Long-Term Effects of Feeding Flaxseed on Performance and Egg Fatty Acid Composition of Brown and White Hens

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ABSTRACT Two hundred fifty-six 18-wk-old Shaver White and ISA-Brown pullets were fed commercial diets containing either 0 or 10% flaxseed in order to study the long-term effects of feeding flaxseed on hen performance and egg production parameters. Performance was monitored over 10 consecutive 28-d periods. Flaxseed was introduced gradually at 28 wk of age and was maintained until hens were 53 wk of age, when flaxseed was gradually eliminated from the diet. Feed intake was less ($P < 0.05$) for hens fed flaxseed compared to those consuming the control diet. Flax-fed hens were also lighter ($P < 0.05$) compared to the control birds. Egg production, egg weight, shell weight, albumen height, and shell thickness were not significantly ($P > 0.05$) different for hens consuming 0 and 10% flaxseed; however, yolk weight was reduced ($P < 0.05$) in hens fed flaxseed. Both strains of birds fed flaxseed deposited significantly more n-3 fatty acids into their eggs. Sampling of livers at the end of the trial showed that hens fed flaxseed had a higher ($P < 0.05$) incidence of liver hemorrhages.

(*Key words*: flaxseed, ISA-Brown, Shaver White, linolenic acid, docosahexaenoic acid)

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INTRODUCTION

Previous studies have shown that hens fed diets containing flaxseed deposit significant amounts of n-3 fatty acids into their eggs (Caston and Leeson, 1990; Aymond and VanElswyk, 1995; Ferrier et al., 1995; Cherian et al., 1996). However, reports on hen performance when they are fed flaxseed are in disagreement. Some reports state that there is a decrease in egg production in response to 15% flaxseed (Aymond and VanElswyk, 1995), whereas others report an increase in production when hens are fed 5 and 10% flaxseed (Scheideler and Froning, 1996). Caston and Leeson (1990) report that feeding flaxseed has no effect on yolk weight, whereas Novak and Scheideler (2001) report that percentage wet yolk is decreased. Caston et al. (1994) and Novak and Scheideler (2001) report that feed consumption is significantly greater for hens fed flaxseed, whereas Scheideler and Froning (1996) showed that birds fed flaxseed exhibit lower feed intakes. It is possible that the duration of these trials influenced the accuracy of the effects on production parameters as these trials ranged in length from 4 to 58 wk.

Several studies have also investigated the effect of feeding diets enriched with n-3 fatty acids to different strains of hen (Ahn et al., 1995; Scheideler et al., 1998; Novak and Scheideler, 2001). Ahn et al. (1995) reported that strain did not impact deposition of fatty acids in eggs when hens are fed diets containing α-linolenic acid. However, Scheideler et al. (1998) reported that strain had an effect on the amount of palmitic, stearic, and oleic acids deposited in eggs when hens are fed diets containing flaxseed.

Although these studies have investigated the effects of feeding flaxseed to laying hens, few studies have focused on examining the long-term effects of diet or strain on multiple production parameters over extended periods. Also, no experiments have investigated the effects of feeding a flaxseed-based diet on production parameters in a brown-egg strain of hen and determined the impact on egg quality and fatty acid deposition. Consequently, the objective of this trial was to quantify bird performance over an entire laying cycle by using white- and brown-egg strains of layer in order to determine whether they perform equally when fed flaxseed.

MATERIALS AND METHODS

Birds and Housing

Two hundred fifty-six Shaver White (white) and ISA-Brown (brown) pullets were obtained at 18 wk of age. Birds were housed individually in laying cages in a room maintained at 20°C, and a step-up lighting schedule was

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Abbreviation Key: DHA = docosahexaenoic acid; LNA = linolenic acid.

