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Effects of treating cottonseed meal with a solution of ferrous sulphate on laying hen performance and discolourations in eggs

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ABSTRACT

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The efficacy of detoxifying cottonseed meal (CSM) by treatment with ferrous sulphate heptahydrate (FSH), in solution or as a dry dietary ingredient, was examined. In Experiment 1, Dekalb G-Link (DGL) hens were fed on a diet containing 300 g CSM kg^{-1} with iron treatment at 1470 mg iron kg^{-1} diet, and in Experiment 2, Hubbard Golden Comet (HGC) hens were fed on a similar diet treated with 1300 mg iron kg^{-1}. Treatment of CSM with FSH in solution slightly depressed food intake and egg production initially, but the performance of hens gradually improved to that of non-CSM controls; treatment with crystalline FSH lowered performance to a considerably greater degree without much sustained improvement. Both treatment methods were effective in preventing the gossypol-related brown yolk discolouration, although in Experiment 2, the solution method produced eggs of slightly better quality.

INTRODUCTION

For many years, treatment of cottonseed meal (CSM) with soluble iron salts has been suggested as a satisfactory way of preventing the brown yolk discolouration effect of gossypol in hens eggs (Waldroup et al., 1970). In recent studies, however, treatment with crystalline ferrous sulphate heptahydrate (FSH), while alleviating the brown yolk egg quality problem, depressed egg production (Panigrahi and Morris, 1991). This depression, which occurred at a supplemental dietary iron concentration of 850 mg kg^{-1}, seemed to be due to a specific effect of excess dietary iron. However, in an earlier study, treatment with FSH in solution at only 100 mg iron kg^{-1} diet had also reduced laying performance (Panigrahi et al., 1989). In view of the report of

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Bressani et al. (1966), that addition of water to CSM itself produced a 50% reduction in free gossypol content, and that of Clawson et al. (1975) that the performance of rats was improved when CSM was treated with ferrous sulphate in solution, the significance of these results was unclear. It was considered possible that unless CSM is dried following treatment with ferrous sulphate in solution, a deterioration of the feed owing to mould growth may result, leading to a lowering in animal performance. Consequently, there was some uncertainty concerning the most appropriate method of treating CSM with iron for inclusion in practical laying hen diets.

In the present study, the effects of treating CSM with FSH in solution or as crystals were further examined in experiments using two batches of CSM with different free gossypol and cyclopropenoid fatty acid (CPFA) contents. Because previous studies had indicated that the depressive effect on egg production of a crystalline iron-treated CSM diet may vary with the genotype of the hen (Panigrahi and Morris, 1991), the experiments were conducted with different breeds.

MATERIALS AND METHODS

The CSMs (CSM-1) and (CSM-2) were produced in China and contained 320 g and 350 g crude protein kg^{-1} and 140 g and 167 g crude fibre kg^{-1}, respectively. However, CSM-1 contained 1226 mg free gossypol kg^{-1}, determined according to the American Oil Chemists Society (1973), 68.2 g residual lipid kg^{-1} and 290 mg CPFA kg^{-1}, determined according to Hammonds et al. (1971) (4.3 g CPFA kg^{-1} cottonseed lipid), whereas the corresponding values for CSM-2 were 1086 mg free gossypol kg^{-1}, 69.2 g residual lipid kg^{-1} and 361 mg CPFA kg^{-1} (5.2 g CPFA kg^{-1} cottonseed lipid). The CSM-1 and CSM-2 were used in Experiments 1 and 2, respectively.

Experiment 1

The diets are shown in Table 1. The CSM-1 was treated with FSH in crystalline or in the solution form, at a 4:1 weight ratio of iron to the free gossypol present, based on analysis of the CSM. The supplemental dietary iron concentration was 1470 mg kg^{-1}. To evaluate whether the adverse effects of FSH on laying performance were caused by its interaction with a specific component of the CSM diet, a control diet was also treated with crystalline FSH. The diets were: 0CSM, control diet (no CSM or cottonseed lipid); 0CSMFe(c), 730 g of crystalline FSH (ground to pass through a 0.2 mm screen) mixed with the 0CSM diet in a horizontal mixer for 30 min; CSM, 300 g CSM-1 kg^{-1} diet; CSMFe(s), 730 g of FSH, dissolved in 1 l of distilled water, mixed with 30 kg of CSM-1 for 30 min before incorporation into a 300 g CSM-1

