

In the name of Allah

بسم الله

THE USE OF COTTON SEED MEAL
IN THE DIET OF LAYING HENS¹

استفاده از کنجاله پنبه دانه در جیره
مرغان تخمگذار

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ABSTRACT

خلاصه

An experiment was carried out to determine the maximum level of free gossypol which could be tolerated by laying hens without any adverse effects on their performance. The possibility of inactivating gossypol using iron sulfate was also investigated.

The results showed that laying hens could tolerate up to 440 ppm free gossypol without any significant adverse effects on egg production, egg weight, feed consumption, and feed conversion.

Supplementation of diets containing free gossypol with ferrous sulfate did not improve egg production, egg weight, and feed conversion.

When freshly laid eggs were examined, there was no effect of 140 ppm free gossypol on yolk and white color. However, this level of free gossypol caused egg discoloration when eggs were stored for one month

در یک آزمایش، حداکثر میزان گوسپول قابل تحمل توسط مرغان تخمگذار بدون ایجاد اثرات سوء در تولید تخم مرغ، وزن تخم مرغ، مصرف غذا، راندمان تبدیل غذا و تغییر رنگ تخم مرغ و نیز امکان خنثی کردن گوسپول توسط سولفات آهن مورد بررسی قرار گرفت.

نتایج این آزمایش نشان میدهد که مرغان تخمگذار قادرند تا ۴۴۰ قسمت در میلیون گوسپول آزاد را بدون هیچگونه اثرات سوء معنی داری در تولید تخم مرغ، وزن تخم مرغ، مصرف غذا و راندمان تبدیل غذا تحمل کنند. اضافه کردن سولفات آهن به غذاهای حاوی گوسپول آزاد منجر به بهبود تولید تخم مرغ، وزن تخم مرغ و راندمان تبدیل غذا نگردید. بررسی تخمهای مرغهای تازه نشان داد که مقدار کمتر از ۱۴۰ قسمت در میلیون گوسپول آزاد در جیره، تاثير چندانی در رنگ سفیده و زرده نمیگذارد مگر اینکه تخم مرغها برای مدت یکماه یا بیشتر نگهداری شده باشند. در اینصورت گوسپول آزاد اضافه شده منجر به بی رنگی تخم مرغ میگردد.

مقادیر ۱۲۰ و ۲۴۰ قسمت در میلیون سولفات آهن موجب گردید که مقاومت مرغان تخمگذار به مقدار گوسپول آزاد، تا ۲۸۰ قسمت در میلیون افزایش یابد.

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or longer.

Iron sulfate at the levels used (120 and 240 ppm) was effective in increasing the tolerance level of free gossypol by laying hens to 280 ppm. Iron sulfate partially decreased the percentage of discolored eggs produced by free gossypol during the period of storage.

INTRODUCTION

The available data on the effect of free gossypol on the performance of the laying hens are contradictory. Information obtained from one experiment (7) indicated that 160 ppm free gossypol in the layers ration decreased egg production and feed consumption, but increased feed conversion. However, data from another experiment (8) showed that laying hens could tolerate up to 200 ppm free gossypol without any adverse effects on these parameters. Heywang and Bird (8) reported that free gossypol up to 120 ppm did not affect the egg weight, but the egg weight decreased when the level of free gossypol was increased to 240 ppm and 360 ppm. Narian *et al.* (12) reported that free gossypol up to 200 ppm had no adverse effects on the egg weight.

In Iran, demand for fresh egg is high and egg discoloration which develops during storage is not serious enough to restrict the use of cotton seed meal in the diet at present. There are indications that egg discoloration due to presence of gossypol in the diet might occur in both fresh eggs and those stored under refrigeration for one week or longer (1,9,10). The discoloration of eggs includes an olive green and salmon color of the yolk which is mostly due to reaction of free gossypol and iron content of the yolk, and pink color of the white as a result of passage of iron from yolk and its combination with conalbumin (15, 16).

In addition to gossypol, cyclic fatty acids (sterculic and malvalic) of the cotton seed oil are also responsible for egg discoloration (11, 18).

An important factor in discoloration of the white and yolk is the pH within eggs (2). Incorporation of gossypol in the diet causes an increase in the pH of the white because of ammonia formation during storage (5,11).

