Effects of moderate (5%) levels of linseed in layer diets

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SUMMARY

The study evaluated the potential of a diet formulation for layers, with a moderate level (5%) of linseeds, to produce omega 3 polyunsaturated fatty acids (alpha linolenic acid - alpha LNA and docosahexaenoic acid -DHA). The experiment was conducted on 108 Lohmann Brown layers (35-42 weeks of age) assigned to three groups. The diets for experimentals groups (E1 and E2) differed from the control (C) diet by the inclusion of 5% linseed or 20.2% full fat soy. The diets for group C and E1 contained 27 ppm vitamin E, while the diet for group E2 contained 250 ppm vitamin E. Feed intake, forage quality preservation in time, egg production, egg weight and egg components weight have been monitored throughout the experiment. Eighteen eggs per group were collected randomly (weeks of age 35, 37, 39 and 42) and average samples of egg yolk were formed (3 eggs/sample). The samples were assayed for the gross chemical composition, pH (determined one week after the harvesting of eggs which were kept in a refrigerator at 4^0C), fatty acids profile and vitamin E concentration. The 5% dietary linseeds treatment produced eggs enriched in alpha LNA and DHA without affecting layer performance. The determinations performed on week of age 37 show that both alpha LNA and DHA were in significantly higher concentrations ($p \leq 0.05$) in the eggs from the linseed treated groups than in the eggs from group C. The 250 ppm vitamin E in the diet for group E2 preserved the quality of the feed and increased vitamin E concentration in the egg yolk. The eggs from group E2 were used in a clinical study conducted at the Parhon National Institute of Endocrinology, Bucharest. The volunteers which consumed 6 eggs per week for six weeks had significantly lower serum triglycerides levels ($p \leq 0.002$) in the end of the survey than at the beginning of it.

Keywords: linseeds, eggs, alpha LNA, DHA, vitamin E

INTRODUCTION

The hen egg is perceived by the Romanian consumer as a readily available food, with a proper feeding to cost ratio for large categories of population. However, there is an increasing proportion of the population (higher education, informed people living in urban areas) whose feeding preferences go towards

foods which can protect their health. In an attempt to meet this desire the Romanian egg producers displayed over the past 4-5 years an increased concern to improve the feeding properties of the hen eggs.

The polyunsaturated fatty acids (PUFA) are among the egg nutrients which are important to human health, particularly the omega 3 polyunsaturated fatty acids (ω: 3-PUFA). These acids are essential for a proper development, they assist in preventing and curing heart diseases, hypertension, arthritis and several types of cancer (Simopoulos, 2000; Sim, 2006). Recent studies reveal the importance of the presence of omega 3 polyunsaturated fatty acids in the diets for children and elder persons. There are reports according to which the children who drank milk enriched in ω: 3-PUFA have an obvious improved sight capacity, a higher neurological development and higher intellectual abilities (Bourre, 2005). Flood et al. (2007) initiated a study on the impact of the fatty acids on the eye diseases. Although the essential role of these acids, which are not synthesized in the human body, is established, the modern diets don't meet the daily requirement (Meyer et al., 2003).

The feeding value of the egg can be influenced by the formulation and composition of layer diets (Huyghebaert, 1995). Hen egg enrichment in ω: 3-PUFA, compared to the standard eggs, can be done by feeding the layers diets which include marine products (fish oil), vegetal oils (linen, canola, safflower), oleaginous seeds (linen, sunflower, canola) or by-products (wheat bran). Linseeds *(Linumm usitatissimum)* are one of the feed ingredients rich in alpha-linolenic acid (alpha-LNA). It is well established that the use of flax seeds in layer diets changes the fatty acids profile of the egg yolk (Caston and Leeson, 1990), particularly that of the linolenic acid. Ferrier et al. (1995) consider that a 10- 20% dietary inclusion rate of the linseeds produces a significant increase of the level of alpha-LNA and of docosahexaenoic acid (DHA) in the egg yolk. The inclusion level in layer diets should take into account that the linseeds contain non starch polysaccharides (mucilage) which increase the gust viscosity in monogastric animals, depressing thus nutrient availability (Novak and Scheideler, 2001).

