

the first natural fatty acid to be successfully estimated by this means.

It may also be added that a cyclopropane ring behaves, more or less, like an olefinic double bond toward heat as well as some reagents (8). In Formula II for sterculeic acid a cyclopropane ring is shown conjugated with a C=C bond. This type of grouping is known to behave like a typical diene conjugated system (3, 10), and this may explain the striking gelation properties of *Sterculia foetida* oil.

Summary

Sterculia foetida oil has been found to contain 71.8% of sterculeic acid and minor proportions of oleic, linoleic, and saturated acids. The saturated component consists mostly of myristic and palmitic acids.

The oil consists of traces of tristearin (0.8%) and a major quantity of tristereulin (31.4%) together with different amounts of the glycerides of the type GS₂U, GSU₂, and GU₃ of other fatty acids.

Feed Value and Protein-Quality Determinations on Cottonseed Meals

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THE USE of cottonseed meal in rations for poultry and swine has been limited in the past by toxicological considerations. While most observers have directed their attention to the gossypol content of cottonseed meal as the criterion for its toxicity ever since the work of Withers and Carruth dating back to more than 40 years ago (1-4), several groups of investigators have questioned the analyzed gossypol content as a true indicator of cottonseed toxicity (5-12). Since practically all of these observations had been made on the rat, it was decided to determine if the conclusions reached were likewise applicable when some of the very same cottonseed meals used in our rat studies were appraised in practical feeding experiments on the chick. In addition, a series of nine coded cottonseed meals tested in a Collaborative Study¹ were evaluated for their protein quality in the rat and in the chick. The effect of incorporation of a constant gossypol level supplied by different samples of cottonseed pigment glands or pure gossypol was likewise determined in the rat.

Experimental

In the first six experiments day-old sexed chicks (breeds to be specified later) were fed a commercial broiler ration for a period of 24 to 48 hrs., after which they were weighed, inoculated with modified live virus Newcastle vaccine, wing-banded, separated into groups having similar average starting weights, and placed on test. The chicks were reared for three to four weeks in a back-warmer type, electrically-heated battery brooder and were then housed in wire-floored batteries. The number of chicks on a given diet in the various experiments varied from 11 to 24; the larger groups were divided into two groups at the time of transfer to the batteries so that no

¹ The results obtained in this Collaborative Study were reported by the participants at the Fourth Conference on Cottonseed Processing as Related to Nutritive Value of the Meal, Southern Regional Research Laboratory, U.S.D.A., New Orleans, La., Jan. 14-16, 1957.

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more than 12 birds were housed in a single battery section. All of the chick experiments were carried out in air-conditioned rooms. Feed and water were supplied *ad libitum*, and all birds were weighed weekly.

The composition of the basal mixture, which constituted 65% of each ration in the first four chick experiments (practical feeding tests), is given in Table I. The remaining 35% of each ration con-

TABLE I
Composition of Basal Mixture Comprising 65% of Each Chick Ration^a in Experiments 1, 2, 3, and 4

Ingredient	Percentage in ration
Ground milo.....	28.0
Ground yellow corn.....	25.5
Dehydrated alfalfa meal.....	3.0
Fish meal.....	2.0
Steamed bone meal.....	2.0
Dried whey.....	2.0
Ground oyster shell.....	1.5
Iodized salt.....	0.5
Aurofac.....	0.5
Total.....	65.0

^a Each ration contained the following in mg. per kg. of ration: Vitamin A and D feeding oil (2250 I.U. A; 300 I.C. D₃ per gram) 4400, methionine 500, choline chloride 440, manganese sulfate 176, niacin 26, calcium pantothenate 11, riboflavin 4, and menadione 1.

sisted of either 35% test cottonseed meal or 17.5% test cottonseed meal plus 17.5% control soybean meal; the control ration contained 35% soybean meal.

