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Full Length Research Paper

Essential fatty acid content of eggs and performance of Layer Hens fed with different levels of full-fat flaxseed

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An examination of the earlier studies in the USA and Canada demonstrate that flax seeds are good source of omega 3 fatty acids. This experiment studies the effect of providing laying hens, with various levels of roasted and unroasted locally produced flax seeds. A key components of this experiment involved attentively observing the laying hens performance, and determining the fatty acids content of the eggs produced. Five levels of flax seeds 0, 5, 10, 15 and 20 dry weight %, roasted or unroasted, were fed to 200 pullets in 5 replicates (4 birds/cage). The results indicated that feeding 5 or 10% roasted flax seed supported good egg production. Birds fed higher levels of unroasted flax seed had the lowest feed consumption. Livability, egg weight, yolk color and specific gravity values were not significantly affected by feeding flax seeds. Feeding 15% unroasted flax seeds maintained higher omega-3 levels: that is docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and alpha-linolenic acid ($C_{18:3n3}$) levels in egg, whereas feeding 5 or 15 weight % unroasted flax seeds resulted in the highest level of linoleic acid ($C_{18:2n6}$) in the egg. Roasting the seeds did not improve the omega-3 content of the egg. Feeding flax, regardless of heat treatment, marginally increased the amount of cholesterol. The saturate palmitic acid ($C_{16:0}$) was lower at 15% flax inclusion. We conclude that 10% flax seed added to feed supports good egg production. However, 15% inclusion of unroasted flax may relatively lower the egg production rate but would support an excellent profile of omega 3 fatty acids in the egg.

Key words: Docosahexaenoic acid, eicosapentaenoic acid, docosapentaenoic acid, -linolenic acid, production, flax seeds.

INTRODUCTION

Fish products are high in n-3 fatty acids such as eicosapentaenoic acid (EPA), 20:5, n- 3 and docosahexaenoic acid (DHA), 22:6, n-3 but fish may not be readily available everywhere. In addition, a fishy taint in meat and egg products might occur, which is unacceptable. Therefore, other sources for n-3 fatty acids are needed (Novak and Scheideler, 2001).

Brown-seeded flax (*Linum usitatissimum* L.) is one of the most concentrated sources of omega-3 unsaturated fatty acid available in natural feedstuffs for poultry (Caston and Leeson, 1990; Jiang et al., 1991). Common varieties of flaxseeds have very high concentration of polyunsaturated fatty acids, especially -linolenic acid (Genser, 1994).

Despite the beneficial effect of using flax seed in layer diets, there has been some concern about toxicity associated with flax seed. Aymond and Van Elswyk (1995) reported a decrease in egg production in hens consuming 15% flax seed. Scheideler et al. (1995) showed that egg production was depressed with the addition of 10% flax seed to the diet, which could be the result of anti-nutritional factors contained with full-fat flax seed. Components such as cyanogenic glycosides (linustatin, neolinustatin and linamarin; Oomah et al., 1992) generate prussic acid and thiocyanate that can be detrimental to the animal. Cyanogenic compounds can be removed from flax seeds by boiling the seeds in water, wet autoclaving or by acid treatments, followed by autoclaving (Mazza and Oomah, 1995). The current experiment studied the effect of different levels of roasted or unroasted flax seeds on the performance and on the egg fatty acids components of hens.

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Table 1. The proximate analysis of the flax seed.

Description	Unit	Results
Moisture	%	5.68
Crude Protein	%	25.91
Ether Extract	%	34.95
Crude Fiber	%	4.00
Ash	%	2.52
Salt as NaCl	%	0.12

*All the results are expressed as is on dry matter.

Table 2. The fatty acids composition of flax seeds[1] .

