Pilot Plant Studies on Extracting Cottonseed with Methylene Chloride

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The practical feasibility of using methylene chloride to extract oil, aflatoxin and gossypol simultaneously from cottonseed flakes was demonstrated in a 56-hr experimental run using a pilot-scale, continuous extractor. Nine different trials varying in extraction time, solvent:flake ratio, flake preparation method and blending with 5% ethanol were evaluated. Residual oil contents were lower than typically achieved in extraction with hexane. Aflatoxin contents of the meals were reduced by 73–92% of the level in cottonseed meats, making possible the upgrading of a large portion of cottonseed meal that otherwise would exceed current action levels. Because gossypol also was extracted, it was possible to produce cottonseed meal that was well suited for use in poultry feeds, especially when a blend of 5% ethanol in methylene chloride was used. Meal desolventized easily, and residual levels of methylene chloride were generally less than 12 ppm. The oil was refined and bleached to acceptable quality standards, and no residual aflatoxin was detected in alkali-refined oil.

Two recent reviews (1,2) on alternative solvents for extraction of oilseeds have indicated that methylene chloride (dichloromethane) has some very desirable characteristics making it attractive as an oils extraction solvent. These include improved solvating properties, low boiling point, low specific heat, low latent heat of vaporization and low solubility of water. The National Fire Protection Association has ranked methylene chloride as a practically nonflammable substance. The possibility of using methylene chloride to extract oil from soybeans was recognized in the 1940's (3) and demonstrated in an early prototype of a popular, continuous, shallowbed extractor (4), but the ready availability of relatively inexpensive hexane made the use of methylene chloride less attractive. However, after the OPEC oil embargo of 1973, prices escalated considerably and there have been periodic short supplies of hexane, which have made methylene chloride more competitive and attractive.

Certain advantages in extraction selectivity of methylene chloride also have been recognized recently by the cottonseed crushing industry. In addition to extracting oil, methylene chloride has been shown to reduce gossypol and aflatoxin contents of cottonseed meal (5,6). Gossypol is a red-black pigment found in most cottonseed kernels at 0.8–1.5% concentration that may be toxic to monogastric animals. Consequently, cottonseed meal is fed only to ruminants (cattle and sheep) unless severely heat treated to inactivate gossypol by covalently binding it to lysine residues in protein. This destroys some lysine and other essential amino acids, and lowers feed conversion rates. Aflatoxin, a toxin produced by the mold *Aspergillus flavus*, is toxic to all animals, and oilseed meals containing more than 20 ppb total aflatoxin are not normally permitted in food or feed. In July 1982, a revised action level was granted by the U.S. Food and Drug Administration allowing cottonseed meal with up to 300 ppb total aflatoxin to be used in feeds for beef cattle, swine and poultry in the U.S. (7). Cottonseed meal used in feeds for dairy animals still must contain 20 ppb aflatoxin or less. Meals exceeding 300 ppb cannot be sold for feed and normally are relegated to fertilizer at greatly discounted prices. If cottonseed meal could be degossypolized, it could compete with soybean meal in poultry and swine feed markets at prices up to 18% higher than prices currently traded at; if cottonseed meal derived from aflatoxin contaminated seed could be made "consumable," it could command prices 15–70% higher than current prices, depending upon whether the reduced level was less than 20 or less than 300 ppb.

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MATERIALS AND METHODS

Extraction trials. Aflatoxin contaminated cottonseed was cleaned, delintered and hulled/separated into meats using production-scale equipment at an operating oil mill. Meats were rolled into flakes in our pilot plant by one of two different methods involving either single or double rolling. In single rolling, the meats were conditioned by adding sufficient water to increase their moisture content to 12% and heating to 88 C in a stack cooker. The meats were immediately rolled into flakes averaging 0.28 ± 0.041 mm (0.0112 ± 0.0016 in.) in thickness. In double rolling, meats were adjusted to 12% moisture, rolled to 0.56 mm (0.020 in.), heated to 88 C and rerolled to 0.28 ± 0.041 mm. After rolling, the moisture content of the flakes was about 10% and they contained 260–693 ppb aflatoxin (Table 1).

