Effects of graded replacement of soybean meal by sunflower seed meal in laying hen diets on hen performance, egg quality, egg fatty acid composition, and cholesterol content

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Primary Audience: Nutritionists, Egg Production Managers, Feed Company Managers

SUMMARY

Protein feed sources are becoming more and more limited worldwide. Soybean meal, as the primary and most reliable protein source, is becoming increasingly expensive; therefore, there is a need to look for economic alternative protein sources. This trial was conducted to evaluate the effects of graded replacement of soybean meal by sunflower seed meal (SSM) on the performance, egg quality, egg fatty acid composition, and cholesterol content in Rugao laying hens (a local breed). One hundred sixty 28-wk-old laying hens were randomly assigned to 4 groups of 40 birds. Each group was made up of 4 replicate subgroups, with 10 laying hens each. All birds were acclimated to the basal diet for 1 wk. Experimental diets contained 0 (control), 8.26, 16.52, and 24.84% SSM (ME, 8.50 MJ/kg; CP, 33.00%; Ca, 0.26%; P, 1.03%) replacing soybean meal. Feed and water were offered ad libitum for 6 wk. No significant difference ($P >$ 0.05) was observed among treatments in the performance and egg quality of laying hens. The concentrations of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in yolks were consistent among the diets, except that the percentage of C17:0 decreased in the yolks of birds fed the experimental diets ($P < 0.05$). However, after 6 wk of feeding, the egg yolk cholesterol concentrations of birds in the experimental groups were lower than those of birds in the control group ($P < 0.05$). Thus, it could be feasible to use SSM in laying hen diets as an available ingredient replacing soybean meal, without affecting performance or egg fatty acid content while reducing the cholesterol level in the yolk and the production cost.

Key words: cholesterol, egg quality, fatty acid composition, laying hen, sunflower seed meal

2012 J. Appl. Poult. Res. 21:367–374
http://dx.doi.org/10.3382/japr.2011-00437

DESCRIPTION OF PROBLEM

Protein is often one of the most expensive components of poultry diets. Traditionally, soybean meal is used as the preferred protein source in the diets because its nutritional value has been verified worldwide. However, this strategic feed ingredient has to be imported into many European and Asian countries, and its price is influenced by external fluctuations. Increasing ingre-

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dient prices remain the greatest single item that determines profit margins in poultry production. The best strategy to reduce costs is the development of diet formulations using alternative, locally available ingredients, thereby decreasing feed costs. Therefore, cottonseed, rapeseed, sunflower, peanut, fava bean, field pea, and black cumin meals have been suggested as alternative vegetable protein sources for poultry [1–7].

These alternative ingredients, which have potential as low-cost protein sources, are underutilized. As an example, sunflower meal, which can be cultivated 2 or 3 times a year in tropical areas, has an established nutrient profile and is a relatively inexpensive protein source for poultry diets [8]. It has been reported that sunflower seed meal (**SSM**) can be included in poultry diets at relatively high amounts without any adverse effect on performance and egg quality [2, 9, 10]. However, little information is available concerning the fatty acids and cholesterol in eggs when SSM is used in laying hen diets. Therefore, the objective of the current study was to evaluate the effects of graded replacement of soybean meal by SSM in laying hen diets on hen performance, egg quality, egg fatty acid composition, and cholesterol content. Concurrently, the cost benefits of SSM were determined.

