean meal based diet (CTL) or a diet in which corn was replaced by pearl millet (PM) and supplemented with 0, 2, 4, 8 or 12 % of flaxseed (PM-0, PM-2, PM-4, PM-8, and PM-12). [a,b,c,d,e,f] Columns in the same week with different letters are significantly different.

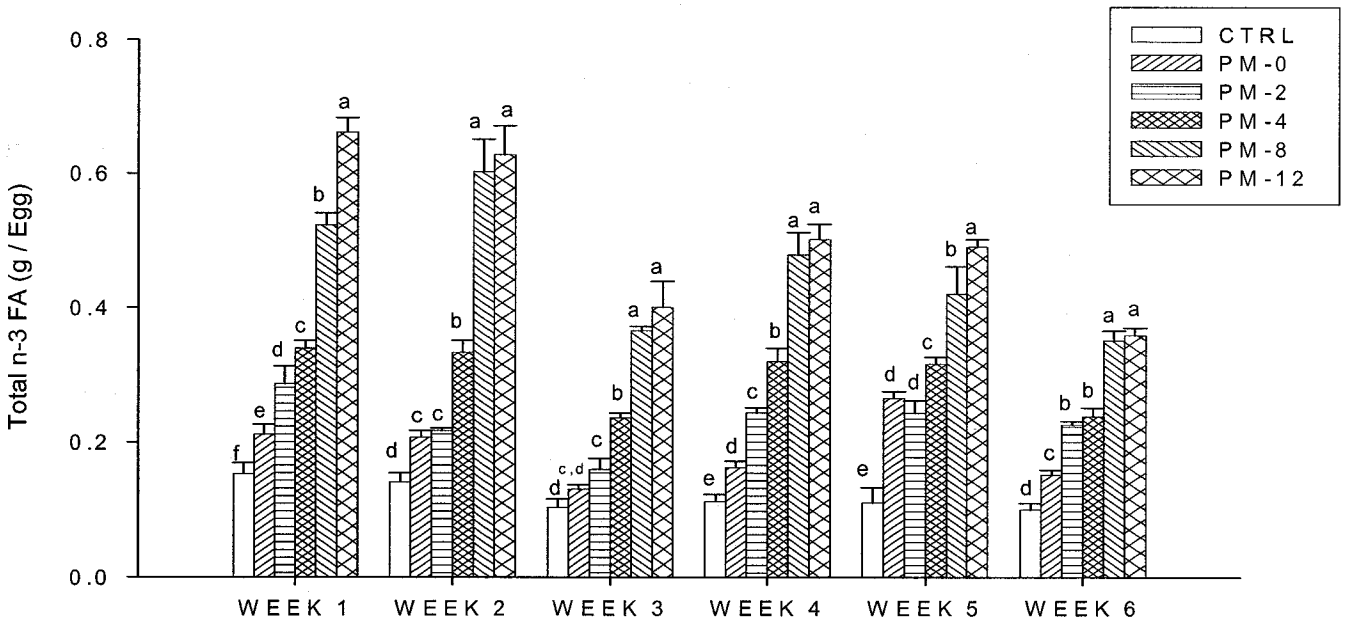
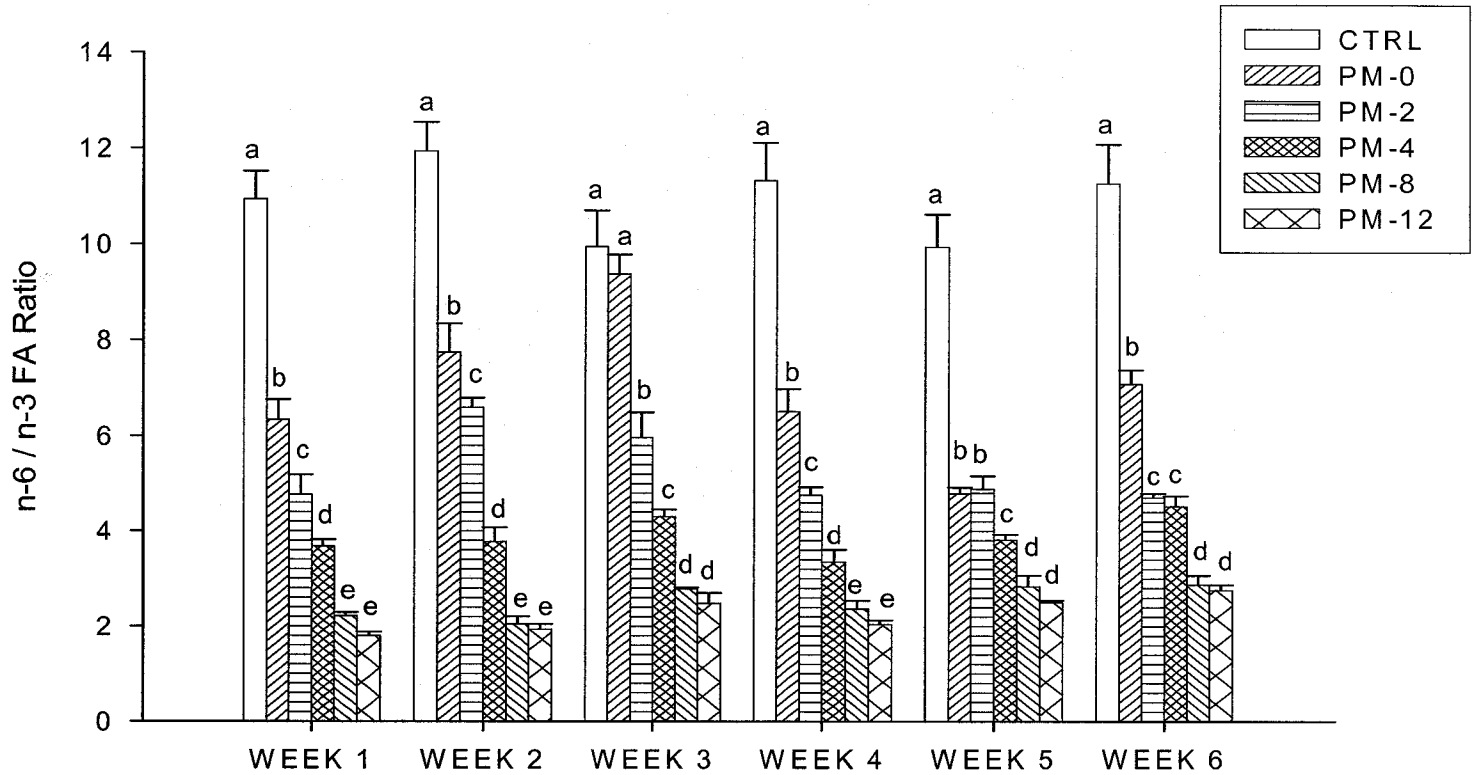