The flakes were extracted in a continuous, shallowbed (0.15 m 6-in. bed depth) extractor manufactured by Crown Iron Works, Minneapolis, Minnesota (Fig. 1) and having a name-plate capacity of one ton per day. Nine different treatments, varying in method of rolling, solvent usage, extraction time and solvent composition, were conducted during a continuous 56-hr period. Eight trials involved using extraction-grade methylene chloride, and a ninth trial involved using a blend of 95 parts by volume extraction-grade methylene chloride and five parts anhydrous ethanol. Solvent usage was varied from 1.6 to 4.0 Kg solvent per Kg flakes. Extraction time was varied from 45 to 90 min. Extraction temperatures were 35 C for trials using methylene chloride and 30 C for the trial

TABLE 1

Composition of Cottonseed Flakes Used in Extraction Studies[a]

Moisture (%)	10.1
Oil (%)	26.3
Total gossypol, AOCS (%)	0.80
Free gossypol, AOCS (%)	0.69
HPLC (%)	0.51
Aflatoxin (ppb)	260–693

[a]As is basis.

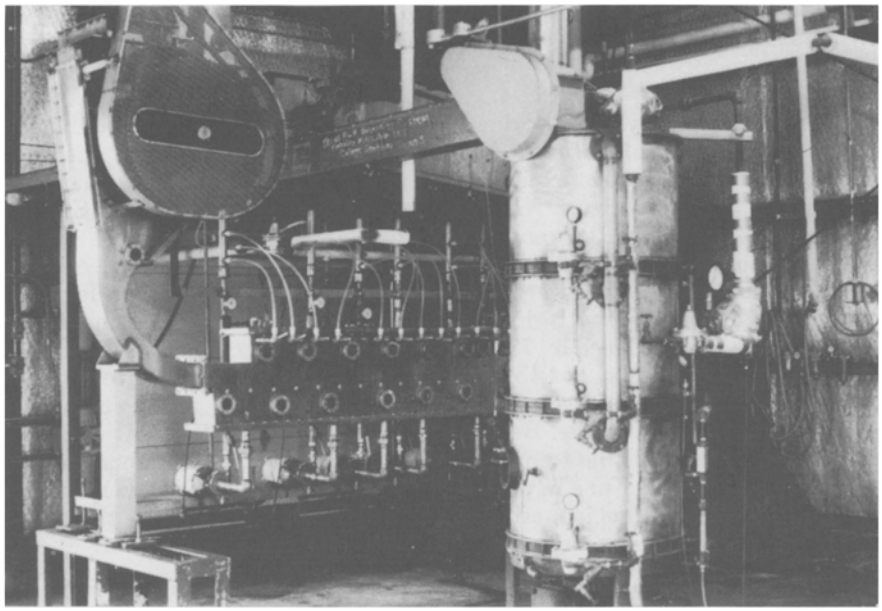

FIG. 1. Pilot plant equipment for evaluating methylene chloride as an extraction solvent.

using the ethanol/methylene chloride blend. Spent flakes were desolventized using a Crown Iron Works three-tray desolventizer without any steam sparging. The dome temperature of the desolventizer was 63 C using methylene chloride and 54 C using the ethanol/methylene chloride blend. Meal exit temperatures from the desolventizer were 95 C using methylene chloride and 91 C using the ethanol/methylene chloride blend.

Analyses. Samples of feed flakes, miscella, spent flakes and meal were taken every 15 min. Each value is the mean of six to 10 observations from samples taken at different times during the extended operating period (at least four hr at steady-state). Proximate analyses and gossypol analyses were conducted by AOCS standard methods. Protein dispersibility index (PDI) was determined in 0.02N NaOH by standard procedures. Available lysine was determined by the method of Carpenter (8).

Residual methylene chloride was determined by a gas chromatographic procedure found to be sensitive to 0.1 ppm methylene chloride. About 10 g of meal was extracted with 25 ml of perchloroethylene under 15 min of vigorous agitation. The extract was allowed to settle, and a subsample was injected directly into a gas chromatograph equipped with a 3 m × 3.2 mm SS column of silicon DC-550 on Chromosorb P and a flame ionization detector. Methylene chloride was eluted isothermally at 80 C. The column was backflushed with helium after perchloroethylene and methylene chloride were eluted.

Aflatoxin was extracted with aqueous acetone (95% acetone) using the appropriate portion of AOCS standard method Aa 8-83 and was quantified by high performance liquid chromatography (HPLC). Aflatoxin was eluted isocratically from a small particle (10 μm) μ-Porasil porous silica gel column (30 cm × 3.9 mm) with benzene:acetonitrile:formic acid (83:12:5) at a flow rate of 1.2 ml/min at a nominal pressure of 70 Kg/cm^2. A fluorescence detector with an excitation filter of 365 nm and an emission filter of 420 nm was used.

