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The Effect of Different Concentrations of Safflower Seed on Laying Hen's Performance, Yolk and Blood Cholesterol and Immune System

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Abstract: Ninety-six 26-wk-old white leghorn layers (w-36 variety) were fed commercial diets containing 0, 4, 7 and 10% safflower seed (SS) and the diets were iso-caloric and iso-nitrogenous. Hens randomly assigned to 4 treatment diets, with 3 replicates and 8 layers in each replicate. The experiment was conducted over a period of 12 wk in order to study the effects of feeding safflower seed on hen performance (egg production, egg weight, egg mass, feed intake, feed conversion ratio (FCR) and weight gain) and egg quality parameters (Hough unit score, yolk color index, yolk index, egg shape, shell weight, shell thickness and density). The yolk of eggs extracted and cholesterol content were determined in each period. Blood samples were collected in non-heparinised tubes from six hens in each treatment by puncturing the bronchial vein in end of experiment and serum was collected after 8-10 hrs as per standard procedures and was stored for subsequent analysis. Hen performance and egg quality parameters were not significantly ($p>0.05$) different among treatments except in specific gravity and body weights. The lowest specific gravity observed in 10% SS (1.0806) and the highest gain weight was in 4% SS (140g) treatment. However the lowest Hough unit and shell thickness observed in 10% SS but the different among treatments were not statistically significant. The mean yolk cholesterol content, blood cholesterol and antibody titer against ND and IBD were not significantly ($p<0.05$) different among treatments but the lowest yolk and blood cholesterol observed in 10 % SS.

Key words: Safflower seed, cholesterol, egg quality, laying hen, egg production

Introduction

Safflower (*Carthamus tinctorius* L.) is an annual, broadleaf oilseed crop adapted chiefly to the small grain production areas of the western Great Plains. Evaluations of safflower in the Great Plains states began in 1925, but the seed had an oil content that was too low for profitable oil extraction. In the following years the Nebraska Agricultural Experiment Station developed varieties with about 35% oil compared to older varieties with less than 30%. Commercial production became concentrated in western Nebraska and eastern Colorado, but is now located in several Western states and Canadian Prairie provinces. California grows approximately 50% of the safflower in the U.S.A., while North Dakota and Montana and grow most of the remaining domestic production. South Dakota, Idaho, Colorado and Arizona also produce safflower, but with much smaller acreages.

Linoleic acid predominated in every lipid class during the whole period of seed development of safflower, while linolenic acid decreased with increasing maturation and it was not detected in mature seeds. Just before the initiation of triacylglycerol accumulation, the fatty acid composition of triacylglycerols changed more rapidly than those of phospholipids and glycolipids

(Ichihara and Noda, 1980). Heinonen *et al.* (1992) reported when the main fats in the rations was safflower, the principal sources of dietary fat in the average Finnish daily diet were edible fats (44%), dairy products and eggs (34%) and meat (15%). The P/S ratio was 0.20 and the ratio of n-6 to n-3 was 4.6. The amount of trans-fatty acids in the average daily diet was 1.7 g, the main source (79%) being edible fats.

The growth response to the safflower meal was increased by the addition of synthetic lysine but the performance of pigs receiving these lysine enriched safflower meal supplements was always inferior to those fed either isonitrogenous soybean meal or fishmeal supplements in both sorghum and wheat based diets. Increasing safflower protein contribution in the safflower meal fishmeal diets resulted in poorer feed conversion ratios and growth rates, particularly when fed to pigs less than 45 kg live weight. (Williams and Danils, 1973).

The substitution of equal amounts of egg yolk high in linoleic acid (produced by hens whose diet contained a large amount of safflower oil) did not result in plasma cholesterol levels which were significantly different than those observed during the intake of egg yolk of average fatty acid composition. (Splitter *et al.*, 1968).