The problem which arises when PUFA-rich feeds are used is how to maintain feeds quality because of their very high potential for oxidizing. This is why the PUFA-rich diets have to be supplemented with antioxidants. Alpha-tocopherol remains the most effective antioxidant to prevent PUFA depreciation, its level increasing with that of PUFA (Valk and Hornstra, 2000; Galobart et al., 2002).

The main goal of this study was to evaluate the potential of a layer diet with a moderate level (5%) of linseeds to produce ω: 3-PUFA-enriched (alpha-LNA and DHA) eggs and to monitor layer performance. The diets were treated with 250 ppm vitamin E and the eggs were used in a clinical study on a group of healthy volunteers at the National Institute of Endocrinology C. I. Parhon, Bucharest, Romania.

MATERIAL AND METHODS

The experiment was conducted on 108 Lohmann Brown layers (age 35-42 weeks) assigned to three groups with 12 replicates each (each replication consisted of a cage with 3 layers in it). The hens were housed on two tiers battery cages. Light bulb lighting was 16 hours every day (04:30-20:30) throughout the experimental period.

The layers had free access to the feed and water. The diet formulation (Table 1) was optimized with the mathematical model of Burlacu (1999). The diets for groups E1 and E2 differed from the control (C) diet by the inclusion of 5% linseed or 20.2% full fat soy. The diets for groups C and E1 were treated with 27 ppm vitamin E and the diet for group E2 was treated with 250 ppm vitamin E.

Table 1. Compound feeds structure

	C	E1	E2
Corn, %	32.12	27.06	27.06
Wheat, %	20	20	20
Peas, %	10	10	10
Gluten, %	3	3	3
Full fat soy, %	-	20.2	20.2
Soybean meal, %	20	3.2	3.2
Linseeds, %	-	5	5
Soybean oil, %	3.3	-	-
Monocalcium phosphate, %	1.2	1.2	1.2
Feed grade limestone, %	8.79	8.76	8.76
Salt, %	0.35	0.35	0.35
Vitamin-mineral premix*, %	1	1	1
Methionine, %	0.18	0.17	0.17
Choline, %	0.06	0.06	0.06
TOTAL	100	100	100
Analyzed			
Metabolisable energy, KJ/kg	11.87	13.05	13.05
Protein %	18.26	18.40	18.40
Fat %	5.70	7.02	7.02
Linoleic acid g% fat	58.15	55.13	54.97
Linolenic acid, g% fat	3.26	6.54	6.61
Vitamin E, ppm	27	27	280

* The premix for E2 diet provided 250 ppm supplemental vitamin E compared to 27 ppm vitamin E in the diet for C and E1 groups

All three diet formulations (Table 1) were isoproteic and in agreement with the feeding requirements recommended for the intensive rearing of this category of poultry. The differences between diets appear in the fat, energy and vitamin

E levels due to the supply of fat ingredients and to the addition of vitamin E (group E2). The diets of groups E1 and E2 had significantly higher levels of linolenic acid (Table 1) than the control group due to the use of 5% linseeds. The linseeds have 53.55% alpha-LNA fat. Full fat soy is a feed ingredient with a high level of PUFA but the higher content of linolenic acid in group C is due to the soybean oil (3%).

Feed intake, forage quality preservation in time, egg production, egg weight and egg components weight have been monitored throughout the experiment.

Feed samples were collected during the period of utilization of the first batches of compound feed (initial, 14 and 28 days from production) and analyzed for rancidity indices, peroxide index and Kreiss reaction.

When the layers were 35, 37, 39 and 42 weeks of age, 18 eggs per group were collected randomly every two weeks and average samples of egg yolk were formed (3 eggs/sample). The samples were dried at 65^0C and assayed for dry matter, protein, fat, ash, fatty acids profile and vitamin E concentration. pH measurement (one week after egg harvesting, the eggs being stored in a refrigerator at 4^0C) was made on liquid samples of egg yolk.