Free gossypol in the meal was also analyzed by an HPLC procedure (9). Free gossypol was extracted with aqueous acetone (70% acetone) using the appropriate portion of AOCS standard method Ba 7-58. Gossypol was eluted from a reverse phase μ-Bondapak C_{18} column (30 m × 3.9 mm) using tetrahydrofuran:0.001 M phosphate buffer, pH 3.5 (60:40) at a flow rate of 1.7 ml/min. Gossypol was detected spectrophotometrically at 254 nm.

In miscella refining, full miscella was concentrated in a rotary evaporator to about 60% oil concentration and refined with alkali using AOCS standard method Ca 9e-52 with 0.25% excess sodium hydroxide. Tests for color, gossypol, phosphorus, free fatty acid and unsaponifiables were AOCS standard methods.

Preliminary feeding study. In a preliminary poultry feeding study conducted at the University of Arizona, cottonseed meals from extraction trials, Trial 2 and Trial 9, were evaluated as feed ingredients for laying hens and broiler chicks at dietary levels of 10, 15 and 20%. Performance was compared to the same levels of a prepress hexane-extracted cottonseed meal obtained from a commercial mill. A basal diet comprised of soybean meal as the primary protein source was used as a control. From the 28-day laying hen study, 120–160 eggs were collected, stored at 7.2 C and broken and examined for green discoloration in yolks after one and two months.

RESULTS AND DISCUSSION

Extraction characteristics. Cottonseed meals from the nine extraction trials were characterized for moisture, residual oil (ether extractables), residual free gossypol, aflatoxin reduction and residual methylene chloride (Table 2). In three trials (Trial 1, Trial 2 and Trial 3), flakes were prepared in the same manner as the conventional practice in most direct solvent extraction cottonseed mills and only the solvent:flake ratio was varied, ranging from 1.6 to 4.0 Kg per Kg of feed flakes. Cottonseed mills employing direct solvent extraction normally

TABLE 2

Effect of Extraction Conditions on Composition of Cottonseed Meal Extracted with Methylene Chloride[a]

Trial	Rolling method	Solvent	Solvent:flake ratio (Kg/Kg)	Extraction time (min)	Residual oil (%)	Residual free gossypol (%)		Aflatoxin reduction (%)	Residual $MeCl_2$ (ppm)
						AOCS	HPLC		
1	Single	$MeCl_2$	1.6	60	0.24	0.094	0.032	81.0	5.6
2	Single	$MeCl_2$	3.2	60	0.24	0.095	0.035	87.5	10.8
3	Single	$MeCl_2$	4.0	60	0.25	0.095	0.031	81.4	9.4
4	Single	$MeCl_2$	3.2	90	0.37	0.082	0.021	92.1	6.9
5	Double	$MeCl_2$	2.6	90	0.64	0.131	0.056	80.2	4.5
6	Double	$MeCl_2$	2.5	75	0.75	0.125	0.048	79.7	4.6
7	Double	$MeCl_2$	2.1	45	1.31	0.112	0.043	83.8	21.6
8	Double	$MeCl_2$	1.8	60	1.32	0.119	0.058	74.3	9.4
9	Double	Blend	2.1	60	0.40	0.049	0.014	91.3	4.2
Statistical analysis[b]					S	S	S	S	S
Least significant difference					0.25	0.020	0.018	7.2	7.4

[a]All meal compositions have been adjusted to 10% moisture basis.

[b]S denotes significant differences between tratments at $P < 0.05$.

use about 1.6 Kg hexane per Kg flakes, which corresponded to about one liter of solvent per Kg of flakes. Because of the much greater density of methylene chloride than hexane, 1.33 g/cc versus 0.69 g/cc, an equivalent usage of solvent volume corresponds to 3.0 Kg methylene chloride per Kg of flakes. In these three trials methylene chloride was extremely effective in extracting oil during the 60 min period, with residual oil contents ranging 0.24 to 0.25%, which were much lower than the 1.25-2.0% normally achieved in direct extraction of cottonseed flakes with hexane in this equipment. Residual free gossypol contents of these three meals, as measured by the AOCS standard method, were much lower (0.09%) than those of typical hexane extracted meal (0.30%), but were not reduced to as low a level as desired (≤0.045%). However, the level of (actual) free gossypol, as measured by HPLC, was only 0.032-0.035%. Therefore, nearly two-thirds of the "free gossypol" content in these cottonseed meals extracted with methylene chloride was other aniline-reactive compounds, some related to gossypol, others not, such as flavonoids. Aflatoxin contents were reduced by 81-88% of the level in the feed flakes. Residual methylene chloride contents of the finished meals also were quite low, ranging from 5-22 ppm. Increasing the solvent:flake ratio had little effect on extraction of oil, free gossypol or aflatoxin.