TABLE 1

Composition of the experimental diets (g kg^{-1} unless otherwise stated)

Constituents	Control diet	300 g CSM kg^{-1} diet
Fish meal	4.8	60.0
Maize	400.0	527.1
Soya bean meal	90.0	
Wheat middlings	100.7	
Sunflower meal	210.0	–
Cottonseed meal		300.0
Meat and bone meal	40.0	–
Maize gluten meal	28.2	
Palm kernel meal	2.4	
Maize oil	42.8	27.2
Limestone	75.8	80.4
Salt	2.3	2.3
Vitamin/mineral/choline premixes[1]	3.0	3.0

Determined analyses of diets	0CSM	0CSMFe(c	CSM	CSMFe(s)	CSMFe(c
Moisture	87.1	80.4	80.2	95.2	88.7
Crude protein	178.0	177.4	187.1	182.9	185.3
Crude fibre	76.2	76.4	49.9	51.6	47.0
Crude fat	73.5	71.5	72.2	67.5	69.7
Ash	128.5	132.4	118.9	15.9	122.6
Calcium	40.9	41.0	38.3	37.6	40.1
Phosphorus	7.0	7.0	5.1	5.1	5.3
Lysine[2]	9.5	9.5	9.5	9.5	9.5
Methionine + cystine[2]	7.0	7.0	7.0		7.0
Metabolisable energy (MJ kg^{-1})[2]	11.4	11.4	11.4	.4	11.4
Free gossypol (mg kg^{-1}		17	262	72	76
Cyclopropenoid fatty acids (mg kg^{-1})[4]		0	87	87	87
Iron (mg kg^{-1})	204	1792	223		1703

[1]For premix composition see Panigrahi et al. (1989).
[2]Calculated values.
[3]Apparent background level.
[4]Calculated on the basis of analysis of the original CSM.
0CSM, control diet; 0CSMFe(c), control diet treated with FSH crystals; CSM, 300 g CSM kg^{-1} diet; CSMFe(s), 300 g FSH solution-treated CSM kg^{-1} diet; CSMFe(c), 300 g FSH crystals-treated CSM kg^{-1} diet.

TABLE 2

Composition of the diets in Experiment 2 (g kg^{-1} unless otherwise stated)

Diets	0CSM	CSM	CSMFe
Cassava root meal	–	45.2	
Fish meal	20.0	25.0	
Maize	531.6	515.8	
Soya bean meal	180.0	–	
Meat and bone meal	2.8	–	
Sunflower meal	152.8	–	
Cottonseed meal	–	300.0	
Dicalcium phosphate	10.4	8.0	
Limestone	84.4	85.2	
Salt	3.4	3.6	
Lysine	–	3.0	
Methionine	0.6	0.9	
Maize oil	11.0	10.3	
Vitamin/mineral premix[1]	3.0	3.0	
Ferrous sulphate heptahydrate	–	–	

Determined analyses of diets	0CSM	CSM	CSMFe(s)	CSMFe(c)
Moisture	104.4	95.2	105.3	95.6
Crude protein	171.5	168.1	170.0	189.0
Crude fibre	69.0	67.0	64.4	62.1
Crude fat	37.1	50.4	49.3	54.3
Ash	127.7	130.4	122.2	119.6
Calcium	37.1	38.8	37.1	36.1
Phosphorus	6.4	6.3	5.9	6.1
Lysine[2]	9.5	9.5	9.5	9.5
Methionine+cystine[2]	7.0	7.0	7.0	7.0
Metabolisable energy (MJ kg^{-1})[2]	11.6	11.6	11.6	11.6
Free gossypol (mg kg^{-1})	18	218	89	74
Iron (mg kg^{-1})	277	324	1567	1608

[1]For premix composition see Panigrahi et al. (1989).
[2]Calculated values.
0CSM, control diet; CSM, 300 g CSM kg^{-1} diet; CSMFe(s), 300 g FSH solution-treated CSM kg^{-1} diet; CSMFe(c), 300 g FSH crystal-treated CSM kg^{-1} diet.

kg^{-1} diet; CSMFe(c), 730 g of FSH, was mixed with 30 kg of CSM-1 for 30 min before incorporation into a 300 g CSM-1 kg^{-1} diet.