TABLE 1. Diet composition (%)

	Diet 1	Diet 2	Diet 3	Diet 4
Corn	58.6	56.4	54.3	53.4
Wheat	1.1	1.0	1.0	1.0
Canola meal	4.0	4.0	4.0	4.0
Soybean meal	19.5	18.1	16.6	15.9
Meat meal	5.0	5.0	5.0	5.0
Animal fat	1.0	0.7	0.3	—
DL-Methionine	0.15	0.15	0.14	0.14
Salt	0.25	0.25	0.25	0.25
Limestone	9.0	9.0	9.0	8.9
Dical PO_4	0.40	0.40	0.41	0.41
Vitamin and mineral premix	1.0	1.0	1.0	1.0
Flaxseed	—	4.0	8.0	10.0
	100.00	100.00	100.00	100.00
Calculated analyses				
ME (kcal/kg)	2,780	2,780	2,800	2,800
CP (%)	18.5	18.5	18.5	18.5
Ca (%)	4.0	4.0	4.0	4.2
Available P	0.45	0.45	0.45	0.45
TSAA (%)	0.74	0.74	0.74	0.74
Lysine (%)	1.02	1.00	0.99	0.98

used. Hens received 15 L:9 D from 18 until 20 wk of age, 15.5 L:5 D until 21 wk of age, and then 16 L:8 D beginning at 22 wk of age and then maintained until the end of the trial. Hens were randomly divided into two dietary treatments, and treatments were replicated 16 times with four adjacently caged hens of the same strain serving as a replicate. Feed and water were provided ad libitum. Feeding and egg collection were conducted daily each morning.

Diets

Birds consumed a standard layer diet (diet 1) from 18 to 28 wk of age. At this time, hens remained on this diet or were offered diet 2 with 4% flaxseed. Birds assigned to the flaxseed-based diet were put on a 3-wk phase-in period during which these hens received 4, 8, and 10% flaxseed (diets 2, 3, and 4) at 28, 29, and 30 wk, respectively. Compositions of experimental diets are listed in Table 1. Hens were maintained on the 10% flaxseed diet until they were 53 wk of age at which time flax was gradually decreased and hens received 8, 4, and 0% (diets 3, 2, and 1) flaxseed at 54, 55, and 56 wk, respectively. At the end of the trial, diets were formulated to provide 4.4% Ca, 0.38% available P, and 16.5% CP. This gradual introduction and subsequent withdrawal of flaxseed mimics commercial practice in Canada. At 56 wk, all birds were fed a standard layer diet containing 0% flaxseed (diet 2), and they were maintained on this diet until the end of the trial when birds were 60 wk of age. Hens assigned to the 0% flaxseed diet were maintained on this diet for the entire duration of the trial. Diets were fed through a full laying cycle of 10, 28-d periods, and production parameters including feed intake, egg quality, and shell quality were monitored at the end of every 28-d period.

Production Parameters

Feed intake was measured for each period and was calculated as a mean for each replicate of four birds. Egg production was recorded daily throughout the experiment. Eggs were collected over 2 d at the end of each period for measurements of weight, albumen height, yolk weight, shell weight, and shell thickness. All birds were weighed individually at the start of the experiment, at 29 wk of age (period 3), 42 wk of age (period 6), 52 wk of age (period 9), and 60 wk of age (period 10).

One hen from every treatment replicate having previously being fed a diet containing 0 or 10% flaxseed was selected at random for liver scoring. Livers were obtained from hens at 59 wk of age, and they were scored on the same subjective scale as reported by Caston et al. (1994). Livers were scored based on a 1-to-5 scale, where a score of 1 denoted no hemorrhaging and a score of 5 denoted excessive hemmorhaging.

Egg Fatty Acid Analysis

Four random samples of eggs from each diet and strain combination were obtained for fatty acid analysis when hens were 49 wk of age. The eggs obtained from each of the four replicates of each diet and strain combination were pooled and homogenized prior to fatty acid analysis. Sixteen samples composed of four eggs each were analyzed one time for 1-d egg production in order to determine fatty acid analysis. Approximately 2 g of the whole egg sample was weighed out into a 50-mL centrifuge tube, and C13 internal standard (synthetic triglyceride with 13 carbons) was added. Lipids were extracted from the eggs using the method of Bligh and Dyer (1959). Approximately 50 to 200 μL of the lower chloroform phase was added to a test tube along with 2.0 mL of boron trichloride in 14% methanol. The tube was then capped,