The composition of the semi-synthetic rations used in the protein-quality evaluations in the chick (replicated experiments 5 and 6) is given in Table II. To obviate the picking, coprophagy, and cannibalism which inevitably occur in such an experiment because of the low level of protein fed, all birds within a given group were removed from the battery-brooder at the first sign of any of such complications and were individually caged even prior to the scheduled time of transfer.

In the protein-quality evaluations in the rat (rep-

TABLE II
Composition of Semi-Synthetic Chick Rations^a Used in
Experiments 5 and 6

Ingredient	Percentage in ration
Test meal to yield 15% protein in diet.....	30.06-37.68
Soybean oil, sufficient to make 4% fat.....	3.63- 3.71
Salts 5 (Briggs <i>et al.</i> , 1943).....	5.00
Dextrose, sufficient to make 100%.....	53.61-61.42

^aAll these semi-synthetic diets were supplemented with the following per kilogram of diet: choline chloride 2,000 mg., inositol 1,000 mg., niacin 100 mg., para-aminobenzoic acid 100 mg., *d*-calcium pantothenate 15 mg., riboflavin 6 mg., α tocopherol 6 mg., thiamine 4 mg., vitamin B₆ 3 mg., folic acid 2 mg., menadione 0.5 mg., biotin 0.2 mg., vitamin B₁₂ 50 micrograms, vitamin A 10,000 I.U., vitamin D₃ 600 I.C.U.

licated experiments 7 and 8) the test cottonseed meals were incorporated into otherwise protein-free semi-synthetic rat diets at levels supplying 9% protein, as shown in Table III.

TABLE III
Composition of Semi-Synthetic Rat Diets Used in
Experiments 7 and 8

Ingredient	Percentage in diet
Vitamin premix.....	20 ^a
Jones and Foster salt mixture.....	4
Agar.....	2
A and D oil (2,250 A; 300 D ₃ /g.).....	1
Wheat germ oil.....	1
Test or control meal, sufficient to supply 9% protein.....	15.44-23.60
Commercial shortening, 10% minus fat contributed by protein source.....	8.63- 9.99
Dextrose, sufficient to make 100%.....	38.87-46.57

^aThe 20 g. of vitamin premix added in 100 g. of diet consisted of the following: Wilson 1:20 liver concentrate (NF 1X) 700 mg., choline chloride 100 mg., inositol 25 mg., para-aminobenzoic acid 10 mg., *d*-calcium pantothenate 5 mg., niacin 4 mg., 2 methyl-1,4-naphthoquinone 1 mg., thiamine hydrochloride 0.8 mg., riboflavin 0.8 mg., pyridoxine hydrochloride 0.8 mg., folic acid 0.2 mg., biotin 0.03 mg., and sufficient dextrose to make a total of 20 g. of premix.

In the ninth experiment 11 groups of eight male rats each were fed stock diet, into which various levels of different cottonseed pigment glands or of pure gossypol were incorporated to supply the same calculated level of gossypol in the diet (0.1%). The composition of the stock diet is as follows: whole ground wheat 21%, meat and bone scraps 19.6%, non-fat dry milk solids 15%, soybean oil meal 15%, ground yellow corn 13%, commercial shortening 10%, salt and yeast mixture 2.5%, alfalfa leaf meal 2%, wheat germ oil 1%, vitamin A and D oil 0.5%, and Wilson 1:20 liver concentrate (NF 1X) 0.4%.

Weanling male rats of the Holtzman strain were fed the stock diet for a period of one to three days, after which they were distributed into various groups according to body weight so that the average starting weights of all groups within a given experiment were the same. All rats were kept in individual wire-bottom cages in an air-conditioned room maintained at 78° ± 1°F. and *ca.* 45% relative humidity. Food and water were allowed *ad libitum*. The rats were weighed daily for the first 14 days and at least twice weekly thereafter. Analyses for free gossypol were made by the method of Pons and Guthrie (13), and those for total gossypol by the method of Pons, Hoffpauir, and O'Connor (14). The method described by Lyman, Chang, and Couch (15) was used for percentage of nitrogen solubility in 0.02 N sodium hydroxide. All analytical data on the coded cottonseed meals used in the Cooperative Study were supplied by the Southern Regional Research Laboratory, U.S.D.A.