Experiment 2

The treatments used were similar to those of Experiment 1, except that the 0CSMFe(c) diet was omitted (Table 2). The supplemental dietary iron concentration was approximately 1300 mg kg^{-1}. The diets were: 0CSM, control diet (no CSM); CSM, 300 g CSM-2 kg^{-1} diet; CSMFe(s), 766 g FSH, dissolved in 2 l of tap water, mixed with 36 kg of CSM-2 in a horizontal mixer

for 30 min, and left to stand for a further 6 days before incorporation into a 300 g CSM-2 kg^{-1} diet; CSMFe(c), 766 g of crystalline FSH mixed with the CSM-2 in a similar manner to the CSMFe(s) diet.

Feeding trials

To minimise deterioration of the diets containing CSM treated with FSH, the feeding trial commenced within 7 days of diet preparation. In Experiment 1, point-of-lay, Dekalb G-Link (DGL) pullets were housed individually in battery cages, arranged in two blocks of two tiers, each cage supplied with an hopper and nipple water drinker. The experimental room was maintained at 20°C and the photoperiod increased gradually from 8 h at 18 weeks to 15 h by Week 26. Pullets were fed on the control diet until all were in lay. They were then allocated to the five dietary groups in a randomised block design with 16 hens per treatment, ensuring an equal number for each treatment in each tier. Hens were fed on the experimental diets for 10 weeks and the following data collected: daily egg output, weekly food intakes, and the overall change in the body weights of hens.

In Experiment 2, 41-week-old Hubbard Golden Comet (HGC) hens were used, with the photoperiod being set at a constant 15 h per day. Hens housed individually were fed on the control diet for 10 days, during which time egg production was monitored to ensure that each laid a minimum of 0.7 eggs per hen per day. Birds were then transferred to the experimental diets, using 17 hens per treatment, for a period of 8 weeks during which their performance was monitored as in Experiment 1.

Egg discolouration study

Eggs were collected from each hen in each experiment between 2 and 6 weeks and examined for changes in colour and pH of yolks and albumen. Tests were carried out under various conditions: on fresh eggs, on eggs subjected to atmospheres containing ammonia (egg contents, in petri dishes, were placed in dessicators containing 25 ml of a 350 g l^{-1} ammonia solution for 30 min), and on eggs stored at 20°C for 1 month and at 5°C for up to 3 months. Brown yolk discolouration was evaluated visually according to a pre-determined colour standard ranging from 0 for none, 1 for very light brown (not objectionable) to 8 for black colour. Yolk mottling was scored on a 0–3 M (mottling) grading system with 3 M representing severe mottling in patches that resembled a discolouration score of 1. Pink albumen and apricot yolk discolourations were evaluated according to 0–8 visual standards.

Statistical analyses

The laying performance data were treated to analysis of variance, using SPSS (1988). In instances where significant treatment effects were observed, the multiple comparison test procedure of least-significant differences ($P<0.05$) was used to determine any significant differences between means. Standard errors were generated using a fixed effects model.

RESULTS

Experiment 1

Effects of CSM on egg production

DGL hens fed on the CSM diet had a 2.9% lower food intake and a 5.4% lower egg output compared with those fed on the 0CSM diet, but these differences were not statistically significant (Table 3).

Effects of FSH treatment of CSM on egg production

The CSMFe(s) diet reduced food intake initially (11.2% lower compared with the 0CSM diet in the first week), and this was accompanied by reduction in egg output during the first 3 weeks; however, food intake and egg output (Fig. 1) increased, and were similar to those in the 0CSM group by Week 10 of feeding. The CSMFe(c) diet produced an immediate and large reduction in food intake (36.2% lower in Week 1) and egg production. These hens also lost body weight – by Week 10, the loss was approximately 115 g compared to a gain of 90 g for hens fed on the CSM diet (Table 3). There was no sustained improvement in egg output as the feeding trial progressed.

Initially, hens fed the 0CSMFe(c) diet had a lower food intake than controls (22.4% lower in Week 1), but recovery was immediate and towards the end of the trial, food intake was similar to controls. The overall depression in food intake and egg output were 7.2 and 4.7%, respectively. Food intake in the 0CSMFe(c) group was always greater than in the CSMFe(c) group, but slightly lower than in the CSMFe(s) group.

Effects of CSM and iron-treated CSM on egg quality

There were no discolourations of any kind in freshly laid eggs from hens fed any of the diets, but subjecting eggs to atmospheres containing ammonia resulted in chocolate-brown yolk colours in the CSM group (Table 4). In cold-stored eggs, the CSM diet produced pronounced brown yolk discolouration, with some yolks becoming black and some albumen reddish-pink after 6 months of cold storage (not shown in Table 4). The yolks of some eggs also developed shades of apricot colours.