In an attempt to increase the extraction of free gossypol, the method of preparing flakes was altered to incorporate a second step of rolling to achieve more complete rupture of gossypol glands which should increase extraction of free gossypol. However, comparisons of analyses of meals, such as Trial 1 with Trial 8, indicated that double rolling of cottonseed for direct solvent extraction did not improve extraction with methylene chloride (Table 2). Meal from flakes prepared by double rolling retained much higher contents of oil, free gossypol and even aflatoxin despite a slightly higher solvent:flake ratio. This may be the result of actually achieving less cell distortion and gland rupture. Good (10) showed larger pieces of soybeans passing through flaking rolls extracted faster and more completely than flakes prepared from smaller pieces. The meats probably were broken into smaller pieces while being rolled at the wide gap setting in the first pass before being rolled to their final thickness at the narrow gap in the second rolling. Single rolling was definitely preferred over double rolling in preparing cottonseed meats for direct extraction with methylene chloride.

Normally, commercial cottonseed extractors require 45-90 min residence time, depending upon the type of extractor used, and increasing the extraction time generally produces meal with lower residual oil content. In comparing Trial 2 with Trial 4, residual oil contents of both meals were quite low, with the shorter extraction time producing the lower residual oil content, 0.24 versus 0.37%. Slightly greater extractions of free gossypol and aflatoxin were observed at 90 min versus 60 min. The lowest levels of residual free gossypol and aflatoxin found in extraction with methylene chloride were observed in Trial 4, in which single-rolled flakes were extracted for 90 min with 3.2 Kg of methylene chloride per Kg of flakes. Under these conditions, 92% of the aflatoxin was extracted, and the defatted meal contained 0.08% free gossypol determined by the AOCS standard method and 0.02% actual free gossypol determined by the HPLC method.

In comparing Trial 5 and Trial 6, in which the less desirable double rolling method was used to produce flakes, more pronounced effects of extraction time might be expected. Slightly greater extraction of oil and aflatoxin were observed at longer extraction time, 90 min versus 75 min. However, residual free gossypol contents were slightly greater after 90 min extraction than after 75 min. In general, the effect of extraction time over the range 45 to 90 min appeared to be minimal, suggesting that extraction capacity might be increased using methylene chloride because extraction with methylene chloride appeared to be more rapid and complete than with hexane in our pilot plant or in commercial practice.

Laboratory trials had indicated that altering the polar-

TABLE 3

Effect of Extraction Solvent on Qualities of Cottonseed Oils

Oil	Factor	Extraction solvent		
		Methylene chloride	Methylene chloride/ ethanol blend	Hexane[a]
Crude	Gossypol, AOCS (%)	0.20	0.13	0.14
	Unsaponifiables (%)	0.75	0.77	0.75
	Phosphorus (ppm)	700	700	500
Refined	Gossypol, AOCS (%)	0.0020	0.0010	0.008
	Aflatoxin[b] (ppm)	ND	ND	ND
	Unsaponifiables (%)	0.61	0.61	0.62
	Phosphorus (ppm)	90	71	70
	Color	79Y-6.9R	79Y-9.9R	57Y-6.6R
	Bleached color	20Y-3.0R	20Y-3.5R	20Y-2.0R
Soapstock	Gossypol, AOCS[b] (%)	1.7	3.0	NA
	Aflatoxin[b] (ppb)	106	218	NA

[a]Obtained from a commercial oil mill.

[b]ND, non-detectable; NA, data not available.