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Table 1: Percent ingredients and nutrient compositions of experimental diets

Ingredients (%)	Composition			
	0% SS	4% SS	7% SS	10% SS
Corn %	66.43	60.42	60.6	58.11
Soybean meal %	20.10	20.05	19.15	18.34
Wheat %	1	3.66	1	1
Safflower seed (SS) %	0	4	7	10
Limestone	8.5	8.5	8.48	8.49
Fish meal %	2	2	2	2
Mineral premix	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
DL-Methionine	0.07	0.08	0.08	0.08
Lysine	0.07	0.07	0.09	0.11
Sodium chloride	0.31	0.32	0.34	0.35
Calculated analysis				
Metabolizable Energy, Mcal/kg	2.82			
Protein %	16.10			
Lysine %	0.84			
Methionin %	0.36			
Met + Cys	0.66			
Nonphytate phosphorous %	0.39			
Calcium%	3.70			
Sodium%	0.17			

*Each Kilogram contains Vit. A (8800000 IU), Vit.B1 (1.477 gr), Vit.B2 (4 gr), Vit.B3 (7.84 gr), Vit.B6 (2.46 gr), Vit. B12 (0.01gr), Vit.D3(2500000 IU), Vit.E(11000 IU), Vit. K3 (220 gr), Folicin (0.25 gr), and Biotin (0.15 gr). *Each Kilogram contains Manganese Oxide (74.4 gr), Ferric Oxide (75 gr) Zinc Oxide (64.675), Cupper Sulphate (6 gr), Selenium Pre Mix (0.2 gr), Calcium Iodate (0.32gr), and Choline Chloride (200 gr).

Materials and Methods

Animals and diets: Ninety-six 26-wk-old white leghorn laying hens (w-36 variety) were housed in cages and were assigned (24 hens per treatment) to four experimental diets (0, 4, 7 and 10% safflower seed). The compositions of experimental diets were shown in Table 1.

The hen's wings numbered and were housed in cages where the temperature and the light were controlled and fed on the experimental diets from 26 to 40 weeks of age. Four diets containing 0, 4, 7 and 10 percent safflower seed were fed for three periods of each 28 days and the first two weeks were for adjustment. Hens were maintained on 16:8 h light: dark cycle and all the diets were iso-caloric and iso-nitrogenous according to NRC (1994). Feed and water were supplied *ad libitum*. The ingredients and chemical composition of the treatment diets are shown in Table 1.

Egg production and egg quality measurements:

Performance data were collected during 12 weeks experimental period. Egg production was recorded daily, feed consumption, egg weight, egg mass and feed conversion ratio (FCR) were recorded every week and body weight gain, antibody titter against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) were recorded at the end of experimental period. For determining of egg quality parameters (Hough unit score, yolk colour index "as measured by Roche yolk colour fan", yolk index, egg shape, shell weight, shell

Table 2: Effect of dietary safflower seed on egg production (%)

SS level%	0% SS	4% SS	7% SS	10% SS	S.E.M.
First period	91.52	90.63	91.61	90.71	2.69
Second period	92.11	90.18	92.74	88.85	2.48
Third period	88.69	88.25	90.38	85.24	2.63
Mean of period	90.77	89.86	91.90	88.12	2.19

Means within each column do not having superscript do not differ significantly (P<0.05).

thickness and specific gravity), three eggs produced by each hen were recorded for 3 consecutive every 28 days. The specific gravity determined with 10 pots containing water with different density (1.06-1.099 mg/cm³). The yolk of eggs separated from white and the fat in yolk was extracted in present of organic solvent and yolks cholesterol was determined by spectrophotometer every 28 days. Blood samples were collected in non-heparinised tubes from six hens in each treatment by puncturing the bronchial vein in end of experiment and serum was collected after 8-10 hrs as per standard procedures and was stored for subsequent analysis.

Statistical analysis: All data were analyzed as a completely randomized design (repeated measurement) by using the general linear models (GLM) procedure of SAS[®] software (SAS Institute Inc., 1991). Duncan's multiple Range test was employed to compare different means at P<0.05.

Results and Discussion

The results on egg production in 3 periods and means of 3 periods for each treatment were shown on Table 2. There was not statistically different among treatments (P<0.05) but the minimum numerically egg production was observed on 10% SS (88.12). However the other performance parameters such as feed intake, egg weight and FCR which were as average 93.57, 89.98, 57.47 and 1.79 respectively did not showed any statistically significant effect. But the maximum gain body weight observed in 4% SS (140) and this increase was statistically significant (P<0.05).

The effect of safflower seed on egg weight was not significantly different and the data represented on Table 3. This result are not agreement with study of Wang 1996, which reported increasing of egg weight with used 8% of safflower oil.