The following chemical methods were used:

- The standard Weende scheme – to assess the gross chemical composition (dry matter, protein, fat, fiber, ash)
- The fat acidity index (amount of KOH milligrams required to neutralize the free fatty acids contained in one gram of fat) was determined by titration with KOH 0.1 N of the free fatty acids contained in the fat solution dissolved in alcohol-chloroform solution.
- The peroxide indicator was determined volumetrically by treating the fat solution in an acid environment with potassium iodide. The iodine freed by peroxides was titrated with sodium thyosulphate.
- In the Kreiss reaction the epihydric aldehyde present in the rancid fat as acetate is released by treatment with HCl (or H2SO4) and combines with fluoroglucine, forming a red coloration whose intensity is proportional to the concentration of this aldehyde.
- pH was measured with a InoLab - Level 1 pH metre.
- Gas chromatography – the fatty acids were determined with a – PERKIN ELMER – Clarus 500 gas chromatograph fitted with injection system in the capillary column and flame ionization detector (FID). The capillary column was polar stationary phase BPX – 70, 60 m × 0.22 m.m.d.i. 0,25 μl film.
- Liquid chromatography – to determine vitamin E. A HPLC-Perkin Elmer was used: UV/VIS Series 200 detector; Hypersil gold -C18, 150 × 4.6mm-5UM column;

A clinical study was performed at the Institute of Endocrinology Parhon, Bucharest to evaluate the effects of these eggs on consumer health. The study involved 62 persons (45 women and 17 men) assigned to two groups: one groups consumed regular eggs and the other consumed ω: 3-PUFA enriched

eggs. Each subject consumed 6 eggs every week for six weeks. Throughout the study the subjects didn't change their feeding habits, so that the effects observed in the end were due entirely to the consumption of eggs. Blood samples were collected from each subject in the beginning of the experiment and assayed for the biochemical profile; leukocyte count; VSH; fibrinogen; inflammatory markers, CRP, TNFα, IL-6. The working protocol and the results were communicated by the team of the Parhon Institute (Manda et al., 2008)

The data were processed to characterise data string homogeneity (descriptive statistics), the significance of difference between data strings of similar nature (ANOVA test).

RESULTS AND DISCUSSION

Forage preservation in time (Table 2) shows that the peroxide index of E1 forage (1.81 ml thyosulphate/g fat) determined 28 days after manufacturing shows fat degradation, as also supported by the Kreiss reaction (positive). The acidity index has also been constantly higher in this group due to the inadequate level of dietary vitamin E (27 ppm).

Table 2. Forage preservation indices

	C	E 1	E 2
Peroxide index (ml thyosulphate 0.1 N/g fat)			
- initial	0.24	0. 76	0.1
- 14 days from manufacture	0.16	0.94	0.65
- 28 days from manufacture	1.02	1.81	1.09
Fat acidity (mg KOH / g fat)			
- initial	16.59	20.04	13.61
- 14 days from manufacture	18.51	25.21	15.14
- 28 days from manufacture	28.66	37.47	24.52
KREISS reaction			
- initial	Negative	Negative	Negative
- 14 days from manufacture	Negative	Negative	Negative
- 28 days from manufacture	Negative	Positive	Negative

The laying percentage and the feed intake were not influenced by the dietary linseed and full fat soybean treatment in diets E1 and E2 (Table 3). Ben and Leeson (2003), who conducted a long-term study on layers (28-53 weeks) with 10% linseeds in the diet, also observed that the laying percentage was not influenced by the diets enriched in ω -3 PUFA from linseeds. The same authors concluded, however, that the dietary 10% linseed level depressed the feed intake and decreased egg yolk weight. Novak and Scheideler (2001), in an other long-term (21-57 weeks) study, used the same 10% dietary linseed level

reported, however, a significantly higher ($p \leq < 0.04$) feed intake in the treated layers compared to the control group; they also reported that during weeks 49-57, the eggs from the layer treated with linseeds were heavier that the eggs from the control layers.

Our results on the feed intake, egg weight and egg components weight (Table 3) show no difference between the control group and the groups treated with 5% linseeds and 20.2% full fat soybeans. These results show that a supplement of just 5% linseeds didn't cause problems due to nutrient digestibility.