ity of the solvent by an addition of anhydrous ethanol improved extraction of free gossypol and aflatoxin. Methylene chloride has a dielectric constant of 9.1 compared to 25.7 for ethanol. Blending 5% by volume of ethanol to methylene chloride (Trial 9) greatly enhanced extraction of aflatoxin, free gossypol and even oil from cottonseed flakes in these pilot plant trials. In comparing trials with similar solvent usage and the same rolling method and extraction time, Trial 8 versus Trial 9, meal extracted with the ethanol/methylene chloride blend contained one-third the residual levels of oil and free gossypol observed with methylene chloride. The extraction efficiency for aflatoxin was also 15% greater using the ethanol/methylene chloride blend than with methylene chloride. In comparing Trial 9 with Trial 6 in which a higher solvent:flake ratio and longer extraction time were used for extraction with methylene chloride, the ethanol/methylene chloride blend produced meals with levels of residual oil and free gossypol that were less than one-half those observed in meal extracted with methylene chloride. Efficiency in extracting aflatoxin was also 10% better when using the ethanol/methylene chloride blend than when using methylene chloride under more extreme conditions. Later laboratory tests have indicated that levels ranging from 1-10% ethanol are effective in enhancing extraction of aflatoxin and free gossypol, and that 5% ethanol may not be optimum. Nevertheless, over 90% reduction in aflatoxin content was achieved in Trial 9. Free gossypol content was reduced to 0.049% as measured by the AOCS procedure, which was very close to the food-grade level of 0.045%. Free gossypol, as measured by HPLC, was much lower, 0.014%. Preparing flakes by single rolling and using the optimum concentration of ethanol should further improve extraction of free gossypol and aflatoxin with equivalent or better recovery of oil than is achieved with hexane under similar processing conditions.

Oil quality. Qualities and compositions of oils extracted with methylene chloride were compared to commercial hexane-extracted oil (Table 3). More gossypol was observed in crude oil extracted with methylene chloride than was extracted with the ethanol/methylene chloride blend or with hexane. Unsaponifiable contents were not different. The phosphorus content of hexane-extracted oil was slightly lower than either experimental oil reflecting less phosphatides.

Good quality refined and bleached oils were obtained after miscella refining and laboratory bleaching. Slightly lower red color and less gossypol were observed in bleached oil that had been extracted with hexane and subjected to miscella refining at a commercial oil mill, compared to bleached oil extracted with either of the experimental solvents and miscella refined with 0.25% excess lye in the laboratory procedure simulating miscella refining. Furthermore, the oil extracted in these pilot trials contained quite a high free fatty acid content (9.6%) because of the five-day period between hulling/separating and the extraction trials. For these reasons, we and representatives of the cottonseed milling industry feel the low red color obtained in the laboratory indicates that acceptable quality refined and bleached oils can be obtained in commercial practice when using methylene chloride or the ethanol/methylene chloride blend as extraction solvents. No aflatoxin was detected in refined oils. Very large quantities of aflatoxin were observed in the soapstock from oils extracted with the experimental solvents, but no attempt was made to account for all of the aflatoxin on the basis of materials balance.

Meal quality. The composition of cottonseed meals extracted with methylene chloride and the ethanol/methylene chloride blend were compared to values of typical cottonseed meals produced by direct hexane extraction and prepress hexane extraction (11,12) (Table 4). Moisture contents of the experimental meals were lower because live steam was not sparged into the meal desolventizer and the finished meal was not humidified. Residual solvent levels were much lower in the experimental treatments than those observed in meals extracted directly with hexane. Greater recovery of methylene chloride from the meal may reduce solvent replacement

TABLE 4

Effect of Solvent on Qualities of Cottonseed Meals

Factor	Methylene chloride	Methylene chloride/ ethanol blend	Prepress[b] hexane	Direct[b] hexane
Moisture (%)	5.1	3.5	10.1	9.6
Residual solvent[c] (ppm)	10	5	NA	400-600
Ash (%)	7.5	7.6	6.4	6.4
Crude fiber (%)	11.8	12.1	13.6	12.4
Residual oil (%)	0.20	0.43	0.58	1.51
Acid hydrolyzed fat (%)	2.2	1.4	2.0	3.9
Protein (%)	44.8	46.0	41.4	41.4
Available lysine (%)	1.56	1.42	1.52	1.60
Protein dispersibility[a] (%)	37.7	16.1	54.4	69.4
Total gossypol, AOCS (%)	0.48	0.48	1.13	1.04
Free gossypol, AOCS (%)	0.100	0.053	0.05	0.30
HPLC[c] (%)	0.038	0.015	NA	NA

[a]In 0.02 N NaOH.

[b]All values as is basis from reference 11 except available lysine from reference 12 and residual solvent and acid hydrolyzed fat from analyses by the authors.

[c]NA, data not available.

