B-5419
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RESEARCH ARTICLE Indian Journal of Animal Research

Dietary Inclusion of Linseed (*Linum usitatissimum*) Oil on Fatty Acid Profile of Egg Yolk in Layer Chicken

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10.18805/IJAR.B-5419

ABSTRACT

Background: Linseed contains exceptionally high content of alpha –linolenic acid (18:3, n-3) considered as unique among oilseeds. Depending upon the hens feeding, the nutrient composition of eggs varies. Egg is considered as a good source of quality protein and holds several vitamins and minerals that are important parts of a healthy diet. Due to its high nutritive value with appropriate price, eggs are taking place in one of the most accepted food in the diet of human.

Methods: A total of 156 numbers of 23 weeks age layer chickens (*Inbrobrown)* were randomly selected and divided into 4 treatment groups; having 3 replicates with 13 hens in each. The hens of control group (T_1) were fed a diet containing no linseed oil. The hens of treatment groups T_2, T_3 and T_4 were fed with a diet contained linseed oil at levels of 1.5%, 2.5% and 3.5 % respectively. Hens were fed the experimental diet for 18 weeks of experimental period (23 to 40 weeks of age). For fatty acid analysis 15 fresh egg samples per treatment were randomly collected at 30th and 40th week of age.

Result: Dietary supplementation of linseed oil increased (P< 0.01) omega 3 fatty acid and decreased the total SFA, MUFA and n6: n3 fatty acid ratio in egg yolk. Further, the hen day egg production was not affected by supplementation of linseed oil up to 3.5% although it lowered (P<0.05) the feed consumption. Production cost was highest in T_4 group and lowest in control group. Hence, it is concluded that from the point of omega 3 fatty acid content and economics of production supplementation of 2.5 % linseed oil in the diet of laying hens can be recommended as production of omega 3 fatty acid enriched egg.

Key words: Chicken, Egg yolk, Fatty acid, Layer, Linseed oil.

INTRODUCTION

Egg is considered as a good source of quality protein and holds several vitamins and minerals. The beneficial effects of omega-3 (n-3) polyunsaturated fatty acids (eicosapentaenoic and docosahexaenoic) acids on growth, health and immune function for humans is well established (Goyal *et al*., 2014; Lee *et al*., 2019). Therefore, supplementation of various n-3 fatty acids into the diet of laying hens has been a nutritional attempt to increase the levels of n-3 PUFA in the chicken eggs (Prakash *et al.,* 2023) and meat (Panda *et al.,* 2015). Fish oil is rich in n-3 PUFA, but there are negative reports of fishy odor eggs and contains heavy metals in eggs (Coorey *et al*., 2015). The dietary microalgae marketed as a source of n-3 PUFA, but the relatively higher cost and uneconomical (Fraeye *et al*., 2012). Linseed oil is cheaply available, which is attained from the dried, matured seeds of the flax plant. It contains exceptionally high content of alpha-linolenic acid (18:3, n-3), so it is considered as unique among oilseeds. As α- linolenic acid is one of two indispensable fatty acids obligatory in the human diet for upholding of good health, use of linseed oil in laying hens' diet to produce omega 3 PUFA-enriched egg is of interest of the researchers and poultry farmers to produce the bio fortified eggs to augment the niche marketing. Taking these aspects, the present study was undertaken to evaluate the effect of dietary inclusion of linseed oil in the diets of commercial layer chicken to determine the fatty acid profile in egg yolk.

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How to cite this article: Hazarika, H., Bhuyan, R., Hazarika, D., Mahanta, J.D., Saikia, B.N., Saharia, J. and Talukdar, P. (2024). Dietary Inclusion of Linseed (*Linum usitatissimum*) Oil on Fatty Acid Profile of Egg Yolk in Layer Chicken. Indian Journal of Animal Research. 1-6. doi:10.18805/IJAR.B-5419

Submitted: 06-06-2024 **Accepted:** 29-11-2024 **Online:**27-12-2024

MATERIALS AND METHODS

The study was conducted after approval from the Institutional Animal Ethics Committee (IAEC), AAU, Khanapara, vide approval No. 770/GO/Re/S/03/ CPCSEA/ FVSc/AAU/IAEC/ 21-22/908 dated 20.08.2022.