Figure 2: Ratio of n-6/n-3 fatty acids in the eggs of hens fed a corn-soybean meal based diet (CTL) or a diet in which corn was replaced by pearl millet (PM) and supplemented with 0, 2, 4, 8 or 12 % of flaxseed (PM-0, PM-2, PM-4, PM-8, and PM-12). [a,b,c,d,e] **Columns in the same week with different letters are significantly different.**



Prologue to Chapter 4

Chapter 4 consists of a manuscript, authored by Keyvan Amini and edited by Dr. Ciro Ruiz-Feria, which is going to be submitted for publication in *British Poultry Science* Journal. All the references mentioned in this chapter are listed under the "References" section at the end of this thesis. The tables included in this manuscript are illustrated at the end of this chapter.

Chapter 4 explains the second experiment of this study, performed to evaluate pearl millet in combination with low levels of flaxseed and natural pigment in laying hens' diets, on flock performance, egg trait parameters yolk pigmentation and n-3 fatty acid content of the eggs as well as n-6/n-3 fatty acid ratio in the eggs.

CHAPTER 4

Omega-3 enriched eggs produced with pearl millet based diets, flaxseed, and natural pigments[1]

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[1] To be submitted for publication to *British Poultry Science*

ABSTRACT

A twelve wk experiment was carried out to evaluate the effects of Pearl Millet (PM) in combination with different low levels of flaxseed (FS) and natural pigment (PG, Oro Glo 15®) on egg fatty acid (FA) profile, laying performance, and yolk pigmentation. The diets were based on PM and soybean meal, with three levels of ground FS (4%, 6% and 8%) and two levels of PG (0.1% and 0.2%) in a factorial arrangement of treatments with six cage replicates per treatment (three birds per cage). All the diets were formulated to be isocaloric and isonitrogenous and to meet or exceed NRC requirements. Egg number and egg mass produced were measured and recorded on a daily basis, whereas BW and feed consumption measurements were recorded every two weeks. At the end of each two wk period, three eggs were randomly collected from each cage to measure egg trait parameters, and then yolks were separated, pooled and lyophilized for fatty acid determination by Gas Chromatography (GC). At the end of the experiment, all the hens were euthanized to determine liver integrity. Egg traits, flock performance parameters, and liver integrity, were not different among treatments, except in wk 8, 10 and 12, when birds fed dietary treatment including 8% FS produced smaller ($P < 0.05$) eggs than hens fed 4% FS. The inclusion of the PG at both levels restored yolk pigmentation. Birds fed a diet containing PM as the sole grain source and 6% FS, consistently produced eggs with more than 350 mg / egg of n-3 FA, which is the lower standard to market eggs as "n-3 FA enriched".

(**Keywords**: laying hens, pearl millet, flaxseed, natural pigment, flock performance, eggs, liver haemorrhage)

INTRODUCTION

The n-3 and n-6 poly unsaturated fatty acids (PUFA) are recognized as essential in human diets. The α-linolenic acid (LNA, C18:3 n-3), representative of the n-3 family, is found in considerable quantities in some oil seeds, such as canola and flaxseed. Linoleic acid (LA, C18:2 n-6) is the most important FA of n-6 series, and is abundant in vegetable oils, such as sunflower, corn, soybeans and cottonseed oils (Dziezak, 1989). Recent studies have shown n-3 fatty acids (FA's) may prevent cardiovascular diseases, diabetes, several auto-immune disorders and some types of cancer. They also play a significant role in neonatal growth (Simopoulos, 2000).