Fatty acid profile of flax seed used	Unit	Result[2]
palmitic acid (saturate)	%	10.77
Palmitoleic acid (monounsaturate)	%	0.14
stearic acid (saturate)	%	9.53
oleic acid (monounsaturate n-9)	%	23.71
linoleic acid (omega-6)	%	15.66
alpha-linolenic acid (omega-3)	%	38.43
docosapenaenoic acid (n-3)	%	0.05
arachidonic acid (omega-6)	%	0.065
eicosapentaenoic acid (n-3)	%	0.52
eicosadienoic acid (n-6)	%	0.06
behenic acid (saturate)	%	0.21
eicosatrienoic acid	%	0.06
lignoceric acid (saturate)	%	0.24

[1]All samples were analyzed based on AOAC 996.06 method.
[2]The results are expressed as relative % of fatty acids in fat.

MATERIALS AND METHODS

Samples of ground flax seeds were subjected to chemical analysis (AACC, 1994) (Table 1). Cholesterol and fatty acids in flax seeds and yolks were determined by Gas Chromotographic method using direct saponification (AOAC, 2000) (Table 2). Lipids were saponified at high temperature with ethanolic KOH. The unsaponifiable fraction, containing cholesterol and other sterols, was extracted with toluene. Sterols were derivatized to trimethylsilyl (TMS) ethers and then quantified by gas chromatography.

Flax seeds were heat treated using large trays exposed to direct heat from a gas stove during which they were turned several times until there was a change in color to dark brown. The temperature of the seeds was supervised by checking the roasting temperature at various intervals. The result being the highest roasting temperature reached at 120°C.

During the early and growing periods, standard management practices were maintained for 300 white leghorn chicks. Starting at week 19, the birds were fed a commercial laying diet containing 17% protein. Since flax seeds are a new and unfamiliar ingredient to chicks, and in order to avoid feed rejection the dietary treatments were introduced gradually in the following pattern: During the first 2 days the birds were fed 10% treatment diets and 90% commercial layer diets. During the next 2 days, the mixture given to the birds was 20% dietary treatments and 80% commercial diets. This pattern of feeding continued in the same manner until the 22nd week of age when the birds started to be fed 100% of the experimental diets. The feeding trial continued for 12 – 28 day periods. The composition of the diets is shown in Table 3.

Each diet was supplied to five cages containing 4 birds each. There were eight 4 week dietary treatment periods and an identical control group that was not fed flax. Performance criteria such as hen-day egg production and egg weight were made, a rotated biweekly. At the end of each 2 week period, three days of egg collection were used for shell quality determination, Haugh unit (albumin height) and yolk color. Specific gravity method was used to measure the shell quality of the eggs (North, 1984). Feed was given ad-libitum daily. At the end of the experiment, 2 eggs from each replicate were collected randomly for cholesterol and fatty acid analysis.

The data was analyzed using ANOVA and SAS General Linear Model procedure (SAS, 1989). Means of the treatments were compared by Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Interaction between the level of the flax seeds in the diet and roasting treatment was significant ($P < 0.05$) in most of the examined parameters.

Birds fed 5 or 10 weight % roasted flax seed had a comparable production rate to the controls which had no flax, while those fed unroasted flax had a lower production rate than controls ($P < 0.05$) (Table 4).

Table 3. The composition of the dietary treatments.

Feed ingredients	Flax level				
	control	5%	10%	15%	20%
Yellow corn	60.1	55.5	53.0	48.5	45.0
Wheat bran	0	1.0	1.0	3.0	3.5
SBM, 48%	25.2	22.9	20.8	18.5	16.5
Fish meal, 67%	3	3.0	3.0	3.0	3.0
Choline Cl, 60%	0.4	0.27	0.25	0.1	0.09
Salt	0.4	0.4	0.4	0.4	0.4
Dl-Methionine	0.2	0.2	0.22	0.2	0.22
L-Lysine	0.1	0.08	0.1	0.1	0.2
Di-Ca P., 18%	0.6	0.6	0.6	0.6	0.6
Limestone	8.31	8.39	8.4	8.35	8.45
Alfalfa	0	1.0	1.0	1.0	1.0
Liquid fat	1.49	1.51	1.03	1.05	0.84
MINVIT premix[1]	0.2	0.2	0.2	0.2	0.2
Flax seeds	0	5.0	10.0	15.0	20.0
Total	100	100	100	100	100