The feeding trial was carried out in the Instructional Poultry Farm, Department of Animal Husbandry and Dairying, College of Agriculture, Assam Agricultural

University, Jorhat from January 2022 to October 2022. A total of 156 numbers of 23 weeks of age (*Inbrobrown*) of layer chickens were randomly divided into 4 treatment groups, having 3 replicates with 13 hens in each. The hens of control group (T_1) were fed a diet with no linseed oil supplementation. The hens of treatment groups T_2, T_3 and T_4 were fed a diet supplemented with linseed oil (LO) at levels of 1.5%, 2.5% and 3.5% respectively. Hens were fed the experimental diet for 18 weeks of experimental period (23 to 40 week of age). Daily recording of eggs, feed consumption and collection of eggs were carried out throughout the experimental period. The experimental diets were prepared as per BIS (2007) standards. Experimental diets were analyzed for the proximate principle (AOAC, 2005). The samples were analyzed for calcium and phosphorus as per method described by Talapatra *et al*. (1940).

For fatty acid analysis 15 fresh eggs samples (5 samples per replicate) per treatment were randomly collected at 30th and 40th week of age. Samples of oil and experimental rations were also analyzed for fatty acid composition. The eggs were subjected extraction of fat and estimation of fatty acid composition using Gas chromatograph. Briefly, the CP-Sil 88 capillary column (50 m × 0.32 mm × 0.2 micron) was used at oven temperature (60°C, 1 min, 25°C/min to 220°C, 10 min) using flame ionization detector and detector temperature was 250°C (Araujo *et al*., 2010).

Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor and Cochran 1989). The means of different treatments were compared with Duncan's multiple range test (Duncan 1955). Significance was considered at P<0.05 levels.

RESULTS AND DISCUSSION

Fatty acid profile in linseed oil and different experimental diets

All the experimental diets of laying hens were iso-nitrogenous and iso-caloric (Table 1). The linolenic acid content (Table 2) in linseed oil was 50.72% and content was lowest in control diet and highest in 3.5% linseed oil supplemented diet. The results of fatty acid compositions in linseed oil were in good agreement with Oliveira *et al.* (2010) and Yi *et al*. (2014). On the other hand, Soliman and Afifi (2020) revealed that linolenic acid content in linseed oil is 54.38% which was higher than the present study and Altacli *et al*. (2022) reported that linseed oil contain 25% linolenic acid which was very much lower than the present study.

Table 1: Chemical composition of experimental diets.

Composition (%)	T_1 (without LO)	T_2 (1.5% LO)	T_3 (2.5% LO)	T_4 (3.5% LO)
CP	18.12	18.18	18.18	18.14
OM	92.47	92.47	92.44	92.43
EE	3.16	3.19	3.23	3.20
CF	5.10	5.96	6.10	6.15
Total ash	7.53	7.53	7.56	7.57
NFE	65.09	65.14	64.93	64.94
Total carbohydrate	70.19	71.10	71.03	71.09
Ca	3.04	3.07	3.09	3.07
P	0.57	0.54	0.54	0.59
ME* (kcal/kg)	2677.90	2684.95	2693.80	2692.85

*calculated value., LO: Linseed oil.

Table 2: Concentration of fatty acid (%) profile in linseed oil (LO) and different experimental diets.

Fatty acid (%)	Linseed oil	T_1(without LO)	T_2(1.5% LO)	T_3(2.5% LO)	T_4(3.5% LO)
Myristic acid (C 14:0)	0.08	0.63	0.43	0.31	0.28
Palmitic acid (C 16: 0)	6.69	16.66	15.36	12.18	11.90
Stearic acid (C 18 : 0)	5.26	7.87	7.15	6.93	6.76
Oleic acid (C 18:1)	21.83	32.36	28.60	28.02	27.10
Linoleic acid (C 18:2)	14.83	33.91	28.22	26.32	24.74
Linolenic acid (C18:3)	50.72	2.21	17.61	22.91	25.96
Arachidic acid (C 20:0)	0.42	0.77	0.43	0.45	0.48
Behenic acid (C 22:0)	-	1.87	0.68	0.68	0.61
Eicosapentaenoic acid (C20:5)	-	0.47	0.49	0.50	0.53
Docosahexaenoic acid (C 22: 6)	-	0.48	0.46	0.45	0.44
Palmitoleic acid (C 16:1)	0.13	0.96	0.53	0.50	0.50

