# PRODUCTION OF EGG ENRICHED WITH OMEGA-3 FATTY ACIDS IN LAYING HENS

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## Abstract

**INTRODUCTION:** This experiment was conducted to study the effects of different levels of flaxseed (0, 5, 10 and 15% of diet) and copper (in the form of cupric sulfate pentahydrate, 0 and 250 ppm of diet) on fatty acids and cholesterol in egg yolk of laying hens.

**METHODS:** A total of 128 White Leghorn layers aged 50 weeks were used in a factorial design and randomly assigned to 8 treatment groups. The experiment lasted 120 days. The birds were fed ad libitum on the experimental diets. Cholesterol concentration and fatty acids in plasma and egg yolk were measured monthly and bimonthly, respectively. Egg yolk cholesterol was extracted using modified Folch procedure and measured using Zak's method.

**RESULTS:** The results showed that experimental diets had no effect on plasma cholesterol. The different levels of flax had no significant effect on egg yolk cholesterol but the presence of copper with flax at levels of 5 and 10 percent decreased cholesterol in egg yolk (mg per gram yolk) significantly ($P<0.05$). The egg cholesterol (mg per egg) in the group treated with 15% flax without copper also decreased significantly ($P<0.05$). The unsaturated fatty acids of egg yolk increased significantly in all experimental diets ($P<0.05$). The highest ratio of omega-3 to omega-6 fatty acids was observed in groups treated with 10% and 15% flax diets.

**DISCUSSION:** This experiment showed that feeding flaxseed to laying hens can increase omega-3 fatty acids of egg yolk.

**Keywords** • Laying hen • Egg yolk • Flax • Copper • Plasma • Cholesterol • Fatty acid

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## Introduction

Healthy nutrition is known to reduce mortality and morbidity due to cardiovascular diseases (CVD).[1] An interventional study has reported a relationship between cholesterol in consumed eggs and plasma cholesterol levels. The relationship between the amount of egg consumption and the incidence of coronary artery diseases (CAD) has also been studied.[2] Studies on fatty acids known as omega-3 show that increased daily intake of linoleic acid in any form has preventive effects against CVD and related mortality and morbidity; it also reduces platelet aggregation in diabetics and is effective against arteriosclerosis.[3]

These fatty acids commonly occur in sea foods, whales, seals, and cod liver oil. Flaxseeds contain an omega-3 fatty acid known as alpha linoleic acid with 18 atoms of carbon and 3 double bonds; when ingested by humans or poultry, it is partly converted to omega-3 fatty acids with 20-22 carbons with a conversion efficiency of 10-15%.[4]

Line, flax, common flax or Linum usitatissimum L. is a one-year grass plant, the seeds of which contain 30-40% oil, 20-25% protein, and 3-10% mucilage.

Flaxseed is a rich source of omge-3 fatty acids. When fed to cattle and poultry, flaxseed displays diarrheic effects due to increasing in bulk and triggering peristaltic intestinal movments.[5]

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Scheideler and Froning[6] reported a significant increase in levels of omega-3 fatty acids in eggs of laying hens fed with chow containing 5-15% flaxseed, compared to controls. Omega-3 fatty acids in egg yolks of control hens and those fed with flaxseed-containing chow comprised 1.2% and 2-7% of yolk fatty acids, respectively.

Caston and Leeson[7] reported that feeding laying hens with chow containing 0, 10, 20 and 30 percent ground flaxseed led to a significant increase in omega-3 and some increase in omega-6 fatty acids in all treatment groups, especially in the group treated with 20% flaxseed. They reported no change in egg cholesterol level in any of the treatment groups. Ceylan and colleagues[8] reported that feeding laying hens with 1.5-3% flaxseed and colza (cannula) oil significantly increased levels of egg linoleic acid (C18:3).

Mighelenj and colleagues[9] reported no significant effect on serum cholesterol levels of laying hens fed with chow containing cannula seed, flaxseed, and fish power at 2.5%, 5%, and 7.5% levels. In a study conducted by Goncuglu and Ergun, [10] feeding laying hens with flaxseed and/or sunflower oil at 0, 1, 2, 3 and 4 percent levels led to significant increase in omega-3 and omega-6 fatty acids of yolk.

Dove[11] observed that adding high levels of copper to chow fed to growing pigs improved their daily weight gain; he attributed this effect to the interaction between copper levels and fat.