Results and Discussion

The results obtained in the first four chick experiments are given in Table IV; the rations in the

individual experiments are listed in the order of decreasing efficiency of feed utilization. Twenty-four cottonseed meals were tested at the 17.5% level, and nine of these were also fed at the 35% level in the ration. There was wide variation in the free (0.22-1.29%), bound (0.12-1.03%), and total (0.05-1.41%) gossypol content of the cottonseed meals being tested. Arrangement of the various diets in the order of their decreasing free, bound, or total gossypol content or their protein or fiber content yielded no apparent quantitative relationship between any of these individual variables and the biological performance.

In order to maintain uniformity of procedure for evaluation of the toxicological potential of the cottonseed meals in the practical feeding tests, no adjustment was made of the protein content of the ration by slight alterations in the amounts of some of the constituents. The protein, fat, and crude fiber content of the respective practical rations did not differ considerably, and there was no apparent correlation between these analytical figures and the body-weight results. In fact, some rations that did have somewhat higher protein content than the rest showed some of the worst final body-weight performances. The best results were not obtained with the diets containing the lowest amounts of free gossypol. On the contrary, there were 12 diets containing 0.02 to 0.07% free gossypol, supplied by feeding 17.5% levels of cottonseed meals whose free gossypol contents were as high as 0.10 to 0.41%. These yielded average body-weight gains of from 1,007 to 1,273 g. in eight weeks.

Feed efficiencies superior to those shown by their own soybean meal controls were given by seven rations containing 17½% levels of cottonseed meals with high free gossypol content. There was no apparent correlation between the efficiency of feed utilization and the free, bound, or total gossypol content of the diet or the percentage of nitrogen solubility in 0.02 N sodium hydroxide of the cottonseed meals tested.

Diets 2, 4, and 8, all containing approximately the same amount of free gossypol, protein, and fat, yielded average body-weight gains after eight weeks of 1,020, 929, and 867 g., respectively, and feed efficiencies of 39.0, 38.5, and 36.8. Indeed diet 2, despite its high free gossypol content (0.07%), gave better body-weight gain results than did 10 diets and better feed efficiency than did eight diets, all of which contained considerably less free gossypol.

Although diet 10 had the same free gossypol content as diet 23 (0.04%), the body-weight gains were 1,273 and 908 g., respectively, and the feed efficiencies were 40.8 and 35.8. Its high free gossypol content notwithstanding, diet 10 showed better body-weight gain than did 16 diets and better feed efficiency than did nine diets, all of which contained considerably less free gossypol. This diet 10 even surpassed the performance of the ration containing 17.5% of the butanone-extracted cottonseed meal control plus 17.5% soybean meal (diet 15) and bettered the results obtained with three soybean meal controls (diets 5, 20, and 27). It should be noted that diet 10 contained a cottonseed meal which had a free gossypol content of 0.22%, a total gossypol content of 0.89%, and a value of 64.6% for nitrogen solubility in 0.02 N sodium hydroxide.

TABLE IV
Effect of Feeding Practical Chick Rations Containing 17.5 and 35% Levels of Cottonseed Meals for Eight Weeks (Experiments 1, 2, 3, and 4)