Eggs enriched with n-3 FA can be obtained by enriching layer feed with flax and/or canola as these easily promote the incorporation of n-3 FA's in the egg yolk (Cherian and Sim 1991; Van Elswyk, 1997b). Dietary FS is a rich source of LNA that has been shown to increase the n-3 FA content of eggs (Caston and Leeson, 1990; Jiang *et al*, 1991, 1992). However, long term supplementation with FS causes reproductive alterations, attributed to phytoestrogenic compounds (Van Elswyk *et al.*, 1994; Aymond *et al.*,1994; Ahn *et al.*, 1995), and is also associated with a high incidence of liver haemorrhages in long term use (Bean and Leeson , 2003). Furthermore, a fishy or fish-related flavour has been reported in the eggs from hens fed a diet with 15% inclusion of FS (Jiang *et al.*, 1992).

Pearl millet (PM) is high in oil content in comparison with other cereals, with an average fat content of more than 5%. According to Rooney (1978) LNA comprises 4% of the total FA's in PM's fat, giving it a higher content of n-3 FA than other cereal grains. PM supplies more n-3 FA for deposition into eggs than corn, but it is not

sufficient to produce "omega-3 enriched eggs" (Collins et al. 1997). By using PM in hens' diet, production parameters and egg flavour are not affected, however it results in reduced yolk pigmentation compared with a corn based feed (Collins *et al.* 1997). It may be possible to develop a nutrition program to produce n-3 FA enriched eggs using a combination of PM, low levels of FS, and PG in order to reduce off-flavour in the eggs, preserve the health and well-being of the hens and at the same time obtain acceptable levels of yolk pigmentation.

In the previous experiment we found that hens fed a diet based on PM and 8% FS supplementation produced eggs with an n-3 FA content averaging 456 mg/egg during a six week experimental period, which is considerably higher than 350 mg/egg required for marketing eggs as "omega-3 enriched eggs". This may open the possibility of lowering the FS content of the diets to levels even lower than 8%, further reducing the detrimental effects of FS on palatability of the eggs as well as health of laying hens. However, yolk pigmentation scores of the egg yolks produced by the birds fed PM-based diet (1.60 ± 0.24) was lower than those produced by feeding corn-based dietary treatment (6.00 ± 0.01), and although the inclusion of FS increased the pigmentation (2.75 ± 0.47 for PM-12 diet), it did not reach the levels produced by feeding corn based diet. The inclusion of 0.2% Marigold petal extract in wheat + barley based diets restores yolk pigmentation from 1.58 ± 0.07 to levels as high as 8.84 ± 0.13 (Karadas *et al.* 2006). Hence, the inclusion of similar quantities of marigold petal extract to a PM based diet may produce comparable results, restoring the pigmentation to marketable levels. This study was carried out to evaluate the effects of PM with added low levels of FS on flock performance, egg trait parameters, liver integrity of laying hens, and on

egg n-3 FA content and n-6/n-3 FA ratio in longer term trial and to evaluate the use of a natural pigment for restoring yolk pigmentation in PM based diets.

MATERIALS AND METHODS

Birds, Housing and Animal Care

Fifty four wk old white leghorn hens of the Shaver White strain (ISA Poultry) were used. The birds were housed in the same commercial type laying cages as of the first experiment, and received the same lighting program. Feed and water were also provided *ad libitum*. All experimental procedures involving animals were conducted according to a protocol reviewed and approved by McGill University Institutional Animal Care and Use Committee.

Dietary Treatments

The diets were based on PM and soybean meal, with three inclusion levels of ground FS (4%, 6% and 8%) and two levels (0.1% and 0.2%) of the natural pigment (Oro Glo 15® [8]) in a 3 by 2 factorial arrangement of treatments (Table 1). Six cage replicates (3 hens per cage) were randomly assigned to each treatment, for a total of 108 hens. All diets contained a fixed level of 4.1% canola oil. The diets were formulated to be isocaloric and isonitrogenous and to meet or exceed NRC (1994) requirements. The pearl millet grain used in the experiment was developed by AERC[9]

8 Kemin Industries Inc., Des Moines, IA, USA
9 Agriculture Environmental Renewal Canada Inc.; www.aerc.ca

(Canadian Grain Pearl Millet Hybrid, CGPMH 1) and was milled by a hammer mill through a 1.5 mm sieve.

Flock Performance Parameters

The experiment lasted for 12 weeks. Feed intake and BW were recorded every two weeks, whereas egg production (number of eggs and egg mass produced) was recorded daily. The flock performance parameters determined included hen-day egg production, egg mass produced per bird per day, feed consumption per bird per day and feed conversion ratio. At the end of the experiment, all the hens were euthanized to macroscopically determine liver haemorrhage score and then 3 histological slides[10] from each liver were prepared using Hematoxylin-and-Eosin (H&E) staining method, to detect the microscopic haemorrhages. Macroscopic scores of liver haemorrhage were scored based on a 1 to 5 scale, where a score of 1 indicated no haemorrhaging and a score of 5 denoted excessive haemorrhaging (Schumann *et al.* 2000).