TABLE 2. Egg production for each 28-d period (%)

Item	Period 1	2	3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)										
0	50.5	94.2	96.7	97.2	95.4	94.0	93.4	91.8	89.1	87.1
10	46.5	95.0	96.9	97.7	96.8	94.9	92.7	92.1	90.9	89.4
Strain										
Shaver White	36.2	92.7	96.5	97.3	96.7	94.7	93.4	91.8	89.1	88.4
ISA-Brown	60.8	96.5	97.2	97.6	95.6	94.2	92.8	92.2	90.9	88.0
Flax	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Strain	**	*	NS	NS	NS	NS	NS	NS	NS	NS
Flax × strain	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Period during which flaxseed was fed.

*$P < 0.05$; **$P < 0.01$.

and methylated in a boiling water bath for 15 min. Tubes were allowed to cool and then vortexed. Approximately 1 μL of the upper hexane layer was then withdrawn into gas chromatography vials for injection. The fatty acid methyl esters were analyzed using a gas chromatograph[2] with a 60-m × 0.32-mm inside diameter, DB-23 capillary column, 0.10-μm film thickness. The quantification of fatty acid methyl esters was based on comparison to a known internal standard.

Statistical Analysis

The experiment was a completely randomized design, and the experimental unit was the replicate consisting of four adjacently caged birds fed as one group. Data were analyzed using the general linear models procedure of SAS software (1992). Main effects of diet, strain, and diet-by-strain interaction were tested.

RESULTS

Egg production, egg weight, shell weight, albumen height, shell thickness, and eggshell deformation were unaffected by feeding flaxseed (Tables 2, 4, 5, 8, and 9, $P > 0.05$). An exception occurred in period 5 when flax-fed hens showed increased ($P < 0.05$) shell thickness (Table 8). However, this was an isolated occurrence and therefore not deemed biologically significant. Flax-fed hens ate significantly less feed during periods 4 and 6 compared to control fed hens (Table 3). Hens fed flaxseed produced eggs with a smaller ($P < 0.05$) percentage of wet yolk compared to control fed birds during periods 5, 6, 7, and 9 (Table 7). Body weights were lighter ($P < 0.05$) for flax-fed hens compared to control fed hens, and there were more ($P < 0.01$) liver hemorrhages detected in hens fed flaxseed (Table 10).

Hen strain had a significant effect on several egg parameters; however, there was no effect of strain on egg production with the exception of periods 1 and 2 (Table 2). Brown hens ate more ($P < 0.01$) feed (Table 3). Egg weight, shell weight, and yolk weight were heavier ($P < 0.01$) for brown hens compared to white hens (Tables 4, 5, and 6). Yolk weight expressed as a percentage of egg weight was not different ($P > 0.05$) between strains (Table 7). Shell thickness was also higher ($P < 0.01$) in brown hens (Table 8). The albumen heights in eggs from brown hens were lower ($P < 0.01$) compared to those from white hens (Table 9). Brown hens also had heavier BW ($P < 0.01$) (Table 10). There was no significant effect of strain on liver hemmorhage score (Table 10).

The fatty acid compositions of eggs are listed in Table 11. Birds that consumed flaxseed deposited higher ($P < 0.0001$) amounts of linolenic acid (LNA), docosahexaenoic acid (DHA), and total n-3 fatty acids into their eggs. These birds also deposited less ($P < 0.001$) total n-6 fatty acids into their eggs. Brown hens deposited more ($P < 0.01$) DHA into their eggs, whereas white hens deposited more LNA into their eggs, albeit not significantly so there was no strain effect on the total n-3 fatty acid content of the eggs.