Al Ankari and colleagues reported significant linear reductions of 20% in serum cholesterol, and 14% in yolk cholesterol of laying hens treated with chow containing copper at levels of 0, 50, 150 and 250 ppm. Balevi and Coskun[12] reported that chow containing 15 ppm copper decreased yolk cholesterol significantly compared to controls, however, no significant difference was observed between yolk cholesterol levels of hens treated with 200 ppm copper and controls (16.64 vs. 16.86). In fact, yolk cholesterol did not decrease in a dose-dependent fashion[5] in response to the increase in diet copper content. The present study was conducted to assess the possibility of enriching eggs of laying hens with omega-3 fatty acids and reducing levels of harmful fatty acids through feeding hens with varying levels of copper and flaxseed.

## Materials and methods

This study was performed on 128 commercial White Leghorn laying hens of Hy-Line strain. The hens were aged 50 weeks and weighed 1.5 kilograms at the start of the experiment. With a factorial design, this study aimed to investigate how varying levels of unground flaxseed (0, 5, 10 and 15 % of diet) at two levels of cupric sulfate pentahydrate ($CuSo_4+5H_2O$), namely 0 and 250 ppm would affect fatty acids and cholesterol in egg yolk of laying hens. Eight treatment groups each with 4 replicates of 4 birds were studied.

The experimental diets were randomly distributed among treatment groups. The experiment lasted four months (four 28-day periods). The laying hens were kept in identical cages and conditions standard for laying hens.

The chemical composition of flaxseed was determined before preparing the diets (Figure 1). The metabolizable energy content and other nutritional characteristics of flaxseed were extracted from the NRC table (1994) and the experimental diets were prepared (Table 1) with computerized calculations based on the table outlining the needs of white Leghorn laying hens (1994, NRC).[13]

Every kilogram of the prepared diet contained 2700 kcal metabolizable energy, 14.72% raw protein, 0.31% calcium, 0.245% absorbable phosphorous, 0.314% methionine, and 1.5-2% linoleic acid. Modified Folsch method[1] was used to prepare yolk extract. Yolk and plasma cholesterol were measured using Zak's colorimetric method. Measurement of yolk fatty acids was conducted using an HPLC machine. The obtained data were analyzed with SAS (version 6) statistical software package using the Randomized Complete Block Design. Comparison of mean values was performed with Duncan's multiple range tests.

BF1 to BF4 correspond to diets containing 5, 0, 10, and 15 percent flaxseed, respectively. CF1 to CF4 correspond to diets containing the same amounts of flaxseed and 250 ppm copper.

## Results

The experimental diets did not alter plasma cholesterol levels significantly (Tables 1 & 2).

The diets containing flaxseed did not change yolk cholesterol levels significantly compared to controls.

However, all copper-containing diets except CF4, were associated with a significant decrease in yolk cholesterol compared to controls ($P<0.05$). All copper-containing diets led to decrease in egg cholesterol content ($P<0.05$).

Various levels of flaxseed did not change yolk fat content (Table 3). However, increased diet flaxseed led to a linear decrease in saturated fatty acids and a decrease in unsaturated fatty acids ($P<0.05$).