In the two FSH-treated CSM groups, brown yolk discolouration did not

TABLE 3

Performance of hens in Experiment 1

Diets	0CSM	0CSMFe(c)	CSM	CSMFe(s)	CSMFe(c)	SE	Probability[1] (<or=to)
Food intake (g per hen day^{-1})	121.6^{a}	112.9^{b}	118.1^{a,b}	118.3^{a,b}	101.4^{c}	3.01	0.0001
Eggs laid per hen day^{-1}	0.95^{a}	0.94^{a,b}	0.92^{a,b,c}	0.88^{b,c}	0.86^{c}	0.023	0.0412
Egg mass per hen day^{-1} (g)	55.7^{a}	53.1^{a,b}	52.7^{a,b}	50.5^{b}	45.5^{c}	1.46	0.0001
Change in egg weights (0–70 days)	+7.37^{a}	+3.91^{b}	+5.43^{a,b}	+3.72^{b}	+0.56^{c}	0.845	0.0001
Change in body weights (0–70 days)	+61^{b,c}	−23^{a,c}	+90^{b}	−6^{b,c}	−115^{a}	37.1	0.0020
Egg output kg^{-1} food (g kg^{-1})	458^{a,b}	470^{a}	446^{a,b}	427^{b}	449^{a,b}	11.4	0.0696
Number of eggs kg^{-1} food	7.8^{a,c}	8.3^{c,d,e}	7.8^{a,d}	7.4^{a}	8.5^{e}	0.22	0.0050

SE, standard error of treatment means.

Values in the same horizontal line without common superscripts are significantly different ($P<0.05$). For diet key, see Table 1.

[1]Residual degrees of freedom = 73.

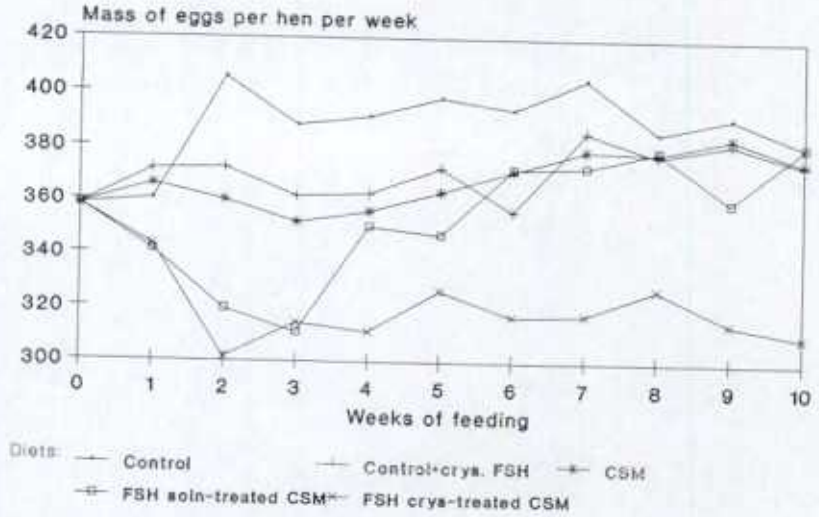

Fig. 1. Mass of eggs laid in Experiment 1.

TABLE 4

Discolourations and pH changes in eggs from Experiment 1

Diets	0CSM	0CSMFe(c)	CSM	CSMFe(s)	CSMFe(c)
Fresh eggs					
Yolk colour	0	0	0	0	0
Albumen colour	0	0	0	0	0
Yolk pH	5.85±0.05	5.90±0.06	5.92±0.05	5.94±0.05	5.94±0.08
Albumen pH	8.79±0.18	8.80±0.11	8.86±0.19	8.82±0.20	9.02±0.16
Yolk colours after ammonia test					
	0.4	0.5	5.82±0.23	0.3	0.5
Eggs stored at 20° C for 1 month					
Yolk colour	0	0	2.9M	0	0
Albumen colour	0	0	0	0	0
Yolk pH	6.5	6.7	6.6	6.7	6.6
Albumen pH	9.5	9.6	9.6	9.7	9.7
Eggs stored at 5° C for 3 months					
Yolk colour	0	0	3.33±2.44	0	0
Albumen colour	0	0	0.83±1.17	0	0
Yolk pH	6.40±0.07	6.45±0.05	7.65±1.00	6.53±0.08	6.54±0.05
Albumen pH	9.18±0.04	9.17±0.06	8.93±0.06	9.19±0.06	9.15±0.09