MATERIALS AND METHODS

Experimental Design

This trial was carried out at the Poultry Institute, Chinese Academy of Agricultural Sciences. In this study, 160 laying chickens (Rugao laying hens) 28 wk of age were randomly assigned to 4 groups of hens, with 4 replicates of 10 birds each (40 laying hens per group), and fed diets supplemented with 0 (control), 8.26, 16.52, and 24.84% SSM (ME, 8.50 MJ/kg; CP, 33.00%; Ca, 0.26%; P, 1.03%) [11] for 6 wk, replacing soybean meal (ME, 9.99 MJ/kg; CP, 43.00%; Ca, 0.33%; P, 0.62%) [12] in the diets. Birds were housed in individual cages at dimensions of 36 × 40 cm, providing 1,440 cm^2/bird. The composition of the experimental diets is presented in Table 1, and the fatty acid composition of the experimental diets containing SSM is shown in Table 2. Water and feed were provided ad libitum during the study. The photoperiod was set at 16L:8D throughout the study. Housing temperature and RH were 20 ± 3°C and 65 to 75%, respectively. All bird handling protocols were approved by the Animal Care and Use Committee of the Poultry Institute, Chinese Academy of Agricultural Sciences.

Sample Collection and Analytical Determination

Body weights of laying hens were determined at the beginning and end of the study. Feed consumption was recorded on a replicate basis at weekly intervals. Daily egg production was monitored during the trial. Egg production is expressed as average hen-day production, calculated from total eggs divided by the total number of hen days. Feed conversion is expressed as grams of feed consumed per gram of egg produced. Eggs were examined for interior and exterior quality. Twelve eggs per group (3 eggs/replicate) were collected at the end of the study to measure egg parameters.

The flotation method was used to determine specific gravity, with a range of salt solutions from 1.068 to 1.100 g/cm^3, in increments of 0.004 gradients. Shell color was measured using a TSS QCR reflectometer [13]. The shape index was calculated according to the following formula: shape index = (height/width) × 100 [13]. Eggs were weighed and the shell strength of uncracked eggs was measured with an Instron testing machine [13]. A constantly increasing load was applied to an egg lying lengthways until it broke. The applied load at the time of breakage was the measured strength. Eggs were cracked onto a flat surface, and the height of the albumen was measured using a digital albumen height gauge [13]. The measurement was taken at 3 points on the thick and flat albumen, which were approximately 1 cm distance surrounding the egg yolk and formed an equilateral triangle. Haugh units were calculated using the formula HU = 100 log(H + 7.57 to 1.7W0.37), where H is the height of the albumen, and W is the weight of the egg [14]. After separating the yolk from the albumen, the yolk was weighed and the mass of albumen was calculated as the difference in egg weight minus the yolk and shell weight. The percentage was calculated as a ratio of egg weight. Yolk color was evaluated

Table 1. Ingredients and nutrient levels of the experimental diets[1]

	Sunflower seed meal, %			
Item	0	8.26	16.52	24.84
Ingredient, %				
Corn	64.74	64.25	63.29	61.91
Soybean meal	19.29	12.90	6.50	
Sunflower seed meal		8.26	16.52	24.84
Corn protein starch	4.00	4.00	4.00	4.00
Limestone	8.48	8.49	8.19	7.68
Calcium hydrogen phosphate	0.60	0.60	0.60	0.60
DL-Met	0.10	0.08	0.06	0.04
L-Lys		0.08	0.16	0.25
Thr		0.02	0.03	0.05
NaCl	0.30	0.30	0.30	0.30
$CaCl_2$	0.12	0.12	0.12	0.10
Phytase	0.01	0.01	0.01	0.01
Vitamin and trace mineral premix[2]	0.23	0.23	0.23	0.23
Zeolite powder	2.14	0.67		
Total	100.00	100.00	100.00	100.00
Nutrient level (calculated)				
ME MJ/kg	11.34	11.34	11.34	11.34
CP, %	16.00	16.00	16.00	16.00
Ca, %	3.30	3.30	3.30	3.30
Total P, %	0.45	0.49	0.54	0.58
Nonphytate P, %	0.26	0.26	0.26	0.26
Lys, %	0.71	0.70	0.70	0.70
Met, %	0.37	0.36	0.36	0.36
Cys, %	0.26	0.26	0.26	0.26
Available Lys, %	0.57	0.58	0.59	0.61
Available Met, %	0.29	0.28	0.28	0.29
Available Met + Cys, %	0.46	0.46	0.46	0.46
Thr, %	0.60	0.60	0.60	0.60
Trp, %	0.16	0.16	0.16	0.16

[1]Values are expressed on an air-dried basis.