[1]The multi vitamin-minerals premix provides the following, per kilogram of mix: 7000000 IU, Alfalfa vit A; 1500000 ICU, vit D3; 30000 IU, vit E; 50000 mg, vit C; 2300 mg, vit K; 1400 mg, vit B1; 5520 mg, vit B2; 2300 mg, vit B6; 12 mg, vit B12; 27600, mg Niacin; 920 mg, Folic acid; 6900 mg, PA; 92 mg, Biotin; 50000 mg, Antioxidant (BHT); 220 mg, Cobalt; 4400 mg, copper; 800 mg, Iodine; 26400 mg, Iron; 44000 mg, Manganese; 180 mg, Selenium.

Table 4. The effect of feeding different levels of roasted or un-roasted flax seed on production traits of single comb white Leghorn hens.

Source of variation	Feed consumption g/b/d	Flax intake g/b/d	Feed conversion Kg/kg	Hen-day egg production, %	Egg weight g	Egg mass g/HD
Interaction	**		*	*	NS	NS
0 T	121.3 ± 5.3	0.00 ± 0.00	2.019 ± 0.26	92.71 ± 8.51	63.91 ± 9.96	60.78 ± 6.07
5 T	121.7 ± 3.3	6.08 ± 0.17	2.041 ± 0.30	93.82 ± 9.40	63.20 ± 10.14	60.82 ± 8.76
10 T	119.6 ± 5.4	11.94 ± 0.57	2.052 ± 0.72	93.35 ± 12.61	64.95 ± 3.68	60.61 ± 9.68
15 T	118.1 ± 9.1	17.59 ± 1.75	2.114 ± 0.39	89.88 ± 13.15	63.66 ± 3.17	57.26 ± 8.98
20 T	120.7 ± 8.9	24.9 ± 1.99	2.106 ± 0.31	91.60 ± 9.90	63.55 ± 3.77	58.21± 7.11
0 U	122.3 ± 4.2	0.00 ± 0.00	2.036 ± 0.21	94.31 ± 7.66	64.67 ± 3.43	60.67 ± 6.07
5 U	126.6 ± 14.6	6.34 ± 0.72	2.248 ± 0.45	89.24 ± 13.26	64.61 ± 3.67	57.66 ± 8.80
10 U	123.5 ± 13.1	12.36 ± 1.38	2.182 ± 0.39	90.51 ± 10.40	63.60 ± 4.15	57.71 ± 8.07
15 U	117.4 ± 5.6	17.58 ± 1.07	2.093 ± 0.33	90.07 ± 10.56	63.62 ± 3.44	57.27 ± 7.89
20 U	115.4 ± 8.9	22.84 ± 2.50	2.076 ± 0.39	88.68 ± 13.31	64.43 ± 4.02	56.94 ± 8.46
P	0.0001	0.0001	0.0118	0.0405	0.1301	0.1574

[1]Means within columns carrying different superscripts are significantly different, $P < 0.05$. NS = Not significant, $P > 0.05$. ** Significant at 1% level of probability, LVL = 0, 5, 10, 15 and 20% of Flax seeds, TRT = T, roasted / U, Un-Roasted flax seeds.

Scheideler et al. (1995) showed that egg production was depressed with the addition of 10% flax seed to the diet, which could be attributed to anti-nutritional factors contained in full- fat seed. Whether roasting the seeds in this experiment was efficient in controlling the anti-nutritional factors in the seeds is open for speculation. Novak and Scheideler (2001) reported that addition of flaxseed to the diet of laying hens did not have any adverse effects on egg production parameters, but flaxseed supplementation can significantly alter weight of yolk solids and yolk and albumen percentages. These factors were not measured in this study.