Hen-day egg production and feed intake

The hen-day production (Table 3) was not found to be significant ($P>0.05$) among all the groups on supplementation of linseed oil at different levels in layers diet when compared to control group. Goncuglu and Ergun, (2004) observed that supplementation of linseed oil at a level of 1, 2, 3 or 4% of the diet had no effect on the laying performance of hens and egg production was similar in laying hens receiving linseed oil up to 4% which was in good agreement with the findings of present investigation. The feed intake (Table 4) was lower in the group supplemented with 3.5% level ($P<0.05$) linseed oil, however upto 2.5 % supplementation of linseed oil did not affect the feed intake. The reduced feed intake in T_4 group might be due to smell on inclusion of high level of oil. Promila *et al.* (2017) reported that feed intake was decreased on inclusion of 3%, 3.5 % and 4% level of linseed oil in hen's diet. Alaqil and Buhaya, (2022) found reduced feed intake on inclusion of linseed oil at 2, 3 and 4% level in hen's diet. However, feed consumption as well as feed conversion ratio was not affected by dietary incorporation of flaxseed oil up to 2 and 3% in broiler chickens (Panda *et al*., 2015 and Bharath *et al*., 2017).

Different fatty acid profile in egg yolk

The level of myristic acid, palmitic acid, stearic acid, oleic acid, palmitoleic acid and behenic acids were lower ($P<0.01$)

Table 3: Per cent hen-day egg production under different treatment groups.

Age in week	Treatment groups				SEM	P value
	T_1 (Control)	T_2 (1.5% LO)	T_3 (2.5% LO)	T_4 (3.5% LO)		
23 - 26	49.35	49.16	49.53	49.35	1.11	0.12
27-30	70.88	70.48	71.15	71.42	1.04	0.09
31-34	68.05	67.99	68.30	68.93	1.02	0.18
35-38	58.00	57.94	57.94	58.86	1.09	0.10
39-40	51.23	51.07	51.67	52.16	1.03	0.13
Mean	60.42	60.26	60.62	61.04	0.65	0.08

SEM; Standard error mean; Significant at $P\leq0.05$; Non significant at $P>0.05$.

Table 4: Daily feed intake (g/hen/day) under different treatment groups.

Age in weeks	Treatment groups				SEM	P value
	T_1 (Control)	T_2 (1.5% LO)	T_3 (2.5% LO)	T_4 (3.5% LO)		
23- 26	111.5	111.8	111.2	110.2	0.08	0.51
27-30	120.4^{a}	120.8^{a}	120.5^{a}	119.3^{b}	0.38	0.04
31-34	123.9^{a}	124.3^{a}	122.8^{b}	119.9^{b}	0.39	0.02
35-38	117.7^{a}	118^{a}	117.9^{a}	115.6^{b}	0.02	0.03
39-40	117^{ab}	118.1^{a}	118.8^{a}	116.1^{b}	0.01	0.02
Mean	118.1^{a}	118.7^{a}	118.1^{a}	116.3^{b}	0.27	0.02

SEM; Standard error mean; Significant at $P\leq0.05$; Non significant at $P>0.05$.

Table 5: Different fatty acid (%) profile in egg yolk of hens at 30th week of age under different treatments groups.

Fatty acid	Treatments groups				SEM	P value
	T_1 (Control)	T_2 (1.5% LO)	T_3 (2.5%LO)	T_4 (3.5% LO)		
Myristic acid (C 14:0)	0.29^{a}	0.28^{b}	0.26^{c}	0.24^{d}	0.001	< 0.01
Palmitic acid (C 16: 0)	27.69^{a}	26.92^{b}	26.01^{c}	25.19^{d}	0.003	<0.01
Stearic acid (C 18 : 0)	14.45^{a}	13.80^{b}	13.20^{c}	12.02^{d}	0.004	<0.01
Oleic acid (C 18:1)	31.37^{a}	27.98^{b}	27.03^{c}	26.50^{d}	0.038	<0.01
Linoleic acid (C 18:2)	12.27^{d}	13.79^{c}	13.89^{b}	14.79^{a}	0.003	<0.01
Linolenic acid (C18:3)	0.48^{d}	4.20^{c}	5.56^{b}	6.49^{a}	0.013	<0.01
Arachidic acid (C 20:0)	0.6^{a}	0.03^{c}	0.03^{c}	0.09^{b}	0.010	<0.01
Behenic acid (C 22:0)	5.89^{a}	4.36^{b}	4.26^{c}	3.70^{d}	0.004	<0.01
Eicosapentaenoic acid (C 20:5)	0.57^{d}	1.42^{c}	1.89^{b}	2.25^{a}	0.003	<0.01
Docosahexaenoic acid (C 22: 6)	1.43^{d}	5.65^{c}	6.17^{b}	6.88^{a}	0.004	<0.01
Palmitoleic acid (C 16:1)	2.31^{a}	1.53^{b}	1.47^{c}	1.40^{d}	0.004	<0.01