Table 3. Layer performance, average values/group

	C	E 1	E 2
Laying percentage (%)	83.08±8.966	83.55±8.208	85.26±6.75
Average daily feed intake, (g CF/layer/day)	142.56±11.07	143.92±8.144	144.53±11.015
Feed conversion ratio, (kg CF /kg egg)	2.181±0.02	2.205±0.047	2.207 ± 0.049
Average egg weight (g)	65.37 ± 0.601	64.93 ± 1.276	65.48 ± 1.476
- yolk, (g)	17.56 ± 0.482	17.45 ± 0.319	17.73 ± 0.433
- egg white, (g)	39.79 ± 1.206	39.70 ± 1.227	39.43 ± 1.135
- egg shell, (g)	7.59 ± 0.43	7.48 ± 0.354	7.82 ± 0.567

a – significant difference from C; b – significant difference from E1; c – significant difference from E2;

No significant differences were observed between groups concerning egg protein, ash and dry matter; differences were noted, as expected, concerning vitamin E concentration in the yolk (Table 4). The fat level was higher in the yolk of the eggs from groups E1 and E2 than in the eggs from group C, but the difference was not statistically significant.

Table 4. Physical-chemical parameters of the yolk (average values/ group)

	C	E1	E2
Dry matter 65^0 C	50.31 ± 1.388	50.43 ± 0.701	50.38 ± 0.768
Dry matter 105^0 C	96.22 ± 0.768	96.66 ± 0.61	96.52 ± 0.571
Crude protein *	31.26 ± 0.534	31.35 ± 0.381	31.818 ± 0.217
Ether extractives*	51.023 ± 0.844	52.32 ± 0.831	51.98 ± 0.523
Ash*	3.202 ± 0.180	3.188 ± 0.151	3.155 ± 0.159
pH- liquid yolk	6.12±0.014	6.225±0.013	6.115±0.026
Vitamin E**	1.133±0.55 [c]	1.236±0.58 [c]	11.611±2.86 [a, b]

* g%g DM 65^0C; ** mg /100 g DM 65^0C; a – significant difference from C; b – significant difference from E1; c – significant difference from E2;

The table above shows that the yolk from E2 eggs has about 10 times higher vitamin E concentration than in the yolk from control and E1 eggs, proportional to the dietary concentrations of vitamin E (Table 1). These results

show a directly proportional relation between the vitamin E concentration in the yolk and in the diet as observed by Melluzzi et al. (2000).

The supplemental 250 ppm vitamin E in group E2 diet might be too high for a level of just 5% dietary linseeds considering that egg white pH (9.24±0.076) in E2 eggs didn't differ from the pH of the egg white from group E1 (9.207±0.107) and group C (9.225±0.047) which had just 27 ppm vitamin E in the diet. The pH results might not be different between E1 and E2 because the determination was done after just one week of storage in a refrigerator. Grune et al. (2001) recommend a minimal supplement of 80 IU vitamin E/ kg for the layer diets enriched in polyunsaturated fatty acids in order to prevent egg fat oxidizing.

All the studies which present the effects of dietary linseeds (particularly the 10% level) in layer diets are in agreement on the fact that these diets produce a higher content of linolenic acid (C18:3n-3). We obtained the same result, but using just 5% dietary linseeds (Table 5).

Table 5. Egg fatty acids level (week 42 of life), g/100 g fatty acids, average values/group