There was a significantly different on egg mass among treatments. The 10% safflower seed showed lowest value (48.49) which was significant with control but non significant with other levels of safflower seed. This reduction in egg mass is due to lower egg production in 10% safflower seed (Table 3).

The FCR and feed intake were not affected with safflower seed and the higher FCR and lowest feed intake were observed in 10% safflower seed 1.82, 90.18 (g/day) respectively (Table 4). This effect may be due to higher crude fibber in safflower seed diet and consequently decreasing density of diet.

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Table 3: Effect of dietary Safflower seed on egg mass production and egg weight

Egg weight (g)				Egg mass (g/day)				SS level%
	Third period	Second period	first period	Mean	Third period	Second period	first period	
Mean								
57.73	60.24	58.11	54.84	52.39	53.40	53.53	50.57	0
58.01	59.91	58.71	55.41	52.00	52.86	52.55	51.24	4
56.69	58.54	56.22	53.43	52.47	54.85	52.51	49.04	7
56.60	58.92	56.65	54.27	49.58	48.86	50.34	49.23	10
0.49	0.52	0.61	0.63	1.35	1.55	1.48	1.62	S.E.M.

Means within each column having common superscript do not differ significantly (P<0.05).

Table 4: Effect of dietary Safflower seed on feed intake and feed conversion ratio (FCR)

FCR				Feed intake (g/day)				SS level %
	Third period	Second period	first period	Mean	Third period	Second period	first period	
Mean								
1.77	1.83	1.73	1.72	92.48	98.05	91.64	86.58	0
1.76	1.80	1.75	1.78	91.69	95.34	95.62	87.58	4
1.80	1.80	1.76	1.73	93.86	98.74	94.33	90.14	7
1.82	1.93	1.80	1.75	90.18	93.66	95.25	86.89	10
0.052	0.066	0.061	0.060	2.17	2.23	2.20	2.35	S.E.M.

Means within each column do not having superscript do not differ significantly (P<0.05).

Table 5: Effect of dietary safflower seed on egg quality (period 1, 28 -32 weeks)

% of yolk weight per egg	Shell thick-ness (mm)	Specific gravity (kg/cm ³)	Yolk Color Index	Yolk Index (%)	Egg shape (%)	Hough unit (%)	SS levels%
25.59	34.33	1.0850 ^{ab}	5.00	39.49	77.30	82.31 ^{ab}	0
26.44	33.83	1.0880 ^a	5.33	43.08	77.25	87.20 ^a	4
27.40	32.83	1.086 ^{ab}	5.17	39.36	77.37	86.99 ^a	7
27.56	31.63	1.0807 ^b	5.50	40.35	78.60	77.82 ^b	10
0.597	0.878	0.0007	0.406	1.259	0.633	1.61	S.E.M.

*Means within each column do not having superscript do not differ significantly (P<0.05).

Table 6: Effect of dietary safflower seed on egg quality (period 2, 32-36 weeks)

% of yolk weight per egg	Shell thick-ness (mm)	Specific gravity (kg/cm ³)	Yolk Color Index	Yolk Index (%)	Egg shape (%)	Hough unit (%)	SS levels%
27.38	34.48	1.0853 ^{ab}	6.00	41.49	77.42	89.87	0
25.88	34.49	1.0863 ^a	5.67	43.15	77.37	92.25	4
26.60	33.34	1.0853 ^{ab}	6.11	41.56	77.39	90.91	7
26.58	33.98	1.0837 ^b	6.00	42.56	77.58	90.12	10
0.475	0.854	0.0011	0.278	1.301	0.476	1.99	S.E.M.

* Means within each column do not having superscript do not differ significantly (P<0.05).

The data on egg quality parameters in 3 periods are shown in Tables 5, 6, 7. Egg quality parameters (egg shape, yolk colour index, yolk index, shell thickness and the percent of yolk weight per egg) were not affected significantly by SS levels in all periods except Hough unit and specific gravity (Tables 5, 6 and 7). The Hough unit score was statistically different in first period and the maximum Hough unit were observed in 4 and 7% SS (87.2 and 86.99 respectively) and the lowest in 10% SS (77.82). The second and third period was not significantly differences but the lowest in each three periods were observed in 10% safflower seed (77.82, 90.12 and 80.39 respectively).