In this paper the effects of various levels of free gossypol and iron sulfate on egg discoloration and performance of laying hens are described.

MATERIALS AND METHODS

The composition of the experimental diets is shown in Table 1. In the control diet (T_1) the main protein source was soybean meal. In other treatments, soybean meal was replaced by different amounts of cotton seed meal to increase the free gossypol content of the diet from zero to 140, 280, 440 and 550 ppm.

Characteristics of the cotton seed meal used in this trial have been reported elsewhere (14) and the gossypol content of the samples was determined according to Pons and Haffpaur (13). Ferrous sulfate was added (0.06 and 0.12% of the diet) to provide 120 and 240 ppm of iron, respectively.

Four hundred 14-month-old White Leghorn hens were selected from the university flock and their egg production and egg weights were recorded for a period of 20 days. From these, 65 hens, with approximately identical egg production rates were selected. They were then divided into five groups of 13 hens housed in individual cages and each group received one of the 13 experimental diets. The experimental diets were fed *ad libitum* for 55 days with water being freely available. The egg production and egg weights were recorded daily and at the end of the experiment the feed consumption, feed conversion, egg production and egg weights were calculated for each hen.

The eggs were stored at 10°C and 3°C for one and three

Table 1. Composition of experimental diets.[†]

Treat- ments	Basal portion (%)	Corn (%)	Soybean meal (%)	Cottonseed meal (%)	Corn oil (%)	Sulka Floc [¶] (%)	Total	Free gossypol (ppm)	Iron (ppm)
T ₁	13	67.0	20.00	-	-	-	100	-	-
T ₂	13	67.8	13.36	5.85	-	-	100	140	-
T ₃	13	68.5	6.68	11.68	0.14	-	100	280	-
T ₄	13	68.0	-	17.35	0.70	0.77	100	440	-
T ₅	13	42.0	-	22.00	6.70	16.30	100	550	-
T ₆	As T ₂ plus 0.06% ferrous sulfate								120
T ₇	As T ₂	" 0.12%	"	"	"	"			240
T ₈	As T ₃	" 0.06%	"	"	"	"			120
T ₉	As T ₃	" 0.12%	"	"	"	"			240
T ₁₀	As T ₄	" 0.06%	"	"	"	"			120
T ₁₁	As T ₄	" 0.12%	"	"	"	"			240
T ₁₂	As T ₅	" 0.06%	"	"	"	"			120
T ₁₃	As T ₅	" 0.12%	"	"	"	"			240

[†]All experimental diets were isocaloric and isonitrogenous and supplied 15% protein and 2835 kcal/kg metabolizable energy.

[§]Basal portion contained: dehydrated alfalfa, 1.5 parts; bone meal, 3 parts; oyster shell, 7 parts; salt, 0.5 parts; Femavit (a source of minerals and vitamins), 1 part.

[¶]Used to give bulk to diets. Sulka Floc purchased from the Brown Company, New York.

months, respectively, after which they were broken and scored on the basis of yolk and white discoloration (Table 2). Several fresh eggs were also broken and scored.

Table 2. Scores indicating degrees of discoloration of the yolk and white.

Score	Description		Acceptability
	Yolk	White	
1	Normal	Normal	Acceptable
2	Slightly discolored	Very slightly pinked	Acceptable
3	One or two relatively large dark spots on the surface of the yolk	Slightly pinked	Relatively acceptable
4	Fifty percent of the yolk surface dark	Completely pinked	Not acceptable
5	Completely dark	Dark pinked	Not acceptable

The data were subjected to the analyses of variance (7) and means were compared by Duncan's multiple range test (3).

RESULTS AND DISCUSSION

Egg production and egg weights were decreased, but feed conversion was increased with increasing levels of free gossypol in the diets (Table 3). However, it was at 550 ppm free gossypol level that these parameters were significantly different from the control ($P < 0.05$). There was no effect of free gossypol on the feed consumption of the laying hens. Results of this experiment support the findings of Heywang (6), Heywang and Bird (8), and Waldroup and Goodner (20), but are not in agreement with those of Heywang and Bird (7) who reported a decrease

Table 3. Egg production, egg weight, feed consumption, and feed conversion of laying hens fed different levels of free gossypol and iron.