Diet No.	Cottonseed Meal				Dietary Levels				Mortality ratio ^c	Final av. wt. gain	Feed Efficiency ^d	
	Meal No. ^a	Type of treatment	Amount in diet	N. Solv. in 0.02 N NaOH	Protein	Fat	Crude fiber	Gossypol				
								Free				Total
First Experiment—New Hampshires												
1	—	Solvent—alkali	17.5	—	25.8	3.8	—	0.03	—	0/20	9.	39.8
2	—	Solvent—acid	17.5	—	26.0	3.7	—	0.07	—	1/20	1020	39.0
3	—	Solvent—commercial	17.5	—	23.2	3.8	—	0.03	—	0/20	1073	38.5
4	—	Solvent—alkali	17.5	—	25.6	3.8	—	0.07	—	0/20	929	38.5
5	—	(Soybean Control A)	(35.0)	—	25.2	3.9	—	—	—	0/20	1052	38.3
6	13	Solvent—commercial	17.5	—	25.8	3.9	—	0.05	—	0/20	984	37.9
7	—	Solvent—alkali	17.5	—	25.8	3.7	—	0.08	—	2/20	857	36.9
8	—	Solvent—carbonate	17.5	—	25.9	3.8	—	0.07	—	0/19	867	36.8
9	2	Solvent—raw flakes	17.5	—	25.6	4.0	—	0.16	—	2/21	331	28.7
Second Experiment—New Hampshires												
10	21	Solvent—alkali	17.5	64.6	24.5	3.0	5.0	0.04	0.16	1/22	1273	40.8
11	—	(Soybean Control B)	(35.0)	—	24.5	3.2	3.9	—	—	0/22	1300	39.9
12	61	Pre-press solvent—comm.	17.5	72.0	24.1	3.2	5.1	0.01	0.14	2/11	1172	39.4
13	19	Solvent—alkali	17.5	71.9	24.1	3.0	5.0	0.04	0.20	1/22	1140	39.3
14	18	Solvent—commercial	17.5	71.5	24.1	3.0	5.0	0.05	0.18	0/22	1123	39.2
15	—	Butanone—Extd. Control	17.5	85.9	23.8	2.9	5.4	<0.01	0.01	0/22	1069*	38.7*
16	19	Solvent—alkali	35.0	71.9	22.8	3.1	6.3	0.08	0.40	0/22	775	35.1
17	18	Solvent—commercial	35.0	71.5	22.3	3.4	6.9	0.09	0.37	4/22	633	34.1
18	1	Solvent—raw flakes	17.5	65.1	23.8	3.2	4.7	0.23	0.25	5/22	227	29.8
Third Experiment—New Hampshires												
19	44	Solvent—alkali	17.5	69.5	23.6	3.5	4.9	0.03	0.19	1/24	1091	40.3
20	—	(Soybean Control C)	(35.0)	—	24.5	3.6	3.5	—	—	0/24	1187	40.0
21	45	Solvent—commercial	17.5	70.0	23.6	3.5	4.9	0.03	—	0/24	1101	39.6
22	51	Solvent—alkali	35.0	69.8	22.8	3.4	5.0	0.02	0.19	0/24	1158	38.9
23	51	Solvent—alkali	35.0	69.8	21.9	3.4	5.9	0.04	0.16	0/12	908	35.8
24	44	Solvent—alkali	35.0	69.5	23.6	3.4	6.5	0.05	0.32	0/12	863	33.7
25	45	Solvent—commercial	35.0	70.0	23.6	3.6	5.3	0.05	0.38	2/12	763	31.8
Fourth Experiment—White Rocks												
26	58	Solvent—alkali	17.5	68.0	23.5	3.0	4.9	0.01	0.16	1/22	1199	44.2
27	—	(Soybean Control D)	(35.0)	—	24.2	3.2	3.7	—	—	0/22	1217	43.5
28	64	Solvent—commercial	17.5	65.2	23.7	3.0	4.2	0.01	0.18	0/12	1212	43.4
29	—	Solvent—commercial	17.5	87.0	24.0	3.2	4.5	0.03	0.14	0/12	1081	43.0
30	67	Expeller—commercial	17.5	41.1	23.3	3.6	4.7	<0.01	0.16	1/22	1207	42.9
31	54	Solvent—alkali	17.5	70.6	22.8	3.0	5.2	0.02	0.18	2/22	1182	42.1
32	—	Solvent—commercial	35.0	87.0	23.7	3.4	5.3	0.06	0.28	0/12	1018	42.1
33	47	Solvent—commercial	17.5	74.9	23.3	3.0	4.7	0.02	0.18	1/22	1201	41.8
34	66	Expeller—commercial	17.5	36.5	23.2	3.5	4.9	0.01	0.19	0/22	1260	41.1
35	58	Solvent—alkali	35.0	68.0	22.8	2.9	6.2	0.03	0.33	2/22	993	39.7
36	47	Solvent—commercial	35.0	74.9	22.4	2.9	5.7	0.05	0.36	0/12	913	39.0
37	54	Solvent—alkali	35.0	70.6	21.3	2.9	6.6	0.04	0.36	0/12	956	37.1