Egg Quality Parameters

Eggs were collected for two consecutive days with biweekly intervals, and three eggs per each cage replicate were randomly selected for measuring egg weight, yolk weight, shell weight and thickness. Albumen weight was calculated accordingly. Yolk pigmentation was determined using the Roche® color fan on a 1-15 scale.

[10] Accel Lab, Boisbriand, Quebec, Canada

Fatty Acid Analysis

Three yolks from each cage replicate were pooled at the end of every two weeks and lyophilized. The yolk FA analysis and the FA compositions of dietary treatments were performed using the method described in the previous experiment (Table 2).

Statistical Analysis

For the flock performance parameters and egg fatty acid analysis results, cage served as experimental and sampling unit and PROC-GLM procedure of SAS (2003) was used to process the data.

Statistical model used:

$Y_{ijk} = \mu + FS_i + PG_j + FS*PG_{ij} + e_{ijk}$

Where:

FS_i : i = 1,2,3

PG_j : j = 1,2

e_{ijk} : k = 1,2,3,4,5,6

Fixed effect parameters of the model:

a) μ is the overall mean

b) FS is the fixed effect of i^{th} level of flaxseed

c) PG is the fixed effect of j^{th} level of natural pigment

d) $FS*PG_{ij}$ is the interaction effect of i^{th} level of flaxseed and j^{th} level of natural pigment

Random effect parameter of the model:

σ^2_e is the random residual variation of the model

For egg trait results, egg served as sampling unit and cage was the experimental unit. Data were analysed using PROC-MIXED procedure of SAS (2003).

Statistical model used:

$Y_{ijkl} = \mu + FS_i + PG_j + FS*PG_{ij} + Cage_{ijk} + e_{ijkl}$

Where:

FS_i : i = 1,2,3

PG_j : j = 1,2

$Cage_{ijk}$: k = 1,2,3,4,5,6

e_{ijkl} : l = 1,2,3

Fixed effect parameters of the model:

c) μ is the overall mean

d) FS is the fixed effect of i^{th} level of flaxseed

e) PG is the fixed effect of j^{th} level of natural pigment

f) $FS*PG_{ij}$ is the interaction effect of i^{th} level of flaxseed and j^{th} level of natural pigment

Random effect parameters of the model:

c) $Cage_{ijk}$ is the random effect of cage

d) σ^2_e is the random residual variation of the model

Treatment means were separated using the least square means function of SAS. Significant differences among treatment means were separated using the Scheffe's multiple comparison test. Statistical differences were declared at $P < 0.05$.

RESULTS

Flock Performance and Egg Parameters

The dietary treatments did not affect BW and flock performance parameters, including hen-day egg production, egg mass produced per bird per day, feed consumption and feed conversion ratio (FCR). In the same way, different levels of FS or PG in the diet did not have significant effects on albumen and shell weights. Only in wk 4, inclusion of 8% FS increased shell thickness compared to diets containing 4% FS and also higher dietary content of PG increased shell thickness. In week 8, 10 and 12 of the experiment, diet treatments containing 8% FS, significantly lowered egg weight compared to diets with 4% inclusion of FS (Table 3) and in week 10 and 12, inclusion of 8% FS in diets had the same effect on yolk weight (Table 4). Liver haemorrhage scores were not different among treatments at the end of the trial. Throughout the experiment, addition of 0.2% PG in the feed caused the hens to produce eggs with consistently higher pigmentation scores compared with 0.1% PG diets (Table 5). In general, we did not find interaction effects of FS and PG on any of the flock performance parameters, egg traits, n-3 FA content and n-6/n-3 FA ratio in eggs.

Fatty Acids

The n-3 FA content of the eggs increased with increasing levels of FS, except at wk 6 and 12, when the n-3 FA of the eggs produced by hens fed the diet containing 4% and 6% FS were not different (Table 6). Inclusion of different levels of FS also affected n-6/n-3 FA ratio in the yolks. The n-6/n-3 FA ratio was consistently higher in the eggs produced by hens fed 4% FS than in those produced by hens fed 8% FS (Table

7). Increasing FS in the feed from 6% to 8% improved the n-6/n-3 FA ratio, except in wk 4 and 8, when n-6/n-3 FA ratio in the eggs produced by feeding 6% FS and 8% FS were not significantly different. Different inclusion levels of PG did not affect the n-3 FA content or n-6/n-3 ratio in the eggs.

DISCUSSION

The effects of PM (Kumar *et al.*, 1991; Collins *et al.*, 1997; Abd-Elrazig and Elzubeir, 1998) and FS (Cherian and Sim, 1991; Jiang *et al.*, 1991; Novak and Scheideler, 2001) on flock performance and on egg FA composition have been previously reported. However, there are no reports on the effects of concurrent usage of PM, PG and low levels of FS on flock performance, yolk pigmentation and n-3 FA content of the eggs.