[2]Premix provided the following per kilogram of diet: vitamin A (retinyl palmitate), 7,715 IU; vitamin D_3 (cholecalciferol), 2,755 ICU; vitamin E (DL-α-tocopheryl acetate), 8.8 IU; vitamin K (menadione sodium bisulfate complex), 2.2 mg; vitamin B_{12} (cobalamin), 0.01 mg; menadione (menadione sodium bisulfate complex), 0.18 mg; riboflavin, 4.41 mg; pantothenic acid (D-calcium pantothenate), 5.51 mg; niacin, 19.8 mg; folic acid, 0.28 mg; pyridoxine (pyridoxine hydrochloride), 0.55 mg; manganese (manganese sulfate), 50 mg; iron (ferrous sulfate), 25 mg; copper (copper sulfate), 2.5 mg; zinc (zinc sulfate), 50 mg; iodine (calcium iodate), 1.0 mg; selenium (sodium selenite), 0.15 mg.

with a Roche Yolk Color Fan, which included 15 colorimetric blades according to their intensity of yellow [13]. The colors of yolk were scored and expressed in grades. Shell thickness was a mean value of measurements at 3 locations on the eggs (air cell, equator, and sharp end), determined in fresh shell without membranes by a digital micrometer [13].

At the end of the study, 12 eggs per group (3 eggs/each replicate) were analyzed for fatty acid composition of egg yolk lipids. Total lipid was extracted from the egg yolks using the method of Noble et al. [15]. The mass of total lipids was determined gravimetrically. The fatty acid methyl esters were analyzed by gas-liquid chromatography using a capillary column (60 m × 0.22 mm internal diameter, coated with BPX70 with a film thickness of 0.25 μm) [16] in a CP9001 instrument [17] connected to an EZ Chrom Data System [18] to determine the fatty acid profile of the lipids. Identification of the peaks was confirmed by comparison with an external standards mixture of fatty acid methyl esters [19].

The 48 eggs (12 eggs/group, 3 eggs/replicate) selected for fatty acid analysis were also used to analyze the yolk cholesterol content. The cholesterol content of the egg yolks was determined according to the methods of Hammad et al. [20]

Table 2. Fatty acid composition (% of total methyl esters of fatty acids) of experimental diets[1]

	Sunflower seed meal, %			
Fatty acid[2]	0	8.26	16.52	24.84
Saturated				
C14:0	0.06	0.05	0.05	0.04
C16:0	13.32	12.27	12.42	11.97
C17:0	0.08	0.07	0.08	0.07
C18:0	1.87	2.15	2.09	2.14
C20:0	0.43	0.51	0.41	0.51
ΣSFA	15.78	15.48	15.05	14.73
Monounsaturated				
C16:1	0.24	0.15	0.15	0.14
C18:1	30.67	29.90	31.38	31.70
ΣMUFA	30.98	30.05	31.53	31.85
Polyunsaturated				
C18:2n-6 (linoleic acid)	51.65	52.91	52.31	52.52
C18:3n-3 (linolenic acid)	1.60	1.56	1.11	0.90
ΣPUFA	53.24	54.47	53.42	53.42
ΣPUFA/ΣSFA	3.38	3.52	3.55	3.63

[1]All the values are analyzed.

[2]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ΣSFA = total saturated fatty acids; ΣMUFA = total monounsaturated fatty acids; ΣPUFA = total polyunsaturated fatty acids.

and Kaya et al. [21]. Yolks were separated from the albumen, and a 0.1-g sample of yolks was weighed into a tube. Yolk lipids were extracted with isopropanol (4 mL), vortexed for 2 to 3 min, and then centrifuged at 907 × g at 4°C for 10 min. The yolk cholesterol concentration (mg of cholesterol/g of egg yolk) was determined in the filtered samples by UV spectrophotometry by using commercial kits and calculated by the method of Boehringer Mannheim GmbH Biochemica [22].