Each colour and pH figure is the mean of readings from ten eggs; SEM are indicated as ±. Yolk colour scores for eggs from the CSM group refer to chocolate-brown discolouration, while those for the CSMFe groups refer to apricot discolouration. M denotes mottling. All albumen colour scores refer to pink discolouration. For diet key, see Table 1.

develop to any significant degree on exposing eggs to gaseous ammonia, indicating that complete inactivation of the free gossypol had been achieved by treatment of the CSM with FSH. Observations on cold-stored eggs also showed no brown colour development in the CSMFe(s) and CSMFe(c) groups, although many of these eggs had apricot-coloured yolks and pink-coloured albumen (CPFA-related effects). A detailed description of the egg discolouration effects of these diets was reported previously (Panigrahi and Hammonds, 1990).

Experiment 2

Effects of CSM on egg production

These are summarised in Table 5. HGC hens fed on the CSM diet had a 13.8% lower food intake and a 8.6% lower egg output ($P=0.0524$) than those fed the 0CSM diet. The efficiency of lay was higher in the CSM group than in the 0CSM ($P=0.07$) and the CSMFe(s) groups.

Effects of FSH treatment of CSM on egg production

Hens fed on the CSMFe(c) diet had lower food intakes and egg output than those fed on the 0CSM and CSM diets, although there were small improvements as the feeding trial progressed (Fig. 2); however, hens fed on the CSMFe(s) diet had a higher food intake than those fed on the CSM diet, with food intake and egg output of the former improving to control levels by Weeks 5 and 8 of feeding, respectively. There was also a significant increase in the body weight of these hens compared with those fed on the CSMFe(c) diet.

Effects of CSM and iron-treated CSM on egg quality

The CSM diet produced slightly brown yolks in freshly laid eggs, the colour intensity increasing slightly during storage at 20°C. On cold storage of eggs for 2 months, most of the yolks became severely swollen and brown, with areas of orange colour developing on their surfaces. Yolks from fresh eggs became chocolate-brown in colour after exposure to ammonia gas.

There were no signs of brown colour in yolks of fresh eggs in either of the FSH-treated CSM groups. However, it was noticeable that eggs in the CSMFe(s) group had the more yellow yolks (scored −0.5 in Table 6), and from this standpoint, were considered more acceptable. After exposure of the contents of eggs to ammonia gas, yolks in the CSMFe(c) group resembled those in the 0CSM group, but eggs in the CSMFe(s) group were, again, slightly more yellow. On cold storage of eggs, brown colour did not develop in yolks in either of the iron-treated CSM groups, but significant CPFA-related increases in yolk pH and apricot yolk and pink albumen discolourations took place.

TABLE 5

Performance of hens in Experiment 2

Diet	0CSM	CSM	CSMFe(s)	CSMFe(c)	SE	Probability[1] (<or=to)
Food intake (g per hen day^{-1})	126.5^{a}	109.0^{b}	122.1^{a}	99.9^{b}	3.51	0.0001
Eggs laid per hen day^{-1}	0.88^{a}	0.83^{a,b}	0.83^{a,b}	0.76^{b}	0.030	0.0541
Egg mass per hen day^{-1} (g)	55.8^{a}	51.0^{a}	51.8^{a}	45.9^{b}	1.78	0.0025
Change in egg weight (0–56 days)	+2.16^{a}	+0.88^{a,b}	+0.22^{b}	−1.32^{c}	0.511	0.0001
Change in body weight (0–56 days)	+82^{a}	−103^{b}	+3^{a}	−149^{b}	31.0	0.0001
Egg output kg^{-1} food (g kg^{-1})	441^{a,b}	468^{b}	424^{a}	459^{a,b}	10.2	0.0247
Number of eggs kg^{-1} food	6.9^{a,d}	7.6^{b,c}	6.8^{a}	7.6^{d,c}	0.19	0.0037

SE, standard error of treatment means. Values in the same horizontal line without common superscripts are significantly different ($P<0.05$). For diet key, see Table 2.

[1]Residual degrees of freedom = 64.

