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Flaxseed Oil for Omega 3 Fatty Acids Enrichment in Eggs and Performance in Laying Chickens

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ABSTRACT

Background: The beneficial effects of omega-3 fatty acids on growth, health and immune function for human is well established. Supplementation of various n-3 fatty acids into the diet of laying hens is a nutritional attempt to increase the levels of n-3 PUFA in the eggs. Therefore, the present study was carried out to determine the effect of dietary supplementation of flaxseed oil (SFO) as a source of omega-3 polyunsaturated fatty acids, on the fatty acid composition of egg, laying performance and egg stability upon storage in laying hens.

Methods: Laying hens of 30-week age (n=168) were selected and randomly distributed into two dietary groups consisting of 14 replicates each having 6 birds in each replicate. Iso-nitrogenous and iso-caloric two diets were prepared with and without FSO. Daily recording of eggs, feed intake and egg collection was carried out throughout the experiment. The eggs were subjected for fat extraction and estimation of fatty acid composition.

Result: The higher (P<0.05) feed intake and lower (P<0.05) egg production in groups fed FSO supplemented diet compared to those groups fed control diet. Further, improved (P<0.05) feed conversion ratio among the groups fed control diet compared to those groups fed FSO supplemented diet. Eggs contained higher (P<0.05) α-linolenic acid, eicosenoic acid, n3 fatty acids in groups fed FSO supplemented diets compared control diet. The stability parameters did not differ by supplementing FSO compared to groups fed control diet. Therefore, it is concluded that FSO can be supplemented up to 6% for feeding of laying hens for producing higher omega 3 fatty acids in eggs. Further, supplementation of 6% FSO did not affect the egg quality parameters upon storing for 21 days at 4°C.

Key words: Chicken eggs, Flaxseed oil, Omega-3 fatty acids.

INTRODUCTION

The beneficial effects of omega-3 (n-3) polyunsaturated fatty acids (PUFA) 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 (Oliveira *et al*., 2010) and meat (Panda *et al*., 2015). Dietary supplements for n-3 PUFA are fish oil, flaxseed, or microalgae (Alagawany *et al*., 2019) have been extensively studied. Though fish oil is rich in n-3 PUFA, but there are negative reports that laying hens fed with fish oil-added diet produced fishy odor eggs and contains heavy metals in eggs (Coorey *et al*., 2015). The dietary microalgae have been recently marketed as a source of n-3 PUFA, but the relatively higher production cost and uneconomical (Fraeye *et al*., 2012). The dietary flaxseed oil has been used to produce n-3 enriched eggs or meats in the poultry industry (Oliveira *et al*., 2010; Petrovic *et al*., 2012). Nevertheless, most of the studies shows that the supplementation of SFO is lower, even less than 1% in the diet (Lee *et al*., 2021). However, it has been reported that the supplementation of FSO linearly increases the omega 3 fatty acids in eggs. Further, as PUFA in layer diets increases the susceptibility of eggs to lipid oxidation (Cherian *et al*., 2007) increases. Therefore, the present study was conducted to determine the performance, egg stability and fatty acid content of eggs in laying hens fed higher quantity FSO.

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MATERIALS AND METHODS

White Leghorn layers (F-line) 30-week age (n=168) were housed and randomly distributed into two dietary groups consisting of 14 replicates each having 6 birds in each replicate. The experiment was carried out from 33 to 40 weeks of age at ICAR-Directorate of Poultry Research, Hyderabad from August 2021 to February 2022. Iso-

nitrogenous and iso-caloric two diets were prepared with and without FSO (6%; Table 1). Daily recording of eggs, feed intake and egg collection was carried out throughout the experiment. The crude protein levels in both the diets were analysed by the Kjeldahl method after acid hydrolysis using AOAC (2005).

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 µm) was used at oven temperature (60°C, 1 min, 25°C/min to 160°C, 28 min, 25°C/min to 190°C, 17 min, 25°C/min to 220°C, 10 min) using flame ionization detector and detector temperature was 250°C (Araujo *et al*., 2010). The FSO and oil extracted from egg yolk was subjected for estimation of fatty acid composition. Further, the eggs were stored during the experiment at weekly interval (14 eggs from each treatment, each from each of the replicate) for estimation of egg quality parameters of eggs (stored for 1, 2 and 3 weeks) during the experiment for determining the egg weight, density, Hough unit, shell weight and shell thickness.

The variations in data of different parameters were analyzed using one-way analysis of variance procedure of SAS version 9.2 (2008; SAS Institute Inc., Cary, North Carolina, USA). The model included the different treatments were designated as the fixed factors and response variables were taken as the dependent variables.

RESULTS AND DISCUSSION

The FSO was subjected for estimation of fatty acid composition and was fond to have contain α-linolenic acid (C18:3n-3) (52.52%), Linoleic acid (C18:2n-6) (14.27%), Oleic acid (C18:1n-9) (21.13%), Stearic acid (C18:0) (5.58%) and Palmitic acid (C16:0) (6.15%) of total fatty acids in FSO. The values of fatty acids recorded in FSO in the present study are in agreement with the reported values of Lee *et al.* (2021).