**FIGURE 1.** Chemical analysis of flaxseed used in experimental diets (percentage)

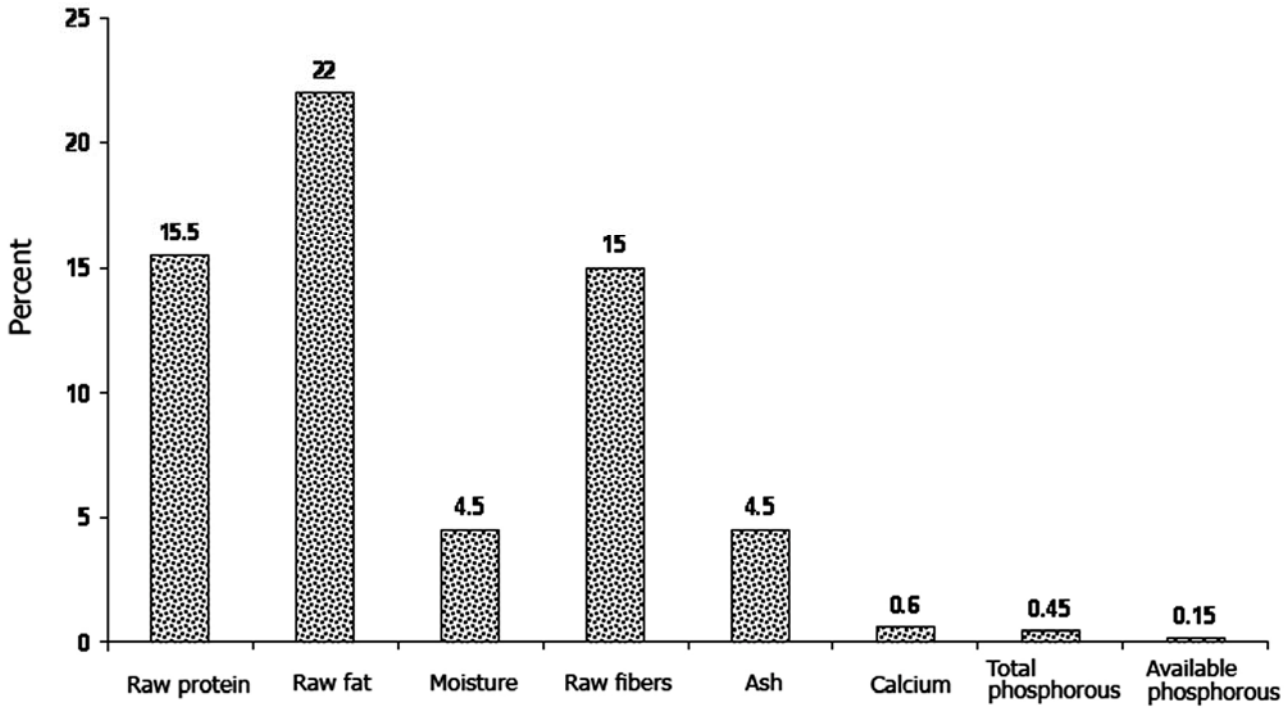


**TABLE 1.** Composition of different experimental diets fed to laying hens

| *Experimental diets* | BF1 | BF2 | BF3 | BF4 | CF1 | CF2 | CF3 | CF4 |
|---|---|---|---|---|---|---|---|---|
| *Diet composition* | 59.7 | 56.4 | 53.1 | 49.8 | 59.7 | 56.4 | 53.1 | 49.8 |
| Corn | 15.1 | 14.3 | 13.5 | 12.6 | 15.1 | 14.3 | 13.5 | 12.6 |
| Soy kernel | 10 | 10 | 1010 | 10 | 10 | 10 | 10 | 10 |
| Barley | 0 | 5 | 10 | 15 | 0 | 5 | 10 | 15 |
| Flaxseed | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Fish powder | 3.4 | 2.6 | 1.8 | 1.1 | 3.4 | 2.6 | 1.8 | 1.1 |
| Bran | 7.9 | 7.8 | 7.7 | 7.65 | 7.9 | 7.8 | 7.7 | 7.65 |
| Shell crust | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 | 0.69 |
| Dicalcium phosphate | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin-mineral supplements | 0.05 | 0.04 | 0.03 | 0.03 | 0.05 | 0.04 | 0.03 | 0.03 |
| DL-methionine | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| ADEB vitamins | - | - | - | - | 0.0008 | 0.0008 | 0.0008 | 0.0008 |

**TABLE 2.** Comparison of mean cholesterol levels in egg, yolk and plasma in the case and control groups

| Variable | BF1 | BF2 | BF3 | BF4 | CF1 | CF2 | CF3 | CF4 |
|---|---|---|---|---|---|---|---|---|
| Blood cholesterol (mg/dl) | $^{ab}$ 153.8 | $^{ab}$ 153.7 | 146.5$^{ab}$ | 158.7$^{ab}$ | 172.6$^{a}$ | $^{ab}$ 152.5 | $^{ab}$ 157.2 | 132.1 $^{b}$ |
| Yolk cholesterol (mg) | 13.8 $^{a}$ | 12.7 $^{b}$ | 11.5 $^{b}$ | $^{c}$ 10.6 | $^{c}$ 10 | 9.8 $^{c}$ | $^{cd}$ 8.7 | $^{cd}$ 8.6 |
| Egg cholesterol (mg/egg) | 200.2 $^{a}$ | 251.6 $^{a}$ | 22.7 $^{bc}$ | $^{bc}$ 229.9 | 200 $^{d}$ | 213.7 $^{cd}$ | 210 $^{bcd}$ | 198 $^{c}$ |

The CF1 diet was not different from the control diet BF1 in respect of the percentage of fat and saturated fatty acids content. However, saturated fatty acid levels in other groups fed flaxseed and copper approximated or equaled those in corresponding control groups, while the percentage of unsaturated fatty acids in groups fed copper was lower than in controls. Flaxseed levels up to 10% of diet were associated with a significant increase in yolk omega-3 and omega-6 fatty acids ($P<0.05$); higher flaxseed levels did not lead to further increase in yolk omega-3 and omega-6 fatty acids.