^a The numbers in this column correspond to the numbers of the cottonseed meals evaluated for toxicity and protein quality in the rat by Eagle *et al.* (12).

^b Calculated.

^c Mortality ratio = number dead/number started.

^d Feed efficiency = g. gained/100 g. of feed eaten.

* As of 7 weeks, 3 days; ration exhausted.

TABLE V
Evaluation of the Protein Quality of Coded Cottonseed Meals Fed to Chicks as the Sole Protein Source in Semi-Synthetic Rations at the 15% Protein Level for Eight Weeks (Averages of Replicated Experiments 5 and 6)^a

Meal No.	Identification	Cottonseed Meal				Dietary Levels				Indices ^f of:					
		N. Soly. in 0.02 N NaOH	Gossypol		Crude fiber	Gossypol		Mortality ratio ^b	Av. wt. gain	Protein efficiency ^c	Feed efficiency ^d	Caloric efficiency ^e	Protein efficiency	Feed efficiency	Caloric efficiency
			Free	Total		Free	Total								
CM 49	Solvent—chemically treated (Soybean control)	83.7	0.04	0.84	3.1	0.01	0.30	1/40	772	2.27	34.9	10.08	105	106	110
S/B	Pre-press solvent	—	—	—	1.0	—	—	1/40	671	2.17	33.0	9.18	100	100	100
CM 45	Solvent	65.9	0.04	0.75	5.5	0.02	0.28	2/40	290	1.73	25.4	7.49	80	77	82
CM 19	Pre-press solvent	70.0	0.28	0.96	4.2	0.10	0.36	4/40	285	1.70	25.6	7.51	78	78	82
CM 6	Screw press—low speed	68.7	0.03	0.75	5.0	0.01	0.27	3/40	324	1.68	25.3	7.44	78	77	81
CM 21	Hydraulic	46.8	0.02	0.62	4.9	0.01	0.24	4/40	284	1.65	25.4	7.40	76	77	81
CM 16	Screw press—low speed	65.1	0.14	1.11	4.8	0.06	0.44	1/40	189	1.29	19.3	5.67	60	59	62
CM 36	Pre-press solvent	39.1	0.05	1.10	3.1	0.02	0.37	3/40	143	1.29	19.2	5.41	60	58	59
CM 10	Screw press—high speed	52.4	0.04	1.00	4.5	0.01	0.34	2/40	154	1.29	18.2	5.30	59	55	58
CM 13	Screw press—high speed	30.1	0.03	1.00	4.7	0.01	0.37	5/40	94	0.97	14.2	4.17	45	44	46

^aThe sexed chicks used in Experiment 5 were Arbor Acre White Rocks; those in Experiment 6 were Lancaster Crosses.
^bNumber dead/number started on test.
^cProtein efficiency = g. gained/g. protein eaten.
^dFeed efficiency = g. gained/100 g. feed eaten.
^eCaloric efficiency = g. gained/100 calories consumed.
^fSoybean control = 100.