DISCUSSION

Trials conducted in the past have focused on how flaxseed-based diets effect production parameters and egg fatty acid composition using white-egg strains (Jiang et al., 1991; Caston and et al., 1994; Scheideler et al., 1998; Novak and Scheideler, 2001). However, there have been no reports regarding the long-term layer performance using a brown-egg strain fed flaxseed. Leeson et al. (1998) studied the organoleptic qualities of eggs from ISA-Brown hens fed 10 or 20% flaxseed and stated that diet had no effect on egg production or egg weight. Whether or not the egg production and egg weight were maintained at every point throughout the trial was not studied. In the current study, there was no long-term effect on egg production or egg weight as a result of feeding flaxseed to brown or white hens. Novak and Scheideler (2001) reported that flaxseed did not affect eggshell quality, and the results from the current study are in agreement. Our results showed that diet had no consistent effect on shell quality parameters in either strain.

[2]Varian 3800, Varian Canada Inc., Mississaugh, ON, Canada.

TABLE 3. Feed intake (g/bird per d)

Item	Period 1	2	3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)										
0	97.8	104.2	109.9	110.6	106.2	103.7	97.2	100.0	101.3	101.8
10	97.6	105.4	110.2	106.4	105.4	99.1	97.3	101.8	107.3	104.6
Strain										
Shaver White	91.0	97.6	103.0	102.0	99.0	94.4	91.5	95.4	99.1	97.6
ISA-Brown	104.4	112.1	117.1	115.0	112.7	108.4	103.0	106.4	109.5	108.8
Flax	NS	NS	NS	*	NS	**	NS	NS	**	NS
Strain	**	**	**	**	**	**	**	**	**	**
Flax × strain	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Period during which flaxseed was fed.
*$P < 0.05$; **$P < 0.01$.

TABLE 4. Egg weight (g)

Item	Period 1	2	3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)										
0	53.1	58.5	60.9	61.6	62.4	61.0	62.6	63.3	64.1	64.5
10	52.5	57.7	60.8	61.6	61.9	60.8	61.7	63.1	63.7	63.7
Strain										
Shaver White	50.3	55.6	58.2	59.5	60.2	59.2	60.3	61.6	62.4	62.2
ISA-Brown	55.3	60.6	63.5	63.7	64.2	62.6	64.0	64.7	65.5	65.9
Flax	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Strain	**	**	**	**	**	**	**	**	**	**
Flax × strain	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Period during which flaxseed was fed.
**$P < 0.01$.

TABLE 5. Shell weight (g)

Item	Period 3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)								
0	6.7	6.6	6.6	6.5	6.5	6.7	6.7	6.7
10	6.7	6.5	6.7	6.6	6.4	6.7	6.7	6.7
Strain								
Shaver White	6.5	6.3	6.4	6.3	6.2	6.4	6.5	6.4
ISA-Brown	6.9	6.8	6.9	6.8	6.7	6.9	7.0	7.0
Flax	NS	NS	NS	NS	NS	NS	NS	NS
Strain	**	**	**	**	**	**	**	**
Flax × strain	NS	NS	NS	NS	NS	NS	NS	NS

[1]Period during which flaxseed was fed.
**$P < 0.01$.

Novak and Scheideler (2001) investigated the long-term effects of feeding flaxseed to DeKalb Delta and Hy-Line W-36 hens. They reported that strain had a significant effect on feed intake, BW, egg production, and egg weight. This report is in agreement with the current study, as our results show that brown-egg hens produce significantly heavier eggs, consume significantly more feed, and have a significantly higher BW compared to white-egg hens ($P < 0.001$). However, our results show that there was no difference in egg production between white and brown hens. Flaxseed at 10% did not effect egg production regardless of hen strain, which is in agreement with results reported by Jiang et al. (1991), Caston et al. (1994), and Novak and Scheideler, (2001) who all reported that feeding 10% flaxseed had no detrimental effects on egg production.