This experiment lasted for twelve weeks (the experimental period which is also used by other researcher (Abd-Elrazig and Elzubeir, 1998; da Silva Filardi, *et al.* 2005) to investigate the possibility of reducing the inclusion level of FS in PM-diets to levels even lower that 8% of dietary treatment, and to evaluate the liver integrity of hens in longer term use (12 weeks) and also evaluate a natural pigment (marigold petal extract) for restoration of yolk pigmentation.

Different inclusion levels of FS and PG in the diets did not affect eggshell quality parameters, except in week 4, when hens fed diets containing 8% FS produced eggs with heavier shells compared to those fed diets containing 4%FS; in the same week, hens fed diets containing 0.2% PG produced heavier eggshells compared to those fed 0.1% PG. However, this increase was an isolated and sporadic event. These results

agree with the findings of our first experiment and with the results reported by Caston *et al.* (1994) in which increasing levels of 0, 10% and 20% FS did not have any significant impact on shell weight. Mazalli *et al.* (2004a) obtained comparable results after feeding 3% flax oil or 9% flaxseed to the birds. In this study, in wk 8, 10 and 12, birds fed 8% FS produced smaller eggs compared to hens fed 4% FS, indicating that FS may manifest a negative effect on egg weight after several weeks of use. We also found that in wk 10 and 12, diet treatments containing 8% FS had a lower yolk weight compared to diets with 4% inclusion of FS, which may explain the reduced egg weight (Scheideler and Froning, 1996; Whitehead *et al.*, 1993). Van Elswyk (1997) suggested that the possible cause of the reduction in egg weight as a result of feeding FS to hens could be the lower serum estradiol, which may limit lipid availability for yolk formation. Whitehead *et al.* (1993) postulated that the phyto-estrogenic compounds contained in FS, and/or the changes in circulating estradiol as a consequence of either the latter substances or an effect of n-3 FA per se could be the reason for reduced yolk and consequently egg weight. Different inclusion levels of FS did not have any significant impact on albumen weight which concurs with the findings of the previous experiment and the results reported by Mazalli *et al.* (2004 a). We did not find any effect of FS level on hen-egg production per day or on daily egg mass produced per bird. Aymond and Van Elswyk (1995) reported decreased production in hens fed 15% FS compared to hens fed a control diet or diets containing 5% FS over a 5 wk period in 22-wk-old hens. This does not contradict with our results in which low levels of 4 to 8% FS did not have any negative effect on egg production.

There were no differences in BW due to dietary treatment. Novak and Scheideler (2001) reported lower BW in young 21-wk-old hens fed diets with 10% FS. This effect has been attributed to anti-nutritional factors in FS that may reduce the digestion and absorption of feedstuffs providing energy (Gonzalez-Esquerra and Leeson, 2000 a; Ortiz *et al.*, 2001; Rodriguez *et al.*, 2001). Mature birds seem to be less susceptible than younger birds to the anti-nutritional factors found in FS (Klosterman, 1974; Jiang *et al.*, 1991; Scheideler and Froning, 1996). We used 54 wk old birds in this study. Increasing levels of FS in this experiment did not have any significant impact on feed efficiency (FCR), which agrees with the reports of Baucells *et al.* (2000) and Mazalli *et al.* (2004a), and is in agreement with the results of our previous experiment, indicating that the combination of PM and low levels of FS in layers diets has no detrimental effect on flock performance. We did not observe any significant liver haemorrhage in 12 wk trial period, which confirms that dietary levels of FS lower than 10% are safe to be used as an ingredient in layers diets.

Pearl millet, compared with corn, contains insufficient quantities of xanthophylls, pigment that imparts a golden yellow color in yolks. This causes lower pigmentation scores in eggs produced by hens fed PM, compared to those produced by birds fed corn. In this experiment, inclusion of 0.1% or 0.2% of the PG to PM-based diets restored yolk pigmentation. Addition of 0.1% PG in the feed restored the yolk color score to an average of 7.43 (Roche® pigmentation score) during the 12 wk experimental period, while inclusion of 0.2% PG increased the average score to 9.89. Different egg markets demand different egg pigmentation scores, but both inclusion levels of PG, produced eggs with acceptable coloration, either of which could meet the

requirements of the target market. According to Leeson and Summers (2005) a pigmentation score of 7-8 will usually be accepted for grade A eggs. In the previous experiment, we found that higher levels of FS in combination with PM slightly improved yolk pigmentation, although it remained lower than the pigmentation obtained through feeding a corn based diet. The results of this study indicate that there is no interaction effect of FS and PG on yolk pigmentation.