Statistical Analysis

Data were compared in a completely randomized design by the GLM procedure of SAS software [23]. Means were compared by Duncan's multiple-range test when probability values were significant ($P < 0.05$).

RESULTS AND DISCUSSION

Performance

The production performance of hens is shown in Table 3. Diets supplemented with 8.26, 16.52, and 24.84% SSM had no significant effects on BW gain, egg production, daily egg mass, daily feed intake, or feed conversion ($P > 0.05$).

Similar results were reported by Tsuzuk et al. [2] that increasing inclusion levels of SSM did not affect daily feed intake and feed conversion.

Table 3. Effects of graded replacement of soybean meal by sunflower seed meal on the performance of laying hens[1,2]

	Sunflower seed meal, %					
Item	0	8.26	16.52	24.84	SEM	Significance
BW gain, g	117.38	92.63	110.50	69.69	22.349	0.629
Egg production, %	72.52	70.24	70.24	73.71	4.815	0.941
Daily egg mass, g/bird	33.45	30.64	31.55	31.10	2.753	0.716
Daily feed intake, g/bird	110.12	119.27	111.99	111.77	4.215	0.212
Feed conversion, g of feed/g of egg	3.79	3.84	3.82	3.70	0.256	0.984

[1]Results are means with n = 4 per treatment.

[2]There are no significant differences in each row ($P > 0.05$).

To the contrary, Karunajeewa et al. [24] attributed an increase in feed consumption to the higher fiber level of rations containing SSM. Vieira et al. [25] observed that sunflower meal in laying hen diets increased feed consumption but that feed conversion was poorer. However, Uwayjan et al. [26] found that the inclusion of 30% sunflower seed in diets did not affect feed conversion in laying hens but that feed consumption was reduced, which might have been due to the increase in the energy content of the diet.

Uwayjan et al. [26] observed a reduced laying rate when laying hens were fed sunflower seed. Rose et al. [27] reported a reduction in the production and weight of eggs by hens fed diets in which SSM protein was substituted for all the protein provided by soybean meal. Nevertheless, no significant difference in performance was observed in this experiment, probably because the experimental diets were formulated to meet laying hen requirements [28].

Egg Quality

Feeding diets supplemented with SSM did not influence average egg weight, egg specific gravity, shell strength, shell color, shell thickness, shell percentage, albumen percentage, yolk percentage, yolk color, or Haugh units ($P > 0.05$; Table 4).

Similarly, Tsuzuki et al. [2] found no effect of inclusion levels of sunflower seed on yolk color or Haugh unit values. The addition of 30% sunflower seed in laying hen diets supplemented with 0.1% lysine and 0.01% methionine increased egg weight and did not change the Haugh unit values, although yolk color was less intense [26], probably because of the reduced content of corn in the rations. However, Karunajeewa et al. [24] observed that hens fed diets containing sunflower seed laid eggs with lower Haugh unit values than did hens fed SSM with or without supplementation with sunflower oil. It was speculated that sunflower seed might contain an antinutritional factor that could cause a reduction in albumen quality. No negative effects were observed from feeding sunflower seed-supplemented diets in the performance and egg quality of laying hens. Therefore, we concluded that SSM was an excellent feed ingredient for laying hen diets.

Egg Fatty Acid Composition

The fatty acid composition of egg yolk lipids after 6 wk of feeding is shown in Table 5. Results showed that the concentrations of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (**PUFA**) in yolks were generally similar among dietary groups ($P > 0.05$), except that the percentage of C17:0 decreased in the yolks of birds in the experimental groups ($P < 0.05$).