With the exception of the 20% flax addition, feed consumption of the birds fed different levels of roasted or unroasted flax seeds decreased significantly ($p < 0.01$) with increasing amounts in the diet (Table 4). However,

Table 5. The effect of feeding different levels of roasted or unroasted flax seed on some egg characteristics and livability of single comb white leghorn hens[1].

Source of variation	Livability, %	Haugh unit	Yolk color	Specific gravity
Levels	**NS**	**NS**	**NS**	**NS**
0	100.0[a]	92.79a	3.066a	1.088a
5	100.0[a]	93.39a	2.980a	1.089a
10	99.9[a]	92.49a	3.163a	1.088a
15	100.0[a]	92.15a	3.066a	1.088a
20	99.9[a]	92.34a	3.033a	1.089a
P =	0.2887	0.4254	0.3697	0.9881
Treatments	**NS**	**	**NS**	**NS**
T	99.96[a]	92.06a	3.014a	1.089a
U	99.95[a]	93.20b	3.110a	1.088a
P =	0.8696	0.0098	0.0956	0.9344
Interaction	**NS**	**NS**	**NS**	**NS**
0 T	100.0 ± 0.0	92.53 ± 4.76	3.042 ± 0.92	1.088 ± 0.006
5 T	100.0 ± 0.0	92.12 ± 3.99	2.850 ± 0.96	1.088 ± 0.007
10 T	100.0 ± 0.0	92.39 ± 3.94	3.128 ± 0.77	1.088 ± 0.006
15 T	100.0 ± 0.0	91.66 ± 4.24	3.000 ± 0.88	1.089 ± 0.007
20 T	99.8 ± 1.8	91.62 ± 4.11	3.048 ± 1.00	1.089 ± 0.006
0 U	100.0 ± 0.0	93.04 ± 4.42	3.090 ± 0.92	1.088 ± 0.006
5 U	100.0 ± 0.0	94.66 ± 16.90	3.111 ± 0.86	1.089 ± 0.006
10 U	99.8 ± 1.2	92.59 ± 4.17	3.198 ± 0.81	1.088 ± 0.007
15 U	100.0 ± 0.0	92.64 ± 5.50	3.132 ± 0.83	1.088 ± 0.006
20 U	100.0 ± 0.0	93.07 ± 4.49	3.018 ± 0.93	1.088 ± 0.006
P	0.0840	0.4800	0.5805	0.7956

[1]Means within columns carrying different superscripts are significantly different, P < 0.05. NS = Not significant, P > 0.05, ** Significant at 1 % level of probability; LVL = 0, 5, 10, 15 and 20% of Flax seeds, TRT = T, Roasted / U, Un-Roasted flax seeds.

when calculations were based on flax consumption per bird per day, there was a gradual increase in flax consumption as level of flax seeds increased in the diet. This certainly reflected on the omega-3 fatty acids content of the egg (Tables 4 and 6). Van Elswyk (1997) reported a linear increase of linolenic acid in response to increasing levels of ground and whole flax seeds. The effect of flax inclusion on egg mass, egg weight, specific gravity, yolk color, haugh unit and livability were not significant (P>0.05) (Table 5) . Bean and Leeson (2003) in a long term study found that egg production, egg weight, shell weight, albumen height and shell thickness were not significantly (P<0.05) different for hens consuming 0 and 10% flax seed. The highest level of flax seed in their experiment was 10%.

Incorporation of 15% unroasted flax seed significantly increased the level of omega-3 fatty acids (DHA, DPA, EPA and alpha- linolenic acids) in the yolk fat by 194% DHA (2.9 - fold), 300% DPA (4- fold), 450% EPA (5.5-fold) and 876% alpha-linolenic acid (9.8-fold) compared with controls (Table 6). Linoleic acid (an omnipresent omega-6 fatty acid) increased by 49% in birds fed 15% unroasted flax seeds while the linoleic:alpha- linolenic acid ratio reduced from 16:1 in the control birds to 2.4:1 in the flax fed birds (Table 6). These changes in the fatty acids profile of the egg yolk (primarily an increase in omega-3 fatty acids) were somewhat unique and very important to human health (Leaf, 1999).