It is well established that dietary PUFA's can cause major changes on egg FA profile (Summers *et al.* 1966; Noble *et al.* 1990; Mazalli *et al.* 2004b). In this study, the n-3 FA content of eggs was expectedly increased with increasing levels of FS, except at wk 6 and 12, when the yolk n-3 FA content of eggs produced by diets containing 4% and 6% FS were not significantly different. As expected, different inclusion levels of the PG did not affect the deposition of n-3 FA in the eggs. Supplementation of 4% FS to PM-based diet was not sufficient to consistently supply enough n-3 FA to obtain the required 350 mg of n-3 FA per egg, necessary for the production of "omega-3 enriched egg" (Scheideler and Lewis, 1997). However, hens fed 6% FS, consistently deposited enough n-3 FA in their egg yolks to be considered as "omega-3 enriched eggs". Higher levels of FS in diets may produce off-flavours in the eggs (15% FS; Jiang *et al.* 1992) and are associated with high incidence of liver haemorrhages in laying hens in long term use (Bean and Leeson, 2003). Therefore a dietary treatment containing PM as the sole grain source and FS with inclusion levels as low as 6%, can eliminate the incidence of liver haemorrhages and production of off-flavours in the eggs and meanwhile provide hens with enough n-3 FA for production of "omega eggs". In the previous experiment, we observed a general decline in total n-3

FA content of the eggs after wk 3, which was attributed to the lower feed consumption of the birds as a result of high ambient temperature recorded in corresponding days. In this experiment, the recorded ambient temperature was homogeneous; we observed a stable feed consumption per bird per day, and a rather constant n-3 FA content of the eggs after wk2. The hens' efficiency in depositing n-3 FA in the yolk is dependent on age. Scheideler *et al.* (1998) reported that hens younger than 35 wk deposit 25% to 50% less n-3 FA in their eggs than older birds. Caston and Leeson (1990) reported that hens fed 0%, 10%, 20% and 30% of dietary FS deposited 21, 247, 478 and 618 mg n-3 FA in their eggs, respectively. The inferior performance in terms of egg n-3 FA deposition can primarily be ascribed to the lower age (32 wk) of the birds. We used 54 wk old birds, which may explain the high amount of n-3 FA in the yolks, using PM based diets with rather low levels of FS.

In this study, we also observed a dose-related improvement in n-6/n-3 FA ratio in response to increasing levels of dietary FS. The n-6/n-3 FA ratio was consistently lowered in the eggs produced by hens fed 8% FS compared to those produced by hens fed 4% FS. Increasing FS in the feed from 6% to 8% also improved the n-6/n-3 FA ratio, except in wk 4 and 8, when 8% FS in the feed did not reduce the n-6/n-3 FA ratio in the eggs compared to 6% FS diets. As expected, dietary level of PG in diets did not have a significant effect on n-6/n-3 FA ratio in the eggs. Clinical studies indicate that the ingested ratio of n-6/n-3 FA is important for maintaining cardiovascular health (Lands, 1992; Okuyama, 2001; Simopoulos, 2003). Both n-3 and n-6 FA are essential for humans. The n-3 and n-6 FA compete for the same metabolic enzymes, thus the n-6/n-3 ratio will significantly influence the eicosanoid hormones (e.g. prostaglandins,

leukotrienes, thromboxanes, etc.), and will alter the body's metabolic function. The ideal dietary ratio of n-6: n-3 has been reported to be lower than 5:1 (Simopoulos, 2003). Therefore, the eggs produced as a result of feeding PM and low levels of FS to the hens, can constitute an optimum food for human consumption.

In general, these results confirm that for the purpose of n-3 FA enriched egg production, the use of PM as the sole grain source in a diet containing 4.1% of canola oil reduces the requirements for FS to levels as low as 6% in diet of laying hens. In a corn based diet, it is necessary to use inclusions of 10-15% of FS to obtain an n-3 FA enriched egg (Scheideler and Froning, 1996). As previously discussed, the use of high levels of FS inclusion in layers feed is associated with liver haemorrhage and producing off-flavours and therefore reduces market acceptability of the eggs. Therefore reduced inclusion of FS in a PM based diet can eliminate these problems without compromising hens' productivity. The reduced yolk pigmentation as a result of feeding PM can be restored by supplementation of marigold petal extract to hens' diets.

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TABLES

Table 1: Composition (%) and calculated analyses of dietary treatments (FS4=4% flaxseed; FS6=6% flaxseed; FS8= 8% flaxseed; PG1= 0.1% natural pigment; PG2=0.2% natural pigment)

Ingredient	FS4/PG1	FS4/PG2	FS6/PG1	FS6/PG2	FS8/PG1	FS8/PG2
Pearl Millet	58.3	58.3	56.1	56.1	53.9	53.9
Soybean-Meal, 48% CP	20.2	20.2	19.8	19.8	19.4	19.4
Flaxseed	4.0	4.0	6.0	6.0	8.0	8.0
Canola oil	4.1	4.1	4.1	4.1	4.1	4.1
Limestone	6.5	6.5	6.5	6.5	6.5	6.5
Dicalcium Phosphate	1.7	1.7	1.7	1.7	1.7	1.7
DL-Methionine	0.2	0.2	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2	0.2	0.2
Premix [1]	4.4	4.4	4.4	4.4	4.4	4.4
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Oro Glo15® (Natural Pigment)	0.1	0.2	0.1	0.2	0.1	0.2
Inert Filler (Sand)	0.1	0	0.7	0.6	1.3	1.2
ME (MJ/Kg)	2781	2781	2781	2781	2780	2780
CP (%)	16.11	16.11	16.11	16.11	16.12	16.12
Ca (%)	4.23	4.23	4.23	4.23	4.23	4.23
P (%)	0.84	0.84	0.84	0.84	0.84	0.84
Mathionine(%)	0.49	0.49	0.49	0.49	0.49	0.49
Lysine (%)	1.02	1.02	1.02	1.02	1.01	1.01