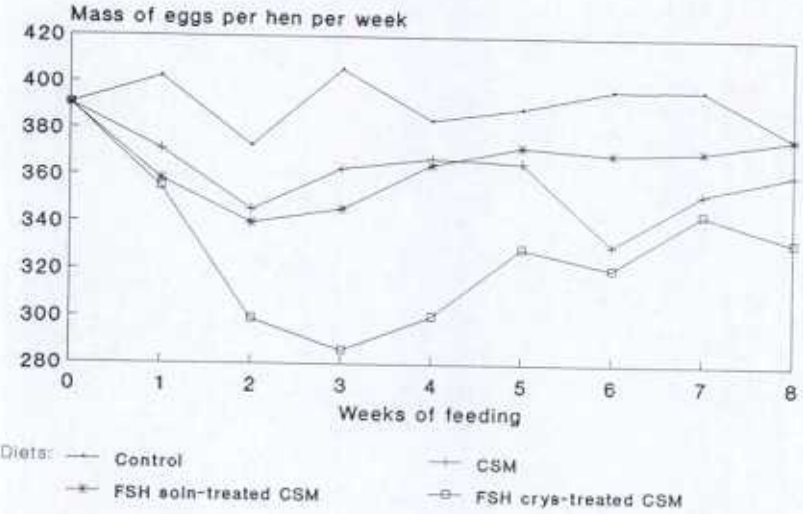

Fig. 2. Mass of eggs laid in Experiment 2.

TABLE 6

Discolourations and pH changes in eggs from Experiment 2

Diets	0CSM	CSM	CSMFe(s)	CSMFe(c)
Fresh eggs				
Yolk colour	0	1.0	−0.5[1]	0
Albumen colour	0	0	0	0
Yolk pH	5.91 ± 0.05	5.90 ± 0.13	5.95 ± 0.05	5.89 ± 0.06
Albumen pH	8.79 ± 0.12	8.93 ± 0.19	8.87 ± 0.11	8.95 ± 0.14
Yolk colours after ammonia test				
	0	6.04 ± 0.36	0	0.36 ± 0.50
Eggs stored at 20° C for 1 month				
Yolk colour	0	1.64 ± 0.46	−0.57[1] ± 0.56	0
Albumen colour	0	0	0	0
Yolk pH	6.42 ± 0.13	6.60 ± 0.10	6.44 ± 0.15	6.52 ± 0.08
Albumen pH	9.62 ± 0.03	9.63 ± 0.05	9.60 ± 0.04	9.60 ± 0.03
Eggs stored at 5° C for 2 months				
Yolk colour	0	3.94 ± 2.04	1.52 ± 1.77	2.13 ± 1.78
Albumen colour	0	0.30 ± 0.45	0	0.10 ± 0.35
Yolk pH	6.32 ± 0.07	7.51 ± 0.78	7.14 ± 0.77	7.50 ± 0.83
Albumen pH	9.24 ± 0.05	8.98 ± 0.19	9.00 ± 0.21	8.95 ± 0.21

[1]0.5 denotes the more yellow colour of these yolks.
Each colour and pH figure is the mean of readings from 17 eggs. SEM are indicated as ±. Yolk colour scores for eggs from the CSM diet group refer to chocolate-brown discolouration, while those for the iron-treated CSM diet groups refer to apricot discolouration. All albumen colour scores refer to pink discolouration. For diet key, see Table 1.

DISCUSSION

The results of this study indicate that the depressive effect of free gossypol may vary with the batch of hen tested, with age or genotype of the hen (Panigrahi and Morris, 1991) being the responsible factor. In 24-week-old DGL hens, a 300 g CSM kg^{-1} diet containing 262 mg free gossypol kg^{-1} and 87 mg CPFA kg^{-1} reduced food intake by only 2.9% (Experiment 1), but in 41-week-old HGC hens, a similar diet containing 218 mg free gossypol kg^{-1} and 108 mg CPFA kg^{-1} produced a 13.8% depression (Experiment 2). These represented daily intakes of 31.0 mg and 23.8 mg of free gossypol, and 10.3 mg and 11.8 mg of CPFA for DGL and HGC breeds, respectively, of which CPFA intakes were not expected to have had any significant influence on the performance of hens (Phelps et al., 1965).