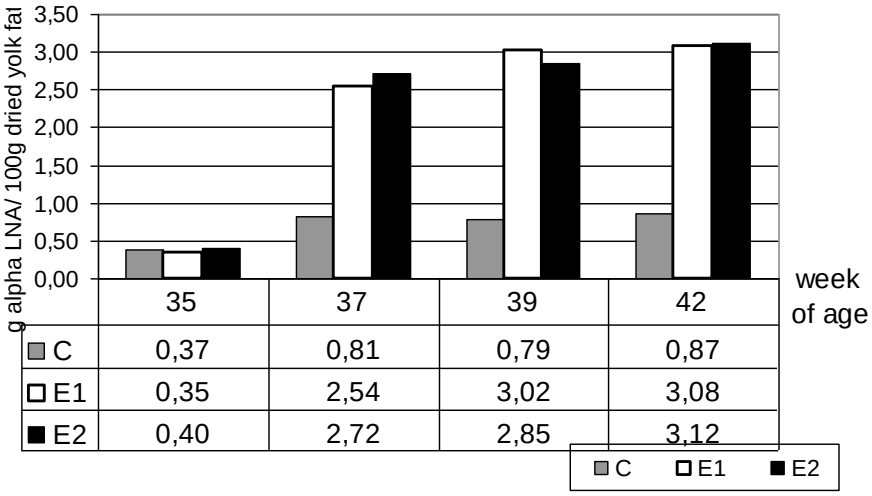
Fatty acids	C	E1	E2
Myristic (C14:0)	0.10 ± 0.113	0.14 ± 0.073	0.13 ± 0.069
Palmitic (C16:0)	21.59 ± 1.011	22.06 ± 1.072	22.24 ± 0.421
Palmitoleic (C16:1)	1.44 ± 0.736 [b,c]	2.09 ± 0.283 [a]	2.04 ± 0.21 [a]
Stearic (C18:0)	11.25 ± 3.048	10.78 ± .386	10.19 ± 1.268
Oleic (C18:1n9c)	31.53 ± 3.121 [b,c]	34.38 ± 1.613 [a]	34.02 ± 1.265 [a]
Linoleic (C18:2n6c)	27.61 ± 1.74 [b,c]	22.25 ± 1.19 [a,c]	23.61 ± 1.04 [a,b]
Linolenic (C18:3n6)	0.87 ± 0.282 [b,c]	3.08 ± 0.499 [a]	3.12 ± 0.451 [a]
Erucic (C22:1n9)	3.65 ± 1.086 [b,c]	2.70 ± 0.383 [a]	2.24 ± 0.402 [a]
Docosahexaenoic (C22:6n3)	1.70 ± 0.586 [b]	2.14 ± 0.397 [a]	2.12 ± 0.468
Other fatty acids.	0.06	0.00	0.00
Σ Saturated fatty acids	32.94	32.98	32.56
Σ Unsaturated fatty acids	66.96	67.03	67.44
Unsaturated. /Saturated	2.03	2.03	2.07
omega3/omega6	0.10	0.25	0.23

a – significant difference from C; b – significant difference from E1; c – significant difference from E2

Table 5 shows the fatty acids profile of the eggs harvested in the end of the experiment (42 weeks old hens). The values for group C are comparable to those reported by Hidalgo et al., (2008) who conducted a study (November – December 2005) of the characteristics of the eggs from hens reared under different housing systems, marketed in northern Italy. The unsaturated fatty acids, palmitoleic, oleic, alpha LNA and DHA are in significantly higher ($p \leq 0.05$) concentrations in the eggs from the experimental groups (E1 and E2) than in the eggs from the control group (table 5). The higher concentration of linoleic acid in the yolk from group C layers is explained by the presence of the

dietary soybeans oil (3.3%) in the diet of this group, the soybeans oil being rich in this essential acid (Panaite et al., 2006). The differences between groups in the yolk concentration of linolenic acid and alpha LAN in are in agreement with the differences between these fatty acids in the diets of the three groups. Omega 3 - PUFA to omega 6 - PUFA ratio was higher in the experimental groups than in the control group, but as Raes et al. (2002) show, the unsaturated to saturated fatty acids ratio is more difficult to manipulate that the individual acids. After 8 weeks of experiment, alpha LNA concentration in the egg was about 3,5 times higher in the experimental groups than in group C, while DHA concentration in the egg was about 1.2 times higher in the experimental groups than in group C. Ferrier et al. (1995) show that 10% and 20% dietary linseeds levels increased alpha LNA concentration and DHA concentration. The fatty acids profile of the experimental groups is comparable, which shows that the higher level of vitamin E (250 ppm) in group E2, didn't influence fatty acids metabolization. Melluzzi et al., 2000) consider that the dietary vitamin E level influence, however, slightly fatty acids profile