Treat- ments	Free gossypol (ppm)	Iron (ppm)	Egg production (%)	Egg weight (g)	Feed consumption (g/bird/day)	Feed conversion (kg feed/kg egg)
T ₁	-	-	71a*	59ab	101ab	2.62cd
T ₂	140	-	67abc	60a	113a	2.62cd
T ₃	280	-	68ab	56abc	102ab	2.72cd
T ₄	440	-	61abc	53abcd	95ab	2.90abcd
T ₅	550	-	52c	48d	94ab	3.88a
T ₆	140	120	69ab	56abc	100ab	2.65cd
T ₇	140	240	70ab	56abc	93b	2.44d
T ₈	280	120	59abc	55abcd	100ab	2.88bcd
T ₉	280	240	65abc	55abcd	96ab	2.69abcd
T ₁₀	440	120	62abc	57abc	100ab	3.16abcd
T ₁₁	440	240	60abc	57abc	93b	2.53cd
T ₁₂	550	120	57bc	55abcd	94ab	2.82bcd
T ₁₃	550	240	59abc	51cd	90b	2.62cd
S.E.			3.94	1.40	0.25	0.23

* Values followed by the same letter in each column are not significantly different ($P < 0.05$).

in egg weight with 300 ppm free gossypol.

Ferrous sulfate at the levels used did not significantly improve the adverse effects of 550 ppm free gossypol on egg production and egg weight, although there was a slight increase in these parameters. However, iron sulfate significantly improved the feed conversion. These results are in agreement with the findings of Waldroup and Goodner (20) who reported a partial improvement in egg production due to 0.5% supplemental iron sulfate.

Irrespective of the period of egg storage, the percentages of unacceptable eggs were increased with increasing free gossypol contents in the diets (Table 4). The effect of free gossypol on yolk discoloration was more pronounced than that on the white. With each particular level of free gossypol in the diet, the percentages of unacceptable eggs were increased with increasing the period of storage. Hens consuming diet that contained 140 ppm free gossypol did not produce discolored eggs when the eggs were examined fresh. These results are not in agreement with the findings of Waldroup and Goodner (20) and Heywang *et al.* (9) who reported that the free gossypol as low as 40 ppm caused yolk discoloration, even in fresh eggs. The data support the reports of Fletcher *et al.* (4) and Waldroup and Goodner (20) with respect to increasing percentages of objectionable eggs due to increased free gossypol contents of diet and period of storage.

Iron at the levels used was partially effective in alleviating the adverse effects of free gossypol in egg discoloration, yet its effectiveness was more pronounced on yolk rather than white discoloration. In most cases, the effect of iron was decreased with increasing levels of free gossypol and period of storage. Iron sulfate was not effective in alleviating the adverse effect of 550 ppm free gossypol on yolk discoloration after three months of storage. Although iron sulfate was used at two levels,

Table 4. Effects of gossypol on discoloration of the yolk and white of eggs used fresh and those stored for one or three months.

Free gossypol in diet (ppm)		Iron (ppm)		Period of storage (month)									
				Fresh					1 (at 10°C)				
				No. of eggs	Accept-able yolk (%)	Accept-able white (%)	No. of eggs	Accept-able yolk (%)	Accept-able white (%)	No. of eggs	Accept-able yolk (%)	Accept-able white (%)	3 (at 3°C)
0	0	0	0	22	100	100	34	100	100	17	100	100	100
140	0	0	0	20	100	100	38	87	100	17	0	85	85
280	0	0	0	21	74	100	31	9	77	19	0	42	42
440	0	0	0	13	23	69	32	5	36	13	0	0	0
530	0	0	0	21	29	76	26	0	35	15	0	13	13
140	120	120	120	20	100	100	23	100	100	17	100	100	100
140	240	240	240	22	100	100	20	90	100	22	91	100	100
280	120	120	120	12	100	100	15	34	100	14	71	98	100
280	240	240	240	16	100	100	36	89	97	20	70	94	94
440	120	120	120	20	95	100	32	69	90	19	5	74	74
440	240	240	240	17	100	100	25	32	82	17	41	92	92
550	120	120	120	18	81	100	21	38	92	18	0	29	29
550	240	240	240	19	53	100	24	25	83	18	0	22	22

it can not be concluded from the present data that the higher level was more effective than lower level. The results support the findings of Heywang (6) and Waldroup and Goodner (20), who reported that iron sulfate was only partially effective in decreasing the percentage of discolored eggs, but do not agree with the findings of Vohra *et al.* (19).