The fact that the depressive effect of the 300 g CSM kg^{-1} diet in HGC hens could not be overcome with time, the food intake during the last 6 weeks of the trial remaining relatively constant at 111 g per hen per day, compared with 120 g per hen per day for the control diet, indicates that CSM poses a toxic rather than a palatability problem in hens. Interestingly, despite lower food intakes, the efficiency of lay, expressed in terms of egg output related to food intake, was highest in the CSM group; this was, however, achieved at the cost of a large reduction in body weight, and may, therefore, suggest that the toxic effect of CSM in hens occurs through a mechanism not directly related to the reproductive process. No evidence has been obtained from the studies carried out at this Institute which would support the suggestion that CSM or free gossypol stimulates lay at low dietary concentrations (Heywang and Bird, 1954; Phelps, 1966).

The egg discolouration effects of screw-pressed CSM and methods for their prevention have been reported recently (Panigrahi and Hammonds, 1990). In the present study, low temperature storage of eggs from hens fed on 300 g CSM kg^{-1} diets resulted in severe brown yolk discolouration, the deposit of gossypol in the yolk being confirmed by exposure of freshly laid eggs to atmospheres containing ammonia (Panigrahi, 1990). Treatment of CSM with FSH at a 4:1 weight ratio of iron to free gossypol, in solution or as crystals, was effective in preventing this discolouration, with the solution method in Experiment 2 producing eggs with slightly more yellow, and hence, more acceptable yolks.

Treatment of CSM with crystalline FSH severely depressed food intake and egg production, with only a marginal improvement taking place as the feeding trials progressed. This finding is consistent with previous observations (Panigrahi and Morris, 1991). Interestingly, in Experiment 1, crystalline FSH treatment of the CSM diet produced a greater depression of performance than similar treatment of a non-CSM control diet, suggesting that the tolerance of hens to supplemental iron may depend on other dietary factors. There is un-

certainty concerning the tolerance level of poultry for iron, with McGhee et al. (1965) reporting that dietary levels as low as 100 mg kg^{-1} may be toxic to young chicks. Excess dietary iron may reduce the absorption of phosphorus (Cox et al., 1931; Deobald and Elvehjem, 1935; McDonald et al., 1981), or interact with the metabolism of copper (Hill and Matrone, 1961) and manganese (Matrone et al., 1959). There are also indications that the toxic effects of excess dietary iron may become critical to hens in the laying period (Panigrahi and Morris, 1991).

Treatment of CSM with FSH in solution slightly depressed food intake and egg production initially, but the effects were rapidly overcome. Thus, the solution method of treating CSM with FSH appears to offer major advantages over the dry salt treatment method. The present results are, however, in contrast to the previously reported adverse effects on egg production of FSH treatment in solution at an iron concentration of only 100 mg kg^{-1} diet (Panigrahi et al., 1989), caused, it is believed, by a deterioration of feed prior to being fed to hens. Feed deterioration may be minimised by drying the feed after iron treatment, as carried out by Clawson et al. (1975), and this aspect warrants further investigation.

Bressani et al. (1966) suggested that addition of water to CSM itself produces a 50% reduction in free gossypol content. This, however, is unlikely to have been the reason for the better performance of hens fed on the CSMFe(s) diet in the present study because egg quality tests indicated that free gossypol had been almost completely inactivated by both iron treatment methods. The colour of CSM is normally yellow-orange (possibly derived from gossypol pigment) with black specks of seed testa; however, it turns darker on treatment with FSH, the change in colour of the feed perhaps representing the complexation of iron with gossypol. Because treatment with FSH in solution imparts a considerably darker appearance to CSM than the crystalline salt treatment, a greater proportion of the iron may bind with gossypol (and perhaps other feed components) in the former, leaving less iron available for toxic interactions in hens. The observation in Experiment 2 that yolks of eggs from the CSMFe(s) group were more yellow than those from the CSMFe(c) group provides some support for this view.

As expected, cold storage of eggs from the iron-treated CSM-based diet groups produced apricot yolk and pink albumen discolourations, effects that are unlikely to occur if unrefrigerated eggs are consumed within 3 weeks of being laid. However, CPFA alter the saturated to unsaturated fatty acid balance in animals by an inhibition of the fatty acyl desaturase system (Raju and Reiser, 1967), and have also been observed to be mitogenic in rat and rainbow trout hepatocytes (Scarpelli, 1974). Although the daily consumption of an egg that could contain up to 1.7 mg of CPFA (Panigrahi and Hammonds, 1990), is unlikely to pose such health hazards to humans, pre-pressed solvent-extracted CSM, with low concentrations of free gossypol and CPFA, would

seem to be of the best type for inclusion in laying hen diets. Further studies are required to determine the optimal quantities of iron and water, and the mixing and drying conditions required, before CSM can be recommended for feeding to laying hens at 300 g kg^{-1} diet.