Caston and Leeson (1990) reported a linoleic: alpha-linolenic ratio of 3:1 in the 10% flax fed birds compared to 37:1 in the control. Oleic acid was lower with birds fed flax diets while Arachidonic acid was better for diets containing 10 and 15% roasted or unroasted flax seeds (Table 7). Caston and Leeson (1990) reported a large increase in omega-3 fatty acids in all levels in the eggs when feeding 10, 20 and 30% flaxseed to laying hens for a 28-day period and collecting eggs for analysis in the last 3 days of the period. Cherian and Sim (1991) fed flax seed to laying hens at 8 and 16% in diets supple-mented with pyridoxine (vitamin B6). They reported increased omega-3 fatty acids in the eggs and in brain tissue of embryos and chicks from the hens fed

Table 6. The unsaturated fatty acids profile of yolks of layers fed different levels of roasted or unroasted flax[1].

Source of variation	Docosahexonoic acid	Docosapento-noic acid	Eicosapentae-noic acid	Alpha-linolenic acid	Linoleic acid
Interaction	**	**	**	**	**
U * 0	0.350 ± 0.014	0.040 ± 0.000	0.020 ± 0.000	0.565 ± 0.007	8.860 ± 0.099
U * 5	0.865 ± 0.007	0.105 ± 0.007	0.050 ± 0.000	2.945 ± 0.021	13.740 ± 0.028
U*10	0.990 ± 0.042	0.135 ± 0.007	0.085 ± 0.007	3.125 ± 0.064	11.900 ± 0.028
U*15	1.030 ± 0.056	0.160 ± 0.000	0.110 ± 0.000	5.515 ± 0.233	13.215 ± 0.332
U*20	0.455 ± 0.064	0.060 ± 0.000	0.035 ± 0.007	1.575 ± 0.021	10.365 ± 0.078
T * 0	0.240 ± 0.028	0.020 ± 0.000	0.010 ± 0.000	0.690 ± 0.042	8.975 ± 0.304
T * 5	0.595 ± 0.007	0.105 ± 0.007	0.050 ± 0.000	3.235 ± 0.035	13.145 ± 0.148
T*10	0.925 ± 0.064	0.120 ± 0.000	0.060 ± 0.000	2.435 ± 0.120	12.225 ± 0.148
T*15	0.395 ± 0.007	0.100 ± 0.000	0.070 ± 0.014	4.495 ± 0.021	13.640 ± 0.042
T*20	0.385 ± 0.021	0.065 ± 0.007	0.040 ± 0.000	3.240 ± 0.028	11.965 ± 0.035
P	0.0001	0.0001	0.0010	0.0001	0.0001

U = Untreated flax seeds with heat, T = Treated flax seeds with heat (roasted) ** significant P < 0.001; 0, 5, 10, 15, 20 = percent level of flax seeds, [1]fatty acids were measured as percent of yolk fat.

Table 7. The Fatty acids profile and cholesterol of layer egg yolks fed different levels of roasted or unroasted flax seeds[1].