It is well known that when C16:0 is released from the synthetase complex, it can be esterified into complex lipids, elongated to C18:0, or desaturated to C16:1 [29]. In the current study, the percentages of C16:0 and C18:0 in the yolks of

Table 4. Effects of graded replacement of soybean meal by sunflower seed meal on egg quality in laying hens[1,2]

	Sunflower seed meal, %					
Item	0	8.26	16.52	24.84	SEM	Significance
Average egg mass, g/hen per day	47.08	45.16	47.98	46.28	1.005	0.285
Shell color	41.17	39.44	40.27	39.82	2.135	0.946
Shell strength, kg/cm^2	3.84	3.48	3.52	3.58	0.208	0.630
Shape index, %	0.77	0.76	0.76	0.78	0.006	0.247
Egg specific gravity, g/cm^3	1.089	1.088	1.089	1.090	0.120	0.274
Eggshell, %	10.77	11.01	10.65	10.94	0.219	0.656
Albumen, %	58.60	57.62	59.36	58.51	0.541	0.211
Yolk, %	30.64	31.38	29.99	30.55	0.501	0.321
Shell thickness, mm	0.31	0.31	0.31	0.31	0.005	0.878
Haugh units	77.80	78.17	75.35	78.57	1.765	0.583
Yolk color, Roche	9.69	9.46	9.67	9.52	0.140	0.602

[1]Results are means with n = 4 per treatment.

[2]There are no significant differences in each row ($P > 0.05$).

Table 5. Effects of graded replacement of soybean meal by sunflower seed meal on egg fatty acid composition (% of total fatty acids) in laying hens[1]

	Sunflower seed meal, %					
Fatty acid[2]	0	8.26	16.52	24.84	SEM	Significance
Saturated						
C14:0	0.37	0.33	0.36	0.35	0.024	0.592
C16:0	27.43	26.09	27.32	26.73	0.558	0.345
C17:0	0.16[a]	0.08[c]	0.08[c]	0.12[b]	0.008	<0.000
C18:0	8.43	9.02	8.19	8.26	0.258	0.145
C20:0	0.04	0.03	0.03	0.03	0.008	0.788
ΣSFA	36.49	35.49	35.94	35.48	0.407	0.303
Monounsaturated						
C14:1	0.10	0.06	0.11	0.10	0.016	0.125
C16:1	5.11	4.06	5.06	4.92	0.397	0.258
C18:1	47.03	49.52	47.06	48.00	0.927	0.243
ΣMUFA	52.25	53.64	52.22	53.02	0.658	0.398
Polyunsaturated						
C18:2n-6 (linoleic acid)	8.88	9.12	9.06	9.12	0.455	0.964
C18:3n-3 (linolenic acid)	0.44	0.25	0.20	0.38	0.069	0.100
C20:4n-6 (arachidonic acid)	2.01	1.49	2.59	2.00	0.363	0.257
ΣPUFA	11.27	10.87	11.84	11.50	0.530	0.637
ΣPUFA:ΣSFA	0.31	0.31	0.33	0.32	0.015	0.558

[a–c]Values within a row with no common superscript differ significantly ($P < 0.05$).

[1]Results are means with n = 4 per treatment.

[2]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ΣSFA = total saturated fatty acids; ΣMUFA = total monounsaturated fatty acids; ΣPUFA = total polyunsaturated fatty acids.

hens fed SSM diet did not vary ($P > 0.05$) except for the percentage of C17:0. Similar results were reported by Cherian et al. [30], who found no change in C16:0 when hens were fed diets rich in n-3 PUFA by adding flaxseed. Others have indicated no change or a small decrease in C18:0 content as diets became richer in n-3 PUFA [30, 31]. Nash et al. [32] found that C18:0 generally increased as linoleic acid (C18:2n-6) content increased. No difference in C18:0 was observed in the yolks of hens in the control and experimental groups ($P > 0.05$), even though the reduction in C18:0 could indicate an additional health advantage for these eggs, because saturated fatty acids are considered hypercholesterolemic [33].