The percent of yolk weight per egg were not affected with different levels of SS in three periods. But Wang (1996) reported that increasing the percent of albumen weight per egg with used 8% safflower oil (Table 5, 6 and 7). Specific gravity of eggs were reduced in SS treatment and the lowest specific gravity of eggs observed in 10% SS (1.076 gr/cm³ at end of experimental) treatment and

the differences was statistically significant in all periods (P<0.05). As the higher crude fiber in SS due to decreased absorption of Ca and P in intestine and resulting lower thickness shell and reducing specific gravity.

The data of egg and yolk cholesterol were shown in Table 8. There was not significantly effect on egg and yolk cholesterol but the 10 percent SS level showed lower cholesterol content (190.65mg/egg and 12.30mg/g), which it was non-significant difference during 3 periods. This result was agreement with Splitter *et al.* (1968) which not reported any affect of different levels of safflower seed on egg cholesterol but disagreement with Weiss *et al.* (1964), when they using 30% level safflower seed for 3 weeks in diet of laying hens and observed that the egg yolk cholesterol, was increased approximately 36%.

Effect of dietary Safflower seed on Cholesterol blood, antibody against ND, IBD and gain weight are represented in Table 9. The blood cholesterol did not

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Table 7: Effect of dietary safflower seed on egg quality (period 3, 36-40 weeks)

% of yolk weight per egg	Shell thickness (mm)	Specific gravity (kg/cm ³)	Yolk Color shape (%)	Yolk unit (%)	Egg Index	Hough Index (%)	SS levels%
27.28	35.08	1.0810 ^a	5.67	41.47	75.33	84.87	0
27.94	35.62	1.0793 ^{ab}	6.78	41.89	75.89	85.20	4
28.30	34.96	1.0787 ^{ab}	6.00	41.03	76.47	84.79	7
28.76	34.24	1.0760 ^b	6.11	39.80	77.44	80.39	10
0.644	0.929	0.0013	0.312	1.411	0.92	2.03	S.E.M.

The egg cholesterol content estimated according to mean of yolk in this period (yolk cholesterol 16.8). Means within each column do not having superscript do not differ significantly (P<0.05).

Table 8: Effect of dietary Safflower seed on Cholesterol blood

SS level %	Yolk cholesterol (mg/g)			Egg cholesterol (mg/egg)*		
	first period	Second period	Third period	first period	Second period	Third period
0	13.10	13.20	12.95	203.05	204.60	200.73
4	13.05	13.00	12.70	202.28	201.50	196.85
7	13.00	12.90	12.65	201.50	199.95	196.08
10	12.55	12.60	12.30	194.53	195.30	190.65
S.E.M.	0.54	0.60	0.575	5.72	3.85	5.47

The egg cholesterol content estimated according to mean of yolk in this period (yolk cholesterol 16.8). Means within each column do not having superscript do not differ significantly (P<0.05).

Table 9: Effect of dietary safflower seed on cholesterol blood, antibody against nd and ibd

SS levels %	0	4	7	10	S.E.M.
Blood Cholesterol (mg /dl)	143.00	142.67	141.33	134.33	8.54
ND titter	8.67	8.33	8.33	7.67	0.33
IBD titter	6812.00	6602.70	6491.70	5826.70	540.13
Gain Body Weight	90ab	140a	99ab	82b	0.015

Means within each column do not having superscript do not differ significantly (P<0.05).

significantly different within control and different levels of SS, but the minimum numerically blood cholesterol observed in SS treatments specifically in 10% SS (134.33mg/dlit) (Table 9).

There was not significant differences in case of ND titter and IBD titter with feeding all levels safflower seed (SS). There was significantly different among level of SS on gain body weight with but the lowest and highest body gain weight was observed in 10% SS (82 g) and 4% SS(140) compare to control diet. It was maybe due to higher fiber content of this diet.

Conclusion: The using of different levels of SS did not effect on performance and production parameters and egg quality except egg mass, specific gravity and gain body weight. However the Hough unit score in first period was reduced but there was not significantly different at second and third periods. The ND and IBD titter, egg and yolk cholesterol and blood cholesterol did not vary.

There was lake of literature to compare our result with them more study should be done with higher levels of safflower seed to investigate its nutritional value. However it can be concluded that, while dietary source are becoming scarcer with time the use of SS and it's by product at lower level are useful.

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