The feed intake was higher (P<0.05) in FSO supplemented groups compared to those groups fed control diet (Table 2 and 3) during both the periods (33-36 and 37 to 40 weeks). Whereas, significantly lower hen day egg production (P<0.05) was recorded in FSO supplemented groups compared to those groups fed control diet. Improved (P<0.05) feed conversion (FCR) ratio among the groups fed

Table 1: Ingredient and nutrient content of experimental diets.

Ingredient (kg/100 kg)	Control	Flaxseed oil
Maize	61.4	35.3
Soybean meal	24.6	21.7
De-oiled rice bran	0.20	23.1
Limestone powder	11.5	11.5
Dicalcium phosphate	1.49	1.43
Salt	0.43	0.41
DL-Methionine	0.09	0.11
L-Lysine	0.00	0.05
Premix*	0.35	0.35
Flax seed oil	0.00	6.00
Nutrient menu		
M.E (kcal/kg)	2618	2618
Protein (%)	16.8	16.8
Lysine (%)	0.83	0.83
Methionine (%)	0.35	0.35
Calcium (%)	4.08	4.08
NPP (%)	0.36	0.36
Sodium (%)	0.18	0.18
Linoleic acid (%)	1.28	1.75

*Premix contained trance minerals 0.10 kg/100 kg, Choline chloride 0.10 kg/100 kg, Toxin binder 0.10 kg/100 kg, Vitamin premix 0.05 kg/100 kg.

Table 2: Effect of feeding diet containing flaxseed oil on feed intake and egg production in IWF line during 33-36 weeks.

Treatment	33 Week			34 Week			35 Week			36 Week		
	EP	FI/b/d	FCR	EP	FI/b/d	FCR	EP	FI/b/d	FCR	EP	FI/b/d	FCR
Control	89.6	100.8^{b}	2.31	86.6	104.9	2.38	92.3^{a}	107.1^{b}	2.25^{b}	90.0^{a}	110.1^{b}	2.41^{b}
FSO	88.9	106.0^{a}	2.48	88.7	107.9	2.49	86.2^{b}	117.1^{a}	2.76^{a}	81.6^{b}	116.2^{a}	2.93^{a}
SEM	1.27	1.380	0.05	1.11	1.606	0.05	1.32	1.991	0.07	1.75	1.691	0.07
P Value	0.78	0.05	0.13	0.34	0.36	0.28	0.02	0.01	0.01	0.01	0.07	0.01

FSO; Flaxseed oil; EP; Egg production; FI; Feed intake; SEM; Standard error mean; FCR; Feed conversion ratio (kg FI /kg Egg mass); Significant at P≤0.05; NS- Non significant at P>0.05.

Table 3: Effect of feeding diet containing flaxseed oil on feed intake and egg production in IWF line during-40 weeks.

Treatment	37 Week			38 Week			39 Week			40 Week		
	EP	FI/b/d	FCR	EP	FI/b/d	FCR	EP	FI/b/d	FCR	EP	FI/b/d	FCR
Control	90.54	106.0	2.36^{b}	86.96	105.1^{b}	2.46	86.96	100.5^{b}	2.29	87.9	104.5^{b}	2.30^{b}
FSO	84.82	111.1	2.66^{a}	90.72	112.8^{a}	2.59	89.64	112.7^{a}	2.49	87.9	113.4^{a}	2.57^{a}
SEM	1.714	1.695	0.07	1.547	1.785	0.06	1.578	2.565	0.08	1.288	1.756	0.05
P Value	0.09	0.13	0.04	0.23	0.02	0.29	0.40	0.01	0.24	0.98	0.01	0.01

FSO; Flaxseed oil; EP; Egg production; FI; Feed intake; SEM; Standard error mean; FCR; Feed conversion ratio (kg FI /kg Egg mass); Significant at P≤0.05; NS- Non significant at P>0.05.

control diet compared to those groups fed FSO supplemented diet. It is well established that the fat is used to decrease dustiness and enhance energy density in poultry diets. However, supplementation of flaxseed oil at 4 per cent improved the body weight gain and feed efficiency of broiler chickens with no adverse effects on carcass parameters

Table 4: Effect of flaxseed oil in the diet of layer birds on egg fatty acid composition.