TABLE VI
Evaluation of the Protein Quality of Cottonseed Meals Fed to Rats as the Sole Protein Source in Semi-Synthetic Rations at the 9% Protein Level for Eight Weeks (Averages of Replicated Experiments 7 and 8)

Meal No.	Identification	Cottonseed Meal				Dietary Level				Indices ^e of:					
		N. Soly. in 0.02 N NaOH	Gossypol		Crude fiber	Gossypol		Mortality ratio ^a	Av. wt. gain	Protein efficiency ^b	Feed efficiency ^c	Caloric efficiency ^d	Protein efficiency	Feed efficiency	Caloric efficiency
			Free	Total		Free	Total								
CM 49	Solvent—chemically treated (Butanone-extd. control)	83.7	0.04	0.84	2.2	0.01	0.18	0/16	175	2.19	21.2	5.36	111	107	110
BUT.	(Soybean control)	—	—	—	0.7	<0.01	0.04	0/16	161	2.13	20.2	5.00	108	103	102
S/B	Solvent	—	—	—	0.5	—	—	0/16	156	1.98	19.8	4.90	100	100	100
CM 19	Pre-press solvent	70.0	0.28	0.96	2.7	0.06	0.21	0/16	120	1.82	17.1	4.32	92	86	88
CM 45	Pre-press solvent	65.9	0.04	0.75	3.1	0.01	0.17	0/16	124	1.77	16.5	4.19	89	83	86
CM 6	Screw press—low speed	68.7	0.02	0.75	2.9	<0.01	0.16	0/16	120	1.74	16.3	4.13	88	82	85
CM 21	Screw press—low speed	46.8	0.02	0.62	3.1	<0.01	0.15	0/16	120	1.71	16.7	4.24	86	85	87
G	Screw press—low speed	37.4	0.01	1.27	1.3	<0.01	0.25	0/8	88	1.49	14.0	3.53	—	—	—
H	Screw press—low speed	40.7	0.01	1.35	1.5	<0.01	0.27	0/8	81	1.44	13.1	3.29	—	—	—
H	Screw press—low speed	39.2	0.01	1.36	1.5	<0.01	0.26	0/8	77	1.38	13.0	3.27	—	—	—
I	Solvent	69.6	0.05	1.60	2.2	0.01	0.35	0/8	80	1.36	12.5	3.09	—	—	—
CM 10	Pre-press solvent	52.4	0.04	1.00	2.7	0.01	0.20	0/16	74	1.33	12.1	3.02	67	62	62
CM 36	Screw press—low speed	39.1	0.05	1.10	2.1	0.01	0.22	0/16	72	1.26	11.4	2.84	64	58	59
CM 16	Hydraulic	65.1	0.14	1.11	3.1	0.03	0.26	0/16	77	1.21	11.6	2.93	61	58	60
F	Screw press—low speed	42.0	0.01	1.35	1.7	<0.01	0.26	0/8	64	1.15	10.9	2.74	—	—	—
CM 13	Screw press—high speed	30.1	0.03	1.00	3.1	0.01	0.22	0/16	42	0.88	8.2	2.09	44	42	43

^aNumber dead/number started on test.
^bProtein efficiency = g. gained/g. protein eaten.
^cFeed efficiency = g. gained/100 g. diet eaten.
^dCaloric efficiency = g. gained/100 calories consumed.
^eSoybean control = 100.

TABLE VII
Effect of Incorporation of a Constant Free Gossypol Level (0.1%) From Various Sources
in the Diet of the Rat (Experiment 9)

Diet No.	Variable in diet	Gossypol content of variable	Amount variable in diet	Calculated gossypol in diet	Average body weight gain after				Feed eff. ^a
					7 days	14 days	21 days	28 days	
85	Cottonseed pigment glands No. 1	38.4	0.261	0.1	21	38	56	75	27.2
86	Cottonseed pigment glands No. 2	36.8	0.272	0.1	20	46	65	78	27.4
87	Cottonseed pigment glands No. 3	29.7	0.337	0.1	20	47	66	84	29.4
88	Cottonseed pigment glands No. 4	37.8	0.265	0.1	21	46	67	89	30.2
89	Cottonseed pigment glands No. 5	35.1	0.285	0.1	23	48	70	91	29.9
90	Cottonseed pigment glands No. 6	27.0	0.370	0.1	25	54	79	91	29.4
91	Pure gossypol A	ca. 100.0	0.100	0.1	27	56	89	108	32.3
92	Pure gossypol B	ca. 100.0	0.100	0.1	31	62	91	114	32.9
93	Pure gossypol C	97.3	0.100	0.1	32	63	94	125	34.9
94	(Stock diet control)	0	0	0	41	84	122	160	37.0

^a Feed efficiency = g. gained/100 g. diet eaten.