Fig 1 Time evolution of egg dried yolk alpha-linolenic acid concentration



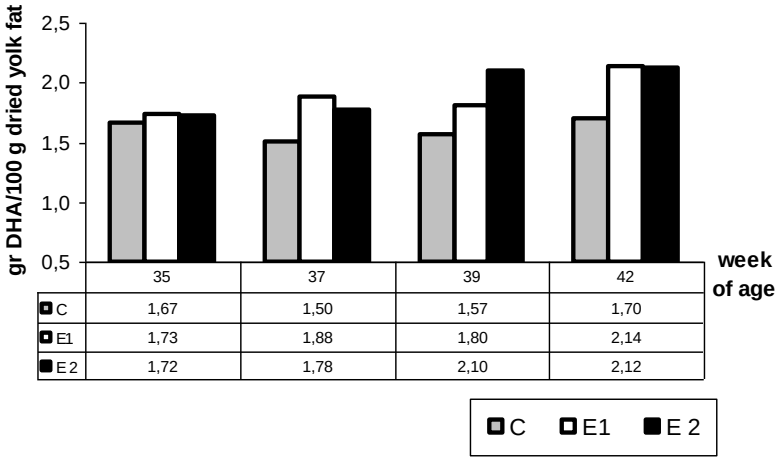
	35	37	39	42
C	0,37	0,81	0,79	0,87
E1	0,35	2,54	3,02	3,08
E2	0,40	2,72	2,85	3,12

Figure 1 shows that starting with week of age 37, alpha LNA concentration in the yolk of the eggs from the experimental groups was significantly ($p \leq 0.05$) higher than in group C. After a significant ($p \leq 0.05$) increase between the first two recordings (weeks of age 35 and 37), alpha LNA level in the eggs of experimental groups increase flattened between weeks of age 37 and 42. The time evolution of alpha LNA concentration in the yolk is modelled by third degree polynomial equations for all three groups. Compared to the measurement from the beginning of the experimental period (week of age 35) the final alpha

LNA concentration was 8.8 times higher for E1 and 7.8 times higher for E2, which exceeded our expectations.

DHA concentration in the yolk of eggs evolved in all groups according to third degree polynomial equations (Fig. 2), just like for alpha LNA. DHA level in the yolk of the eggs from the experimental groups started to be significantly ($p \leq 0.05$) higher than in group C as of the third experimental week. DHA concentration was 23.7% higher for group E1 and 23.25% higher for group E2, compared to the control group, in the end of the experiment than at the beginning of the experiment.

Fig. 2 Time evolution of egg dried yolk docosahexaenoic acid concentration



	35	37	39	42
C	1,67	1,50	1,57	1,70
E1	1,73	1,88	1,80	2,14
E 2	1,72	1,78	2,10	2,12

The above results show that a diet formulation with just 5% linseeds and 20.2% full fat soybeans produced ω: 3-PUFA enriched eggs. This is in agreement with Aymond and Van Elswyk (1995) who, using diets with 5% and 15% linseeds observed an increase of the ω:3-PUFA in the yolk of eggs. Caston and Leeson(1990) used 10, 20 and 30% dietary linseeds over a period of 28 days and observed increased concentrations of ω: 3-PUFA for all levels of supplementation..

Starting with week of age 37, the eggs from groups C and E2 were tested at the Parhon National Institute of Endocrinology, Bucharest, on a group of 62 volunteers who consumed 6 eggs per week for six weeks. The final data (Manda et al., 2008), show that the volunteers who consumed eggs from group E2 had a significantly lower ($p \leq 0.002$) triglycerides level at the final blood sampling (90,967± 10,437 mg/dl) than at the initial blood sampling (112,400 ± 13,215 mg/dl). Shapira et al. (2008), show that the high bioavailability of alpha ALA and DHA from the eggs is proved by the increased serum DHA in he

consumers, by the lower ω:3-PUFA to :6-PUFA ratio and by a significant decrease of plasma triglycerides.

CONCLUSIONS

- A dietary level of just 5% linseeds produced ω: 3-PUFA enriched eggs (alpha LNA si DHA) without affecting layer performance. The higher dietary energy levels didn't affect the feed intake or the laying percentage;
- The significant increase of alpha LNA in the egg influenced positively DHA level and ω:3-PUFA to :6-PUFA ratio in the yolk of the eggs from the linseed treated groups;
- The 250 ppm of vitamin E in the diet for group E2 preserved the quality of the compound feed and increased vitamin E concentration in the egg yolk.
- Linseeds are a rich and efficient feed ingredient for the production of ω: 3-PUFA enriched eggs; however, this fact is somehow ignored by the Romanian poultry producers.

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