**TABLE 3.** Comparison of yolk nutritional indices between the case and control groups

| Treatment group | Fat (%) | Saturated fatty acids (%) | Unsaturated fatty acids (%) | Linoleic acid (ω-6) | Linolenic acid (ω-3) | Oleic acid | Palmitic acid | MUFA* | PUFA* | ω6/ω3 | MUFA/ PUFA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BF1 (Control) | 25.7 | 42.1 | 28 | 27.7 | 10.5 | - | - | - | - | - | - |
| BF2 (5% Flaxseed) | 26.1 | 31.8 | 64.5 | 45.3 | 18.4 | 24.6 | 34.6 | 70.7 | 66 | 2.64 | 1.09 |
| BF3 (10% Flaxseed) | 26.7 | 28.3 | 69.7 | 48.3 | 22.8 | 34.7 | 42.5 | 72.1 | 68.5 | 2.9 | 1.15 |
| BF4 (15% Flaxseed) | 25.7 | 28.2 | 70.3 | 48.9 | 21.5 | 36.7 | 38.6 | 74.8 | 75.8 | 3.2 | 1.1 |
| CF1 (Copper sulfate) | 25.6 | 42.1 | 28 | - | - | - | - | - | - | - | - |
| CF2 (5% Flaxseed + Copper sulfate) | 25.5 | 31.2 | 53.5 | 42.4 | 17.1 | 25.8 | 14.5 | 69.5 | 42.7 | 2.40 | 1.6 |
| CF3 (10% Flaxseed + Copper sulfate) | 26.3 | 29.9 | 59.9 | 43.9 | 18.5 | 31.4 | 16.7 | 60.9 | 41.1 | 2.45 | 1.58 |
| CF4 (15% Flaxseed + Copper sulfate) | 26.8 | 30.3 | 63.1 | 44.1 | 17.3 | 33 | 20.6 | 61.4 | 55.1 | 2.5 | 1.1 |
| P value (Control vs. case group) | 0.5 | 0.04 | 0.03 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

*PUFA: Poly unsaturated fatty acids, MUFA: Mono-unsaturated fatty acids

## Discussion

Plasma cholesterol levels in the groups fed experimental diets with various amounts of flaxseed, with or without copper were not significantly different from each other; neither was there any significant difference between the experimental groups and the controls.

The findings of this study are consistent with those of Beyer and colleagues[14] who concluded that feeding laying hens with flaxseed has no effect on their plasma cholesterol levels.

Two studies reported a linear decrease in plasma cholesterol concentration with the increase in diet copper content [15, 16]. In the current study, however, plasma cholesterol concentration was not influenced by copper levels in diet (Table 2).

Caston and colleagues showed that cholesterol does respond to the rise in diet copper content in a dose-dependent manner; while the strongest decrease in yolk cholesterol was seen at a copper concentration of 150 ppm, no significant difference was seen between copper concentrations of 200 ppm and 0 ppm (control).

The highest yolk cholesterol content was seen in the control group and varying levels of flaxseed did not affect yolk cholesterol level. These findings are in agreement with results reported by Scheideler and Froning[6] who also experimented with different levels of flaxseed in diet, and Lewis[13] who used 4% flaxseed oil in diets of laying hens and reported no significant decrease in yolk cholesterol.

The effect of copper on yolk cholesterol was significant at all flaxseed levels except the 15% level; this is in agreement with findings of Al Ankari and colleagues.[16]

In this study, the results concerning egg cholesterol content were largely similar to those related to yolk cholesterol, with the exception of the significant decrease in yolk cholesterol seen in group BF4 compared to controls.

Flaxseed did not affect yolk fat content at any level, however, saturated fatty acids decreased and unsaturated fatty acids increased in a linear fashion with the increase in diet flaxseed content (Table 3).

These findings are in agreement with the results reported by Ceylan[8] and Froning[6] about the effect different levels of flaxseed exert on the composition of yolk fatty acids without altering yolk fat content.

The results of this study confirmed the original study hypothesis, i.e. the possibility of reducing egg cholesterol content while increasing its useful fatty acids (omega-3 and omega-5).

The 15% flaxseed level was associated with decrease in egg production and increase in food conversion rate (data not made available), hence we propose a flaxseed level of 10%. The proposed flaxseed level significantly increased useful fatty acids in yolk.

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