No inferences can be made from the first four chick experiments as to the protein quality of the individual cottonseed meals incorporated in these practical rations, which contain significant levels of protein from many good sources.

A comparison of the order of increasing average-weight gain of the various groups showed that this order differed widely as the tests progressed during the early stages, that the performance of chicks within certain groups was inconsistent, and that variations between different groups became increasingly more marked, particularly in the case of diets containing the more toxic cottonseed meals. This raises the question as to whether a short-term feeding test of less than a few weeks can provide better than a mere indication of the true feeding potential (algebraic sum of toxicity plus food value) of practical rations containing cottonseed meals which have wide differences, particularly with respect to the former of these highly important variables.

In another series of experiments nine coded cottonseed meals were evaluated for their protein quality in replicated experiments at the 15% protein level in chicks and at the 9% protein level in rats. This was part of a Cooperative Cottonseed Meal Study primarily designed to determine the performance of commercial cottonseed meals in combination with a standard soybean meal in practical chick rations and to develop data on the relationship between processing conditions and protein quality.

Our chick diets containing the cottonseed meals used in the Cooperative Study are listed in Table V in the order of decreasing protein efficiency. Of particular interest was the finding that the pullets frequently weighed more than the cockerels. Of the 20 rations fed in the replicated, protein-quality evaluations on the nine coded cottonseed meals and the soybean meal control, 12 of these resulted in heavier females than males. In three other instances both sexes showed the same average body-weight; and in only five out of the 20 possibilities did the males outweigh the females, in two cases by only a small margin. The original sexing of the chicks was confirmed by autopsy at the termination of the tests. This lowered body weight in the males may be attributed to the greater amino acid requirements of the cockerels for growth and maintenance. The limiting factor in these specially designed chick experiments was the threshold protein level of 15% derived from a single protein source.

The results from the rat studies on the same nine coded cottonseed meals are listed in Table VI, again in the order of decreasing efficiency of protein utili-

zation. Meals E, F, G, H, and I were not part of the Cooperative Study but are included because of their good biological performance despite their low nitrogen solubility values.

An unusual observation in these protein-quality evaluations (Table VI) was the darkening and marked enlargement of the spleens of the rats fed CM 49, subsequently identified as a solvent-extracted, chemically-treated cottonseed meal in the Cooperative Study. The average splenic weight per 100 g. of body weight for the 16 rats fed CM 49 represented a 78% increase over the average spleen:body-weight ratio of the other 160 rats fed in the Cooperative Study. This positive toxicological finding merits further study.

From the chick and rat data it may be noted at the outset that not only does the protein quality of cottonseed meals vary with the type of processing but that even a given method of processing can yield cottonseed meals of greatly different protein quality. The results obtained in the protein-quality evaluations in the chicks were strikingly parallel to those obtained in the rat. Even among the limited number of coded cottonseed meals tested there were many of good to fair protein quality which had unusually poor nitrogen solubility values, and some meals of poor protein quality which had rather high nitrogen solubility figures. This nonconformity becomes even more marked when one compares the analytical values for nitrogen solubility of meals which were found to have the same biologically evaluated, protein quality. Four such meals (CM 45, CM 19, CM 6, and CM 21) of identical protein quality had such divergent nitrogen solubility values as 65.9, 70.0, 68.7, and 46.8%. Again meals CM 10, CM 16, and CM 36 all had practically the same protein quality regardless of whether evaluated in the chick or in the rat, but their nitrogen solubility values were, respectively, 52.4, 65.1, and 39.1%. These data and those previously reported (12) on a series of 25 other cottonseed meals fail to confirm the relationship between the solubility of cottonseed protein in 0.02 N sodium hydroxide and the protein quality of cottonseed meal as claimed by Lyman, Chang, and Couch (15).