From the results of this study it can be concluded that properly processed cotton seed meal of known gossypol content can be used in diets for commercial laying hens, provided the dietary free gossypol content does not exceed 140 ppm. The addition of ferrous sulfate is recommended as a prophylactic against possible gossypol discoloration.

LITERATURE CITED

1. Altschul, A.M., C.M. Lyman, and F.H. Thurber. 1958. Cotton seed meal *In* A.M. Altschul (ed.). Processed plant protein feed stuffs. Academic press, New York.
2. Bandemer, S.L., P.L. Schaible, and J.A. Davidson. 1946. Composition of fresh and storage eggs from hens fed cotton seed and non-cotton seed ration. II. Ammonia nitrogen content. *Poultry Sci.* 25: 446-450
3. Duncan, D.B. 1955. Multiple range test and multiple F tests. *Biometrics* 11: 1-42.
4. Fletcher, J.L., B.F. Barentine, L.J. Dreesen, J.E. Hill, and C.B. Shawver. 1953. The use of ferrous sulfate to inactivate gossypol in diets of laying hens. *Poultry Sci.* 32: 740-742.
5. Frampton, V.L., B. Piccol, and B.W. Heywang. 1961. Discoloration in stored shell eggs produced by hens fed cotton seed meal. *J. Agric. Food Chem.* 9: 59-63.
6. Heywang, B.W. 1957. Failure of ferrous sulfate to inactivate free gossypol in diets for laying chickens. *Poultry Sci.* 36: 715-717.

7. Heywang, B.W., and H.R. Bird. 1950. Supplements of cotton seed meal in diets for chickens. *Poultry Sci.* 29: 486-495.
8. Heywang, B.W., and H.R. Bird. 1954. Egg production, diet consumption and live weight in relation to the gossypol content of the diet. *Poultry Sci.* 33: 851-854.
9. Heywang, B.W., H.R. Bird, and A.M. Altschul. 1955. Relation between discoloration in eggs and dietary free gossypol supplied by different cotton seed products. *Poultry Sci.* 34: 81-90.
10. Heywang, B.W., and G. Vavich. 1965. Comparison of performance of layers fed soybean, glandless or glanded cotton seed meals. *Poultry Sci.* 44: 1240-1244.
11. Kemmerer, A.R., B.W. Heywang, and M.G. Vavich. 1961. Effects of *Sterculia foetida* oil on gossypol discoloration in cold storage eggs, and in mechanism of gossypol discoloration. *Poultry Sci.* 40: 1045-1048.
12. Narian, R.L., C.M. Lyman, and J.R. Couch. 1957. High level of free gossypol in hen diets: effect on body weight, feed consumption and egg production. *Poultry Sci.* 36: 1351-1354.
13. Pons, W.A., Jr., and C.L. Hoffpauir. 1957. Determination of free and total gossypol in mixed feeds containing cotton seed meals. *J. Assoc. Off. Anal. Chem.* 40: 1068-1080.
14. Pourreza, J., and K. Keshavarz. 1983. Evaluation of nutritional value of cotton seed meals produced in Iran. *Iran. J. Agric. Sci.* 12: (In press). (In Persian).
15. Schaible, P.J., and S.L. Bandemer. 1946. Composition of fresh and stored eggs from hens fed cotton seed and non-cotton seed rations. V. Cause of discoloration. *Poultry Sci.* 25: 446-459.

16. Schaible, P.J., L.A. Moore, and J.M. Moore. 1934. Gossypol, a cause of discoloration in egg yolk. *Science* 79: 372.
17. Steel, R.G.D., and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York.
18. Stenstone, F.S., and J.R. Vickery. 1959. Substance in plants of order Malvale causing pink white in stored eggs. *Poultry Sci.* 38: 1055-1071.
19. Vohra, P., Y. Hafez, L. Earl, and F.H. Kratzer. 1975. The effect of ammonia treatments of cotton seed meal on its gossypol-induced discoloration of egg yolks. *Poultry Sci.* 54: 441-447.
20. Waldroup, P.W., and T.O. Goodner. 1973. Tolerance level of free gossypol in layer diets as influenced by iron gossypol ratios. *Poultry Sci.* 52: 20-28.