Source of variation	Arachidonic acid	Oleic acid	Palmitoleic acid	Palmitic acid	Cholesterol
Interaction	**	**	NS	**	NS
U * 0	0.625 ± 0.021	37.175 ± 0.02	4.650 ± 0.070	36.210 ± 0.0	0.685 ± 0.049
U * 5	0.645 ± 0.007	34.180 ± 0.14	5.065 ± 0.007	32.750 ± 0.30	0.960 ± 0.000
U*10	0.715 ± 0.021	36.000 ± 2.23	5.030 ± 0.141	29.855 ± 0.81	0.905 ± 0.007
U*15	0.715 ± 0.035	35.170 ± 0.38	4.320 ± 0.127	29.815 ± 0.70	1.010 ± 0.000
U*20	0.475 ± 0.049	35.110 ± 0.18	5.285 ± 0.658	35.040 ± 1.02	0.845 ± 0.007
T * 0	0.470 ± 0.000	38.845 ± 0.83	4.110 ± 0.141	34.755 ± 1.0	0.738 ± 0.117
T * 5	0.605 ± 0.007	34.665 ± 0.09	3.895 ± 0.035	32.110 ± 0.04	0.878 ± 0.025
T*10	0.700 ± 0.028	32.760 ± 0.66	4.165 ± 0.106	35.125 ± 0.81	0.820 ± 0.043
T*15	0.480 ± 0.014	29.580 ± 0.44	3.570 ± 0.042	34.325 ± 0.01	0.921 ± 0.018
T*20	0.420 ± 0.042	32.860 ± 0.03	4.215 ± 0.007	34.950 ± 0.18	0.838 ± 0.041
P =	0.0010	0.0006	0.3531	0.0001	0.1843

** Significant P<0.001. NS Not significant, P > 0.05, [1]fatty acids and cholesterol were measured as percent of yolk fat.

ground flaxseed. Aymond and Van Elswyk (1995) reported that feeding both 5 and 15% flaxseed caused increased total omega-3 fatty acids in the eggs and that ground seeds caused a greater level of these fatty acids at the 15% level of feeding than used whole seed.

It is worth noting that the alpha-linolenic acid content of the flax seed, used in this study, was much lower (38.43%) (Table 2) than what is commonly reported (52%, Kratzer and Vohra, 1996). This could be why alpha-linolenic acid did not plateau in yolk at 10% addition of flax seed, as reported by some researches. On the other hand, the subsequent observed decline in alpha-linolenate at 20% flax seed in the feed could be due to toxicity, as the consumption of flax seeds increased from 17 gm to 24 and 23 gm roasted or unroasted flax, respectively (Table 4).

Increasing the amount of unroasted flax to 15% of feed reduced the saturate palmitic acid in the egg yolk while it increased at a 20% addition. The percentage of cholesterol in yolk fat increased significantly with increasing levels of flax, compared with controls (Table 8). However, this increase was not consistent and there was no move in this direction. On the other hand, Caston and Leeson (1990) reported that the amount of cholesterol in egg was not influenced by dietary flax.

It is conclude that adding flax seeds to the feed of layers significantly improved the fatty acid content of the egg yolk. More specifically, and importantly, it increased their omega-3 fatty acid content, an important dietary player in the prevention of cardiovascular disease

Table 8. The fatty acids and cholesterol profile of yolks of layers fed different levels of heat treated or un treated flax seeds[1].

Source of variation	Archidonic acid	Oleic acid	Palmit-oleic acid	Palmitic acid	Stearic acid	Cholesterol
Between treatments	**	**	**	**	NS	NS
U	0.635[a]	35.527[a]	4.870[a]	32.734[a]	8.967[a]	0.881[a]
T	0.535[b]	33.742[b]	3.991[b]	34.253[b]	9.506[a]	0.839[a]
P	0.0001	0.0006	0.0001	0.0003	0.2587	0.0648
Between levels	**	**	**	**	NS	**
0	0.548[c]	38.010[a]	4.380[a]	35.482[a]	9.270[a]	0.711[d]
5	0.625[b]	34.422[b]	4.480[a]	32.430[b]	8.402[a]	0.919[ab]
10	0.708[a]	34.380[b]	4.598[a]	32.490[b]	9.778[a]	0.862[bc]
15	0.598[b]	32.375[c]	3.945[b]	32.070[b]	9.438[a]	0.966[a]
20	0.448[d]	33.985[b]	4.750[a]	34.995[a]	9.295[a]	0.842[c]
P	0.0001	0.0001	0.0051	0.0001	0.4425	0.0001

** Significant P < 0.001. NS Not significant, P > 0.05, [1]fatty acids and cholesterol were measured as percent of yolk fat.

(Leaf, 1999). However, the production performance was somehow jeopardized.

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