The results obtained in the experiment, in which a constant level of gossypol (0.1%) was incorporated into stock diet by addition of different levels of cottonseed pigment glands or of pure gossypol, are given in Table VII. Despite a constant contribution of 0.1% gossypol to the diet, the different cottonseed pigment glands varied in their body-weight depressing effect on rats. But all six of the differ-

ent cottonseed pigment gland samples caused greater body-weight depressions than did three samples of pure gossypol. Since all of the 10 diets varied only to the extent that the small amounts of cottonseed pigment glands or pure gossypol were incorporated into the standard stock diet, these differences in body-weight performance were obtained with diets whose protein and fiber levels were constant. The efficiencies of feed utilization were less for the groups fed cottonseed pigment glands than for the groups fed samples of pure gossypol.

If the free (or bound, or total) gossypol were the sole factor(s) involved in cottonseed toxicity, it would appear to be a simple matter indeed to determine by well-controlled experimentation whether a true statistical correlation exists between growth and the free (or bound, or total) gossypol level in the diet. Gallup (5) had stated in 1928 however that the chemical methods available at that time for determination of gossypol and its related compounds did not suffice as a measure of the toxicity of heated cottonseed products. In 1948 Boatner *et al.* (6) reported that cottonseed pigment glands, distinct morphological entities containing the toxic components of cottonseed, added to the diets of chicks produced marked retardation of growth and a high incidence of deaths whereas addition of an equivalent amount of pure gossypol to the diet caused little or no retardation in growth. They likewise found that no correlation was apparent between the nutritive value for chicks of various cottonseed products and their relative contents of gossypol and gossypurpurin. Eagle *et al.* (7, 8, 10) showed that the different acute oral toxicity (LD 50) values for these pigment glands in various animal species bore no relation to their gossypol content.

Reports of feeding tests, in which various levels of pure gossypol were incorporated into the diets of rats, showed that while the body weight depression caused by pure gossypol is proportional to the amount added to the diet, the greater mortality and body-weight effects caused by adding various levels of cottonseed pigment glands to the diet cannot be attributed to their analyzed gossypol content alone (10). Feeding tests on rats likewise have shown that the residual toxicity of cottonseed meals could not be explained on the basis of their analyzed free gossypol content (11, 12). These conclusions are confirmed by the results from the nine experiments on more than 300 rats and 1100 chicks reported in the present paper.

Summary

Twenty-four cottonseed meals fed to chicks in practical feeding rations for eight weeks led to good growth performance and favorable feed efficiency in many cases, despite the unusually high free gossypol content of the rations.

The results from replicated, protein-quality evaluations in chicks fed for eight weeks at the 15% protein level were closely parallel to those from similar experiments in rats fed at the 9% protein level and showed that the percent nitrogen solubility in 0.02 N sodium hydroxide of cottonseed meals is a poor indicator of protein quality.

Constant gossypol levels of 0.1%, supplied by additions of different amounts of cottonseed pigment glands, caused greater body-weight depressions than did the same gossypol level supplied by pure gossypol.

The toxicity of cottonseed pigment glands and of cottonseed meals cannot be accounted for solely on the basis of analyzed gossypol content.

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Studies on Guar Seed Oil (*Cymmosis Psoralioides*)

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PARIKH (1) and Ramakrishnan (2) have studied in detail the nutrient composition of guar seed, a cheap legume available in abundance in certain parts of India, and have found that the seed contains a good percentage of high quality protein along with about 4% of fat. The oil was extracted from dried seeds, and its properties were studied as reported in this paper.

Experimental

The fat was extracted from crushed seeds by petroleum ether (b.p. 40°-60°C.). The characteristics as determined by standard procedures (3) are given in Table I.

The mixed fatty acids (excluding the unsaponifiable fraction) were analyzed spectrophotometrically by the method of Hilditch, Morton, and Riley (4)