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REFERENCES

American Oil Chemists Society (AOCS), 1973. Sampling and Analysis of Vegetable Oil Source Materials. Oilseed By-products. Official and Tentative Methods of the American Oil Chemists Society, Champaign, IL, USA, official method Ba 7. 58.

Bressani, R., Braham, J.E., Elias, L.G., Jarquin, R., Gonzalez, J.M. and Dysli, R., 1966. Effect of mineral salts upon the performance of swine chicks and rats fed gossypol, pigment glands, and cottonseed meal. In: Proc. of the Conf. for the Inactivation of Gossypol with Mineral Salts, 4–5 April 1966 New Orleans, LA, National Cottonseed Products Association, Memphis, TN, pp. 76–82.

Clawson, A.J., Maner, J.H., Gomez, G., Flores, Z. and Buitrago, J., 1975. Unextracted cottonseed in diets for monogastric animals. II. The effect of boiling and oven vs sun drying following pretreatment with a ferrous sulphate solution. J. Anim. Sci., 40: 648–654.

Cox, G.J., Dodds, M.L., Wigman, H.B. and Murphy, F.J., 1931. The effects of high doses of aluminium and iron on phosphorus metabolism. In: Scientific Proceedings of the XXV Meeting of the Society of Biological Chemists. J. Biol. Chem., 92: 11.

Deobald, H.J. and Elvehjem, C.A., 1935. The effect of feeding high amounts of soluble iron and aluminium salts. Am. J. Physiol., 111: 118–123.

Hammonds, T.W., Cornelius, J.A. and Tan, L., 1971. Use of the Halphen reaction for the determination of the cyclopropenoid content of lipids. Analyst, 96: 659–664.

Heywang, B.W. and Bird, H.R., 1954. Egg production, diet consumption and live weight in relation to the free gossypol content of the diet. Poult. Sci., 33: 851–854.

Hill, C.W. and Matrone, G., 1961. Studies on copper and iron deficiencies in growing chickens. J. Nutr., 73: 425–431.

Matrone, G., Hartman, R.H. and Clawson, A.J., 1959. Studies of a manganese–iron antagonism in the nutrition of rabbits and baby pigs. J. Nutr., 67: 309–317.

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D., 1981. Nutrient requirements of laying hens. In: Feeding Standards for Reproduction and lactation. Animal Nutrition, Longman, London, p. 304.

McGhee, F., Creger, C.R. and Couch, J.R., 1965. Copper and iron toxicity. Poult. Sci., 44: 310–312.

Panigrahi, S., 1990. Ammonia and the dietary cottonseed meal-associated brown yolk discolouration in hens eggs. Trop. Sci., 30: 325–342.

Panigrahi, S., Plumb, V.E. and Machin, D.H., 1989. The effects of dietary cottonseed meal and iron-treated cottonseed meal in laying hens. Br. Poult. Sci., 30: 641–651.

Panigrahi, S. and Hammonds, T.W., 1990. Egg discolouration effects of including screw-press cottonseed meal in laying hen diets and their prevention. Br. Poult. Sci., 31: 107–120.

Panigrahi, S. and Morris, T.R., 1991. Effects of dietary cottonseed meal and iron-treated cottonseed meal in different laying hen genotypes. Br. Poult. Sci., 32: 167–184.

Phelps, R.A., 1966. Cotton seed meal for poultry: from research to practical application. World's Poult. Sci. J., 22: 86–112.

Phelps, R.A., Shenstone, F.S., Kemmerer, A.R. and Evans, R.J., 1965. A review of cyclopropenoid compounds: biological effects of some derivatives. Poult. Sci., 44: 358–494.

Raju, P.K. and Reiser, R., 1967. Inhibition of fatty acyl desaturase by cyclopropene fatty acids. J. Biol. Chem., 242: 379–384.

Scarpelli, D.G., 1974. Mitogenic activity of sterculic acid, a cyclopropenoid fatty acid. Science, 185: 958–959.

SPSS, 1988. SPSS/PC+V20 Base Manual for IBM PC/XT/AT and PS2. SPSS, Chicago, IL, USA.

Waldroup, P.W., Goodner, T.O. and Sloan, D.R., 1970. Use of cottonseed meal in diets for commercial egg production. Feedstuffs, April: 20–22.