TABLE 5

Effect of Extraction Solvent on Performance of Laying Hens

Treatment	Egg production rate (%)	Feed conversion (Kg/doz)	Average egg weight (g)	Egg mass output (g/bird/day)	Body weight change (g/bird/day)
Basal soybean meal	81.3	1.53	57.5	46.7	1.01
Hexane extracted cottonseed meal					
10%	85.0	1.54	57.6	49.0	−0.51
15%	77.8	1.66	58.2	45.3	0.00
20%	80.3	1.55	57.8	46.5	−0.51
Meal from Trial 2					
10%	77.8	1.55	56.2	43.7	−1.01
15%	81.3	1.56	57.3	46.5	−1.01
20%	82.8	1.49	57.3	47.4	−2.03
Meal from Trial 9					
10%	79.4	1.60	57.0	45.1	−0.51
15%	75.6	1.64	59.6	45.0	0.17
20%	82.5	1.48	57.3	47.3	0.51
Statistical analysis[a]	NS	NS	NS	NS	NS
Least significant difference	9.4	0.43	3.0	5.3	3.5

[a]NS, no significant difference between treatments at $P < 0.05$.

costs despite the higher initial cost. Ether extractables or residual oils were much lower in the experimental treatments, as already discussed. Ash and crude fiber contents were similar, but protein contents were slightly higher in the experimental treatments. A slightly lower value for available lysine content was observed in meal extracted with the ethanol/methylene chloride blend than in the meal extracted with methylene chloride, which was probably the result of greater binding of gossypol to lysine in the meal desolventizer. Available lysine values in both experimental meals were between those observed in meals from direct extraction with hexane (1.6%) and from screw pressing (1.3%). The much lower values for protein dispersibility in 0.02N NaOH for the experimental treatments reflect greater protein denaturation than occurs during direct hexane extraction or prepress hexane extraction. Protein denaturation by heat is often believed to reduce feed conversion, but denaturation from solvent may not have the same effect. Free gossypol values were much lower using direct extraction with methylene chloride than for direct extraction with hexane and equivalent to prepress hexane extraction.

A preliminary poultry feeding study was conducted with laying hens and broiler chicks using dietary levels of 10, 15 and 20%. No statistically significant differences were observed in performance (egg production rate, feed

TABLE 6

Effect of Extraction Solvent on Performance of Broiler Chicks

Treatment	Daily body weight gain (g/day)	Average feed intake (g/day)	Feed conversion (g feed/g gain)
Basal soybean meal	24.0	41.4	1.74
Hexane extracted cottonseed meal			
10%	23.7	40.4	1.72
15%	23.6	44.6	1.93
20%	23.6	39.7	1.70
Meal from Trial 2			
10%	25.8	43.8	1.71
15%	24.4	44.1	1.82
20%	24.6	44.6	1.82
Meal from Trial 9			
10%	23.7	42.0	1.77
15%	23.7	42.0	1.78
20%	22.7	40.8	1.86
Statistical analysis[a]	NS	NS	NS
Least significant difference	3.9	4.9	0.17

[a]NS, no significant difference between treatments at $P < 0.05$.

conversion, average egg weight, egg mass output and body weight change) of laying hens fed meal extracted with methylene chloride from Trial 2, meal extracted with the ethanol/methylene chloride blend from Trial 9, a commercial prepress hexane-extracted cottonseed meal and a direct hexane-extracted soybean meal (Table 5). Egg production rates were 82.8, 82.5, 80.3 and 81.3% at the 20% replacement level, respectively.

Eggs from hens fed each of the treatments were stored at 7.2 C for periods of one or two months prior to examination for yolk discoloration due to gossypol. Only the hens fed methylene chloride extracted meal from Trial 2 produced eggs which discolored during storage to a degree which would preclude their use in market channels. Eggs stored for two months showed gossypol discoloration of yolks only at the 20% level to the extent of 12% incidence. It was somewhat surprising that the prepress hexane-extracted cottonseed meal did not also produce a high incidence of discoloration, but later analysis indicated that this meal contained only 0.028% free gossypol as measured by the AOCS procedure and had a very low (27.9%) PDI in 0.02N NaOH, indicating more extensive heat treatment than typically achieved in the industry.

In feeding of broiler chicks for four weeks, there were no statistically significant differences in growth, feed conversion or feed intake among chicks fed any of the three cottonseed diets or the soybean basal diet (Table 6).

Methylene chloride, especially when blended with 5% ethanol, was effective in simultaneously extracting oil, aflatoxin and free gossypol from cottonseed flakes making the meal well-suited for feeding poultry. Despite these benefits, commercial use of methylene chloride to extract vegetable oils from oilseeds cannot yet be recommended until concern over health and environmental risks are resolved and approvals are granted by several governmental agencies.

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