In many studies, researchers have shown the benefits of PUFA on human health, especially in relation to heart problems and similarly important diseases [34]. However, we suggest, based on the lack of a significant difference in total monounsaturated fatty acid and PUFA content among the control and experimental diets in this trial, that the n-3 PUFA provided by SSM was below the content needed to produce a change in C18:1 levels because desaturation of C18:0 to C18:1 by Δ^9-desaturase is depressed by PUFA-rich diets [35].

Egg Cholesterol Content

Figure 1 shows the effect of SSM supplementation on egg yolk cholesterol. Egg yolk cholesterol was decreased significantly ($P < 0.05$) by supplementation of SSM in the layer diet. The concentrations (mg/100 g of yolk) of cholesterol in the eggs of hens fed diets containing 8.26, 16.52, and 24.84% SSM were 1,150.91 ± 23.21, 1,017.65 ± 48.96, and 937.17 ± 14.97 mg/100 g, respectively. More recently, the effects of substitutes on egg yolk cholesterol content were observed to differ. The feeding of plant sterols caused no change in total yolk cholesterol content [36]. Conjugated linoleic acid in the diet increased the content of yolk cholesterol in Brown Dwarf layers [37]. Oral administration of pravastatin significantly lowered egg cholesterol in laying hens [38]. Similarly, garlic paste in the diet of laying hens reduced serum and yolk concentrations [39]. In the current study, a significant decrease was found in egg yolk cho-

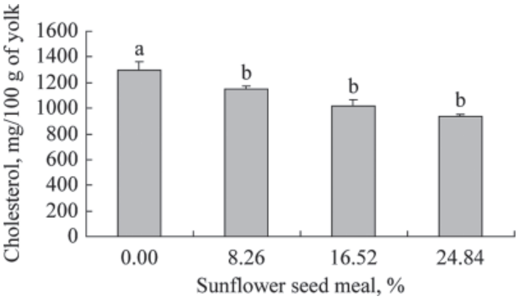

Figure 1. Effects of graded replacement of soybean meal by sunflower seed meal on egg cholesterol content in laying hens. Values without a common letter (a, b) are significantly different ($P < 0.05$). Values are expressed as milligrams of cholesterol per 100 g of yolk, as means of 3 eggs from replicates of all groups (12 eggs/group).

lesterol when soybean meal was replaced with different levels of SSM ($P < 0.05$). This appears to be good news for consumers because a higher circulating cholesterol level is one of the major risk factors for cardiac problems [40, 41].

Production Cost

The market purchase price of SSM was less than one-half the soybean meal price ($0.219 vs. $0.512/kg) during the experimental period (January 2009). Regarding the comparative costs of the diets, the diet cost for the control group was $0.301 per kilogram and those for the SSM diets were $0.285, $0.268, and $0.250/kg, respectively (8.26, 16.52, and 24.84% SSM content). An additional advantage of these diets was that there was no difference in daily feed intake and feed conversion among the groups ($P > 0.05$; Table 3). Therefore, the production cost could be reduced when SSM was used in the diet, compared with soybean meal.

CONCLUSIONS AND APPLICATIONS

1. No significant difference was observed in the performance, egg quality, and fatty acid content of laying hens fed graded levels of SSM.
2. After 6 wk of feeding the experimental diets, egg yolk cholesterol content was significantly lower for groups fed SSM than the control.
3. We suggest, based on the data presented here, that it is feasible to use SSM in the diets of laying hens as a replacement for some or all of the soybean meal without affecting performance, egg quality, or egg fatty acid content while reducing egg cholesterol and production costs.

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Acknowledgments

This work was financially supported by the earmarked fund for Modern Agro-Industry Technology Research System, Special Fund for Agro-Scientific Research in the Public Interest of Ministry of Agriculture (201003011); 333-Project of Jiangsu Province [cx(10)122]; Independent Innovation Program of Agriculture Science and Technology of Jiangsu Province (SBE201038266); Agro-Three Project of Jiangsu Province [SX(2010)138, SX(2010)019], P. R. China.