Several reports have stated that white strains fed flaxseed have significantly lower BW compared to hens consuming a conventional diet (Caston et al., 1994; Scheideler and Froning 1996; Schumann et al., 2000; Novak and Scheidler, 2001). In the current study, the same result was found with white and brown hens. Although the AME_n of the flax diet was slightly higher than the control diet, flax-fed hens had a significantly lower BW. It has been

TABLE 6. Yolk weight (g)

	Period							
Item	3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)								
0	14.9	15.7	16.0	15.7	16.3	16.4	16.7	16.9
10	14.7	15.4	15.4	15.3	15.7	16.1	16.3	16.5
Strain								
Shaver White	14.0	14.9	15.1	15.0	15.5	15.9	16.2	16.3
ISA-Brown	15.6	16.2	16.3	16.0	16.4	16.6	16.8	17.0
Flax	NS	NS	**	*	**	NS	*	*
Strain	**	**	**	**	**	**	**	**
Flax × strain	NS	NS	NS	NS	NS	NS	NS	NS

[1]Period during which flaxseed was fed.
*$P < 0.05$; **$P < 0.01$.

TABLE 7. Yolk weight as a percentage of egg weight

	Period							
Item	3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)								
0	24.5	25.4	25.7	25.8	26.0	26.0	26.1	26.2
10	24.2	25.0	24.8	25.2	25.4	25.5	25.5	25.9
Strain								
Shaver White	24.1	25.0	25.0	25.4	25.7	25.9	25.9	26.3
ISA-Brown	24.6	25.5	25.5	25.5	25.7	25.6	25.7	25.8
Flax	NS	NS	**	*	*	NS	*	NS
Strain	NS	*	NS	NS	NS	NS	NS	NS
Flax × strain	NS	NS	NS	NS	NS	NS	NS	NS

[1]Period during which flaxseed was fed.
*$P < 0.05$; **$P < 0.01$.

TABLE 8. Shell thickness (0.01 mm)

	Period							
Item	3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)								
0	0.349	0.345	0.339	0.351	0.350	0.355	0.354	0.351
10	0.348	0.341	0.349	0.352	0.347	0.354	0.357	0.349
Strain								
Shaver White	0.338	0.333	0.331	0.343	0.339	0.342	0.343	0.338
ISA-Brown	0.358	0.353	0.357	0.360	0.358	0.368	0.368	0.362
Flax	NS	NS	**	NS	NS	NS	NS	NS
Strain	**	**	**	**	**	**	**	**
Flax × strain	NS	NS	NS	NS	NS	NS	NS	NS

[1]Period during which flaxseed was fed.
**$P < 0.01$.

hypothesized that anti-nutritional factors contained in flaxseed may impair the digestion and absorption of energy yielding nutrients (Gonzalez-Esquerra and Leeson, 2000; Rodríguez et al., 2001). Ortiz et al. (2001) concluded that deleterious compounds in flaxseed (linseed) interact with the other dietary ingredients in flax-based diets resulting in decreased dietary AME_n. Therefore, it is possible that anti-nutritional factors in the flax diet caused the decrease in hen weight that was observed in the current study.

Scheideler et al. (1998) reported that neither hen strain nor flaxseed have a significant effect on percentage egg yolk. Novak and Scheideler (2001) reported that strain did effect yolk percentage with Hy-Line W-36 hens producing eggs with significantly larger yolks compared to DeKalb Delta hens. In the current study, brown hens produced eggs with larger ($P < 0.01$) wet yolk percentage (Table 8) compared to those from white hens in period 4; however, this result was an isolated incident and was probably not biologically significant. Yolk weight as a percentage of egg weight was significantly lower in hens fed flaxseed during periods 5, 6, 7, and 9. This finding is in agreement with the results reported by Scheideler and Froning (1996) and Novak and Scheideler (2001) who stated that yolk

TABLE 9. Albumen height (mm)

Item	Period 3[1]	4[1]	5[1]	6[1]	7[1]	8[1]	9[1]	10
Flax (%)								
0	8.5	8.0	7.4	8.0	7.3	7.4	7.1	6.6
10	8.3	7.8	7.3	7.9	7.2	7.4	7.1	6.7
Strain								
Shaver White	9.0	8.5	8.0	8.6	7.9	8.1	7.8	7.3
ISA-Brown	7.8	7.3	6.7	7.2	6.5	6.8	6.5	6.0
Flax	NS	NS	NS	NS	NS	NS	NS	NS
Strain	**	**	**	**	**	**	**	**
Flax × strain	NS	NS	NS	NS	NS	*	NS	NS

[1]Period during which flaxseed was fed.