SEM; Standard error mean; Significant at $P\leq0.01$; Non significant at $P>0.01$.

in T_4 group compared to control group at both 30th and 40th week of age (Table 5 and 6). The present study was good agreement with Promila *et al*. (2017) and Omri *et al.* (2019) who also observed reduced myristic acid, palmitic acid, stearic acid, oleic acid, palmitoleic acid and behenic acid on inclusion of linseed oil or linseed in the layer diets. On the contrary, Petra *et al*. (2012) reported that myristic acid, palmitic acid, stearic acid and oleic acid increased on incorporation of 3% linseed oil. Highest linoleic acid concentration in egg yolk was found in T_1 group followed by T_2, T_3 and T_4 group. It might be due to decreased concentration of the oleic acid in the linseed incorporated diets. Previous studies by Shakoor *et al*. (2020) and Altacli *et al*. (2022) reports also support the results of the present study. Linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentration in the egg yolk was highest in T_4 group followed by T_3, T_2 group and lowest in control group at both 30th and 40th week of age. Increased EPA and DHA might be due to high amount of linolenic acid in the diet as because it is the precursors of EPA and DHA. It is established that the dietary supplementation of flax seed oil linearly increases the percentages of heptadecanoic acid, eicosatrienoic acid and DHA in egg yolks (Seyyed and Hasan, 2018). Further, Bean and Leeson, (2003) reported that brown hens deposited more (P<0.01) DHA into their eggs, whereas white hens deposited more LNA into their eggs.

The omega 3 fatty acid content (Table 7 and 8) and Poly Unsaturated Fatty Acid were significantly (P<0.01) higher in T_4 group followed by T_3 and T_2 group and lowest in T_1 group at both 30th and 40th week of age. It was revealed that inclusion of different levels of linseed oil in the diet of laying hens increased the omega 3 fatty acid content in the egg yolk. Significant reduction of omega 6: omega 3 fatty acid was observed in the groups on supplementation of different levels of linseed oil. It was highest (P<0.01) in T_1 group followed by T_2, T_3 and lowest in T_4 group at 30th and 40th week of age. Similar findings were also observed by Keten (2019) and Petra *et al*. (2012) who also reported that the addition of linseed oil to the diet of laying hens increases the content of n- 3 PUFAs, such as linolenic acid, EPA and DHA. Further, the value total Saturated Fatty

Table 6: Different fatty acids (%) profile in egg yolk of hens at 40th week of age under different treatments groups.

Fatty acid	Treatments groups				SEM	P value
	T_1 (Control)	T_2 (1.5% LO)	T_3 (2.5% LO)	T_4 (3.5% LO)		
Myristic acid(C 14:0)	0.27[a]	0.25[b]	0.24[c]	0.23[d]	0.002	<0.01
Palmitic acid (C 16:0)	28.72[a]	27.14[b]	26.71[c]	26.36[d]	0.004	<0.01
Stearic acid (C 18 : 0)	13.83[a]	13.79[b]	13.70[c]	13.52[d]	0.004	<0.01
Oleic acid (C 18:1)	33.77[a]	30.72[b]	29.94[c]	28.93[d]	0.004	<0.01
Linoleic acid (C 18:2)	10.93[d]	11.07[c]	11.26[b]	11.96[a]	0.003	<0.01
Linolenic acid (C18:3)	0.32[d]	3.23[c]	4.75[b]	5.39[a]	0.003	<0.01
Arachidic acid (C 20:0)	0.11[a]	0.06[b]	0.07[b]	0.10[a]	0.002	<0.01
Behenic acid (C 22:0)	5.54[a]	4.39[b]	2.71[c]	2.57[d]	0.003	<0.01
Eicosapentaenoic acid (C 20:5)	0.36[d]	1.23[c]	1.36[b]	2.01[a]	0.002	<0.01
Docosahexaenoic acid (C 22: 6)	1.34[d]	5.57[c]	6.26[b]	6.33[a]	0.003	<0.01
Palmitoleic acid (C16:1)	2.39[a]	2.19[b]	2.11[c]	1.78[d]	0.003	<0.01

SEM; Standard error mean; Significant at P≤0.01; Non significant at P>0.01.