Fatty acid composition, %	Experimental diets		SEM	P Value
	Control	FSO		
Myristic acid (14:0)	0.394	0.351	0.01	0.22
Palmitic acid (16:0)	26.70	24.85	0.82	0.27
Palmitoleic acid (16:1)	4.935	4.597	0.14	0.25
Heptadecanoic acid (C17:0)	0.181	0.215	0.02	0.48
Cis-10-Heptadecenoic acid (C17:1)	0.218	0.304	0.03	0.16
Stearic acid (18:0)	7.039	6.663	0.28	0.51
Oleic acid (18:1)	46.64	44.92	0.81	0.29
Linoleic acid (18:2 n-6)	12.00	13.47	0.57	0.19
á-Linolenic acid (18:3 n-3)	0.875[b]	3.981[a]	0.50	0.01
Eicosenoic acid (20:1)	0.252[a]	0.103[b]	0.03	0.01
Docoshexaenoic acid (22:6 n-3)	0.712	0.536	0.05	0.07
TSF (14:0+16:0+17:0+18:0)	34.31	32.07	1.10	0.31
Total MUSF(16:1+17:1+18:1+20:1)	52.04	49.09	0.85	0.22
Total n-6 polyunsaturated fatty acids	12.00	13.47	0.56	0.19
Total n3 fatty acids	1.839[b]	4.612[a]	0.48	0.01
Ratio n6:n3	9.030[a]	5.190[b]	0.85	0.02

TSF; Total saturated fat; MUSF; Mono unsaturated fat; FSO; Flaxseed oil; Significant at P≤0.05; NS- Non significant at P>0.05.

Table 5: Effect of flaxseed oil on egg quality parameters that were stored up to 21 days in refrigerator at 4°C.

Days of storage/Egg quality parameters	Experimental diets		SEM	P Value
	Control	FSO		
			0 Day	
Egg wt. g	50.9	49.2	0.53	0.10
Density g/cm^3	1.08	1.08	0.01	0.31
HU	67.8	70.9	1.53	0.33
Shell wt g	4.70	4.71	0.09	0.97
Shell thick, mm	0.39	0.38	0.01	0.26
			7 Day	
Egg wt. g	51.6	49.5	0.96	0.27
Density g/cm^3	1.08	1.08	0.01	0.64
HU	68.3	65.1	3.12	0.61
Shell wt g	4.65	4.66	0.08	0.95
Shell thick, mm	0.40	0.41	0.01	0.76
			14 Day	
Egg wt. g	50.9	48.6	0.78	0.14
Density g/cm^3	1.08	1.08	0.01	0.88
HU	72.9	71.8	1.59	0.74
Shell wt g	4.66	4.75	0.08	0.60
Shell thick, mm	0.38	0.39	0.01	0.44
			21 Day	
Egg wt. g	48.6	48.3	0.55	0.82
Density g/cm^3	1.06	1.05	0.01	0.07
HU	62.9	61.0	1.96	0.63
Shell wt g	4.83	4.54	0.10	0.15
Shell thick, mm	0.40	0.39	0.01	0.24

FSO; Flaxseed oil; HU: Hough unit; SEM: Standard error mean.

(Bharat *et al*., 2017). The increased feed intake and reduced hen day egg production in laying hens at higher levels of FSO (4.5%) supplementation is also reported by Promila *et al*. (2017). However, the body weight gain, feed consumption as well as feed conversion ratio are not affected by dietary incorporation of FSO up to 2 and 3% in broiler chickens (Panda *et al*., 2015).

Egg yolk contained significantly (P<0.05) higher α-linolenic acid, eicosenoic acid, n3 fatty acids in groups fed FSO supplemented groups. The ratio of n6 to n3 decreased (P<0.05) significantly in groups fed FSO supplemented diets compared to those groups fed control diet (Table 4). It is established that the dietary supplementation of FSO linearly increases the percentages of heptadecanoic acid, eicosatrienoic acid and DHA in egg yolks (Seyyed and Hasan, 2018; Lee *et al*., 2021). Similar to our findings, the ratio of n-6 to n-3 fatty acids in egg yolks decreases with increase the supplemental levels of FSO in the diets (Lee *et al*., 2021). The main biological role of α-linolenic acid is to serve as a substrate for the synthesis of EPA and DHA (Liang *et al*., 2017). Laying hens have the ability to elongate and desaturate α-linolenic acid to the functional EPA and DHA (Ehr *et al*., 2017). Therefore, it is evident from the present study that the feeding diets supplemented FSO, which is rich in á-linolenic acid resulted in higher content of EPA and DHA.

In present experiment, none of the egg stability parameters were differed by supplementing FSO compared to those group fed control diet up to 21 days of storage in the refrigerator at 4°C (Table 5). PUFA rich eggs are more susceptible to oxidation as PUFAs have several double bonds (Wang *et al.,* 2017). It has been reported that the lipid oxidation is a process that affects egg yolk lipid stability during storage (Faitarone *et al.,* 2016; Omri *et al.,* 2019) and affects egg quality parameters (Omri *et al.,* 2019). However, the egg quality parameters did not vary and remained comparable with those groups fed control diet.

CONCLUSION

Flax seed oil can be supplemented up to 6% for feeding of laying hens for producing higher omega 3 fatty acids in eggs. Further, supplementation of 6% flax seed oil did not affect the egg quality parameters upon storing for 21 days at 4°C. Supplementation of 6% flax seed oil did not affect the egg production. However, the higher feed intake and poor feed efficiency was recorded in laying hens fed flax seed oil supplemented diet.

Conflict of interest: None.

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