**P* < 0.05; ***P* < 0.01.

TABLE 10. Body weight (grams) and liver score

Item	Bird age (wk) 18[1]	29[2]	42[3]	52[4]	60[5]	Liver hemorrhage score (1 to 5)
Flax (%)						
0	1,414	1,726	1,825	1,894	1,907	1.2
10	1400	1,700	1,760	1,799	1,851	1.7
Strain						
Shaver White	1,222	1,553	1,644	1,700	1,746	1.5
ISA-Brown	1,592	1,873	1,942	1,994	2,012	1.4
Main effects						
Flax	NS	NS	**	***	*	*
Strain	****	****	****	****	****	NS
Flax × strain	NS	NS	NS	NS	NS	NS

[1]Starting weight.

[2]Start of 10% flax feeding.

[3]Middle of 10% flax feeding.

[4]End of 10% flax feeding.

[5]End weight.

**P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

TABLE 11. Egg fatty acid composition (mg/50-g whole egg sample)

	Linolenic acid (C-18:3n3)	Docosahexaenoic acid (C22:6n3)	Total n-3	Total n-6
Flax (%)				
0	38.5	53.3	99.8	832.3
10	306.3	83.7	415.4	748.4
Strain				
Shaver White	178.5	64.3	258.5	805.0
ISA-Brown	166.3	72.7	256.6	775.7
Flax	***	***	***	**
Strain	NS	*	NS	NS
Flax × strain	NS	NS	NS	NS

**P* < 0.01; ***P* < 0.001; ****P* < 0.0001.

percentage decreases in hens fed flaxseed. In the current study, albumen height was influenced ($P < 0.01$) by strain with brown hens producing eggs with consistently lower albumen heights compared to white hens. Although albumen heights decreased in both strains over time, use of flaxseed did not ($P > 0.05$) effect albumen heights in either strain.

Scheideler et al. (1998) reported that hen strain has no effect on the deposition of LNA or DHA into the egg yolk. However, Ahn et al. (1995) reported minor differences in the fatty acid content of eggs from various strains of hens consuming diets enriched with n-3 fatty acids. In the current study, brown hens deposited more ($P < 0.01$) DHA compared to white hens, whereas white hens deposited more LNA into whole eggs compared to brown hens, although this latter effect was not statistically significant. The total amount of n-3 fatty acids deposited into the eggs was comparable for both strains.

Average liver hemorrhage scores from hens consuming flaxseed were higher ($P < 0.05$) compared to those consuming the control, indicating that flaxseed contributed to the development of liver hemorrhages. This result is contrary to reports by Schumann et al. (2000) and Caston et al. (1994) who reported that hens did not have more hemorrhages when consuming flaxseed. In the current study, strain did not affect ($P > 0.05$) liver hemorrhage score. Some liver hemorrhages were apparent in both strains regardless of diet, which is in agreement with Schumann et al. (2000). In some instances, very high liver lesion scores were noted, and one wonders what effect flax feeding might have if used for the entire laying cycle. The reason for hemorrhaging is unknown, although presumably the liver of flax-fed birds contains more long chain unsaturates, which are more prone to oxidative rancidity.

Overall, feeding flaxseed to either white or brown hens produced no detrimental long-term effects on egg production or egg quality. It seemed as though both strains performed equally when fed flaxseed, and both strains of hen deposited comparable amounts of n-3 fatty acids into their eggs when fed flaxseed. However, with the brown producing a significantly larger egg, there are potentially more n-3 fatty acids deposited. Consequently, an n-3—enriched brown egg could provide a greater proportion of a person's daily requirement of n-3 fatty acids. However, there may be some concerns regarding egg flavor, so it would be of interest to conduct taste panels on eggs from brown hens fed flaxseed.

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