Table 7: Total SFA (%), PUFA (%), MUFA (%), Omega 3 PUFA and Omega 6:3 PUFA in egg yolk of hens at 30th week of age under different treatment groups.

Treatment groups	Total SFA	Total MUFA	Total PUFA	Omega 3 PUFA	Omega 6:3 PUFA
T_1 (Control)	48.92[a]	33.68[a]	14.75[d]	2.48[d]	4.95[a]
T_2 (1.5% LO)	45.39[b]	29.51[b]	25.06[c]	11.27[c]	1.22[b]
T_3 (2.5%LO)	43.76[c]	28.50[c]	27.51[b]	13.62[b]	1.01 [c]
T_4 (3.5% LO)	41.24[d]	27.90[d]	30.41[a]	15.6[a]	1.00[d]
SEM	0.002	0.002	0.002	0.020	0.003
P value	<0.01	<0.01	<0.01	<0.01	<0.01

SEM; Standard error mean; Significant at P≤0.01; Non significant at P>0.01.

NB:

Total SFA = Sum of myristic acid, palmitic acid, stearic acid, arachidic acid and behenic acid.

Total MUFA = Sum of oleic acid and palmitoleic acid.

Total PUFA = Sum of linoleic acid, linolenic acid, EPA and DHA.

Omega 3 PUFA = linolenic, EPA and DHA.

Omega 6 PUFA = linoleic acid.

Table 8: Total SFA (%), PUFA (%), MUFA (%), Omega 3 PUFA and Omega 6:3 PUFA in egg yolk of hens at 40th week of age under different treatment groups.

Treatment groups	Total SFA	Total MUFA	Total PUFA	Omega 3 PUFA	Omega 6:3 PUFA
T_1 (Control)	48.47[a]	36.16[a]	12.95[d]	2.02[d]	5.41[a]
T_2 (1.5% LO)	45.63[b]	32.91[b]	21.10[c]	10.03[c]	1.10[b]
T_3 (2.5%LO)	43.43[c]	32.05[c]	23.63[b]	12.37[b]	1.00[c]
T_4 (3.5% LO)	42.78[d]	30.71[d]	25.69[a]	13.73[a]	0.90[c]
SEM	0.002	0.003	0.002	0.005	0.002
P value	<0.01	<0.01	<0.01	<0.01	<0.01

SEM; Standard error mean; Significant at P≤0.01; Non significant at P>0.01.

NB:

Total SFA = Sum of myristic acid, palmitic acid, stearic acid, arachidic acid and behenic acid.

Total MUFA = Sum of oleic acid and palmitoleic acid.

Total PUFA = Sum of linoleic acid, linolenic acid, EPA and DHA.

Omega 3 PUFA = linolenic, EPA and DHA.

Omega 6 PUFA = linoleic acid.

Acid (SFA) and Mono Unsaturated Fatty Acid (MUFA) content in the egg yolk was higher (P<0.01) in T_1 group and lowest in T_4 group in both 30th and 40th week of age. Similar findings also reported by Promila *et al*. (2017) that total SFA were decreasing with addition of linseed oil in the diet of laying hens.

Cost of production

Local available feed materials were used for formulation of different diets. Costs of diet per kg for different experimental diets were (Rs.) 32.16, 34.97, 37.57 and 40.37 for T_1, T_2, T_3 and T_4 diet respectively. Production cost of one egg for different treatment groups were (Rs.) 7.80, 8.67, 9.08 and 9.69 for T_1, T_2, T_3 and T_4 respectively.

CONCLUSION

Supplementation of different levels (1.5%, 2.5% and 3.5%) of linseed oil increased omega 3 fatty acid and total PUFA concentration and decreased the total SFA, total MUFA concentration and n6:n3 fatty acid ratio in egg yolk at both 30th and 40th week of age. Further, supplementation of linseed oil up to 3.5% did not affect the hen day egg production although it lowered the feed consumption. Hence, it is concluded that from the point of omega 3 fatty acid content and economics of production supplementation of 2.5% linseed oil in the diet of laying hens can be recommended as production of omega 3 fatty acid enriched eggs.

Conflict of interest

All authors declare that they have no conflicts of interest.

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