[1] Contained: Calcium 31.6%; Phosphorus 2.8%; Sodium 1.8%; Cobalt 4 mg/kg ; Copper 100 mg/kg, Fe 2215 mg/kg ; Iodine 10 mg/kg ; Manganese 930 mg/kg ; Zinc 730 mg/kg ; Selenium 3 mg/kg ; Vit. A 100000 IU/kg; Vit. D 25000 IU/kg; Vit. E 400 IU/kg

Table 2: Fatty acid composition of layer diets (% of total fatty acids) (FS4=4% flaxseed; FS6=6% flaxseed; FS8= 8% flaxseed; PG1= 0.1% natural pigment; PG2=0.2% natural pigment)

	TREATMENTS					
	FS4/PG1	FS4/PG2	FS6/PG1	FS6/PG2	FS8/PG1	FS8/PG2
Fatty acid						
C16:0	10.60 ± 0.92	10.71 ± 0.53	8.56 ± 0.74	9.46 ± 0.34	9.50 ± 0.54	9.72 ± 0.57
C16:1	0.20 ± 0.02	0.23 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.15 ± 0.01
C18:0	3.90 ± 0.35	3.10 ± 0.15	3.09 ± 0.11	2.97 ± 0.24	3.08 ± 0.13	3.06 ± 0.02
C18:1	39.67 ± 0.89	39.71 ± 0.24	37.51 ± 0.89	37.40 ± 1.15	35.87 ± 0.59	36.30 ± 0.78
C18:2/n-6	32.57 ± 0.70	33.11 ± 0.38	31.23 ± 0.84	32.16 ± 0.66	29.31 ± 0.23	29.24 ± 0.45
C18:3/n-3	7.85 ± 0.48	8.02 ± 0.25	11.30 ± 0.50	10.71 ± 0.31	13.08 ± 0.16	12.53 ± 0.07

Table 3: Biweekly effects of different levels of flaxseed (FS) and pigment (PG) on egg weights (g) [(FS4=4% flaxseed; FS6=6% flaxseed; FS8= 8% flaxseed; PG1= 0.1% natural pigment; PG2=0.2% natural pigment)]

	WEEKS					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Flaxseed (FS)						
FS4	63.57	64.40	64.39	65.04 [a]	64.83 [a]	65.44 [a]
FS6	62.09	64.04	63.17	63.30 [ab]	63.36 [ab]	63.49 [ab]
FS8	62.05	62.87	62.23	60.81 [b]	62.43 [b]	62.38 [b]
SEM	0.60	0.68	0.74	0.94	0.61	0.70
P- value	0.149	0.053	0.138	0.013	0.031	0.014
Pigment (PG)						
PG1	62.23	64.06	62.69	63.24	63.14	63.29
PG2	62.90	64.14	63.83	62.87	63.93	64.26
SEM	0.49	0.55	0.61	0.77	0.50	0.57
P- value	0.3461	0.9211	0.1943	0.7357	0.2701	0.2404

a,b Values in the same column with different superscripts are significantly different

Table 4: Biweekly effects of different levels of flaxseed (FS) and pigment (PG) on yolk weight (g) [(FS4=4% flaxseed; FS6=6% flaxseed; FS8= 8% flaxseed; PG1= 0.1% natural pigment; PG2=0.2% natural pigment)]

	WEEKS					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Flaxseed (FS)						
FS4	17.46	17.77	17.36	17.39	17.69 [a]	17.59 [a]
FS6	17.56	17.40	17.00	16.96	17.22 [ab]	17.09 [ab]
FS8	17.36	17.37	16.98	16.65	16.16 [b]	16.63 [b]
SEM	0.30	0.27	0.25	0.23	0.26	0.25
P- value	0.641	0.5083	0.5011	0.0951	0.017	0.0349
Pigment (PG)						
PG1	17.32	17.30	17.37	16.82	17.40	17.31
PG2	17.66	17.74	16.85	17.18	17.33	16.92
SEM	0.24	0.21	0.20	0.19	0.21	0.20
P- value	0.1911	0.1656	0.0844	0.2022	0.7043	0.1826

a,b Values in the same column with different superscripts are significantly different

Table 5: Biweekly effects of different levels of flaxseed (FS) and pigment (PG) on Roche® Pigmentation Scores [(FS4=4% flaxseed; FS6=6% flaxseed; FS8= 8% flaxseed; PG1= 0.1% natural pigment; PG2=0.2% natural pigment)]

	WEEKS					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Flaxseed (FS)						
FS4	8.36	8.08	8.08	8.19	8.33	8.67
FS6	8.50	8.19	8.75	8.31	8.58	8.72
FS8	8.56	8.44	8.86	8.42	8.78	8.78
SEM	0.10	0.13	0.109	0.14	0.17	0.17
P- value	0.3791	0.1591	0.0911	0.5412	0.1962	0.9001
Pigment (PG)						
PG1	7.40 [b]	7.00 [b]	7.83 [b]	7.87 [b]	7.20 [b]	7.25 [b]
PG2	9.53 [a]	9.48 [a]	9.96 [a]	10.24 [a]	9.92 [a]	10.18 [a]
SEM	0.08	0.10	0.08	0.11	0.13	0.13
P- value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

a,b Values in the same column with different superscripts are significantly different

Table 6: Biweekly effects of different levels of flaxseed (FS) and pigment (PG) on total n-3 fatty acid content of the eggs (g/Egg)[(FS4=4% flaxseed; FS6=6% flaxseed; FS8= 8% flaxseed; PG1= 0.1% natural pigment; PG2=0.2% natural pigment)]

	WEEKS					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Flaxseed (FS)						
FS4	0.301 ^{c}	0.369 ^{c}	0.382 ^{b}	0.367 ^{c}	0.336 ^{c}	0.394 ^{b}
FS6	0.357 ^{b}	0.411 ^{b}	0.402 ^{b,}	0.420 ^{b}	0.400 ^{b}	0.401 ^{b}
FS8	0.431 ^{a}	0.504 ^{a}	0.498 ^{a}	0.505 ^{a}	0.518^{a}	0.503 ^{a}
SEM	0.010	0.010	0.013	0.010	0.011	0.010
P- value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0055
Pigment (PG)						
PG1	0.379	0.423	0.429	0.428	0.438	0.415
PG2	0.381	0.418	0.427	0.411	0.437	0.410
SEM	0.015	0.009	0.011	0.008	0.009	0.007
P- value	0.7768	0.3943	0.5353	0.0649	0.5617	0.3663

a,b,c Values in the same column under the same effect with different superscripts are significantly different

Table 7: Biweekly effects of different levels of flaxseed (FS) and pigment (PG) on n-6/n-3 fatty acid ratio in the eggs [(FS4=4% flaxseed; FS6=6% flaxseed; FS8= 8% flaxseed; PG1= 0.1% natural pigment; PG2=0.2% natural pigment)]

	WEEKS					
	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Flaxseed (FS)						
FS4	3.69 [a]	3.09 [a]	3.32 [a]	3.20 [a]	3.26 [a]	3.08 [a]
FS6	3.36 [b]	2.82 [b]	2.78 [b]	2.63 [b]	2.81 [b]	2.57 [b]
FS8	3.01 [c]	2.64 [b]	2.54 [c]	2.41 [b]	2.45 [c]	2.38 [c]
SEM	0.06	0.06	0.06	0.09	0.08	0.04
P- value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pigment (PG)						
PG1	3.01	2.87	2.90	2.83	2.89	2.73
PG2	3.02	2.82	2.86	2.66	2.79	2.69
SEM	0.05	0.05	0.05	0.07	0.06	0.04
P- value	0.4044	0.4928	0.4948	0.1189	0.3262	0.0537

a,b,c Values in the same column under the same effect with different superscripts are significantly different

CHAPTER 5: GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The results of this study indicate that pearl millet is an acceptable grain to be used in laying hens' diets for production of "Omega Eggs". In the first experiment, corn-based diet and pearl millet- based diets with increasing levels of flaxseed, gave essentially equivalent performances in terms of flock and egg trait results, except that feeding pearl millet drastically reduced yolk pigmentation to levels generally unacceptable to consumers of table eggs. However, pearl millet showed no obvious anti-nutritional effects influencing the productivity of the hens. Another potential disadvantage of PM could be its lower ME content. Different varieties of PM have different ME contents. The analysis of PM used in this study indicated the ME content of 3000 kcal/kg. We used canola oil in the dietary treatments, in order to meet the energy requirements of the hens. The advantage of canola oil is that it has lower n-6/n-3 FA ratio compared to other vegetable oils (NRC, 1994) which makes it a suitable feed ingredient to be used for production of omega eggs.

Moreover, because of its rather high oil and n-3 fatty acid content compared to other common cereals, peal millet can serve as a suitable alternative for production of "omega-3 enriched eggs". The results of two experiments indicate that by total replacement of corn by pearl millet in laying hens' diets, inclusion of 6% flaxseed is enough to deposit sufficient quantities of n-3 fatty acids into eggs to produce omega-3 enriched eggs, without compromising flock performance parameters and egg trait factors, except a significant reduction in yolk pigmentation score in eggs produced. However, inclusion of 0.1% or 0.2%

of a natural pigment (Oro Glo 15®) successfully recovers the egg pigmentation score to acceptable levels, satisfying the yolk coloration requirements for marketing the eggs. Both inclusion levels of Oro Glo 15®, produced eggs with acceptable coloration either of which can be used to meet the requirements of the target market.

Both n-3 and n-6 fatty acids are essential, i.e. humans must consume them in the diet, however, they compete for the same metabolic enzymes and the n-6/n-3 ratio in the food alters the body's metabolic function. This necessitates that n-6 and n-3 FA be consumed in a balanced proportion with the recommended ratio of n-6 to n-3 being lower than 5 to 1. Considering the n-6/n-3 fatty acid ratios of the eggs in this study, the eggs produced as a result of feeding PM and low levels of FS to the hens can constitute an optimum food for human consumption.

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APPENDIX: ANIMAL CARE PROTOCOL