TABLE XI Summary of Properties of 26 Prepress-Solvent Extracted Meals from 11 Mills

Chemical Properties (as received basis)	Range	Average	
	%	%	
Oil content	0.14 - 2.34	0.91	
Free gossypol	0.024 - 0.063	0.044	
Total gossypol	0.66 - 1.28	0.93	
Total nitrogen	6.35 - 7.32	6.72	
Nitrogen solubility-0.5 M NaCl	25.5 - 47.8	38.0	
Nitrogen solubility-0.02N NaOH	65.4 - 83.4	72.2	
Chemical index	53.7 -98.1	76.9	

resulted mainly from removal of gossypol in the prepressed and solvent-extracted oils.

Nitrogen solubility data, which have been suggested as a measure of protein damage, indicated that the major change or reduction in nitrogen solubility occurred during cooking. Very little reduction was noted for prepressing or solvent extraction. The reduction in nitrogen solubility during prepressing is much smaller than that previously reported for normal screw-pressing operations.

Prepressed oils gave lower refining losses and lower refined and bleached color than did the solvent-extracted oils. Bleach color reversion, after storage of crude oils for 30 days at 100°F., was greater for solvent-extracted than for prepressed oils.

A number of meals exhibited the desirable characteristics of low free gossypol content and high nitrogen solubility. Values calculated for chemical indexes of protein quality, as suggested by Lyman and associates (11), indicate that many of the meals should have good protein quality.

#### Acknowledgments

This investigation was made possible by the interest and cooperation of the Dothan Oil Mill Company, Dothan, Ala.; General Vegetable Oil Company, Sherman, Tex.; Helena Cotton Oil Company, Helena, Ark.; Lubbock Cotton Oil Company, Lubbock, Tex.; Ranchers Cotton Oil Company, Fresno, Calif.; Union Cotton Oil Company, West Monroe, La.; and the Western Cotton Oil Company, Abilene, Tex.; Lubbock, Tex.; Pecos, Tex.; and Phoenix, Ariz.

The authors are indebted to Marion F. H. Le Blanc Jr., J. C. Kuck, Julian F. Jurgens, Claire Lesslie, Alva F. Cucullu, and Vidabelle O. Cirino for many of the chemical analyses reported.

#### REFERENCES

ALFERENCES 1. Altschul, A. M., Offic. Proc. 55th Ann. Conv. Natl. Cottonseed Products Assoc., 1951, 32-34, 36. 2. Altschul, A. M., and Thurber, F. H., Cotton Gin & Oil Mill Press, 54, No. 23, 26, 68-71 (1953). 3. Altschul, A. M., Poultry Sci. (in press). 4. American Oil Chemists' Society, "Official and Tentative Methods of Analysis," Ed. 2, rev. to 1951, Chicago, 1946-1951. 5. D'Aquin, E. L., Spadaro, J. J., Vix, H. L. E., Pominski, J., Mo-laison, L. J., and Pollard, E. F., Oil Mill Gaz., 51. No. 10, 17-19 (1947).

(1947).
6. Fats and Oils Situation (U. S. Bureau Agr. Economics), FOS-162,
11 (Aug. Sept. 1953).
7. Fontaine, T. D., "Cottonseed and Cottonseed Products," A. E. Bailey, ed., Interscience Publishers, New York, 1948, pp. 409-465.
8. Haddon, R. P., Schwartz, A. H., Williams, P. A., Thurber, F. H., Karon, M. L., Dechary, J., Guice, W., Kupperman, R., O'Connor, R. T., and Altschul, A. M., Cotton Gin & Oil Mill Press, 51, No. 9, 18-20 (1950)

(1950).

(1900).
9. Jensen, E. A., Condon, M. Z., Karon, M. L., and Altschul, A. M., Cotton Gin & Oil Mill Press, 54, No. 5, 24-25 (1953).
10. Knoepfler, N. B., Vix, H. L. E., and Thurber, F. H., Cotton Gin & Oil Mill Press, 53, No. 6, 16, 18, 61-66 (1952).
11. Lyman, C. M., Chang, W. Y., and Couch, J. R., J. Nutrition, 49, 679-90 (1953).

National Cottonseed Products Assoc., "Rules Governing Trans-actions Between Members," 1953-54 (Dallas) 1953.
 Olcott, H. S., and Fontaine, T. D., Ind. Eng. Chem., 34, 714-16

Olcott, H. S., and Fontaine, T. D., Ind. Eng. Chem., 34, 714-16 (1942).
 Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chemists' Soc., 27, 390-93 (1950).
 Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chemists' Soc., 28, 8-12 (1951).
 Pons, W. A. Jr., Murray, M. D., LeBlanc, M. F. H. Jr., and Castillon, L. E., J. Am. Oil Chemists' Soc. 30, 128-32 (1953).
 Pons, W. A. Jr., Murray, M. D., LeBlanc, M. F. H. Jr., and Castillon, L. E., J. Am. Oil Chemists' Soc. 30, 128-32 (1953).
 Reuther, C. G., Le Blanc, M. F. H. Jr., and Batson, D. M., J. Am. Oil Chemists' Soc., 30, 28-32 (1953).
 Thurber, F. H., Vix, H. L. E., Pons, W. A. Jr., Crovetto, A. J., and Knoepfler, N. B., J. Am. Oil Chemists' Soc. (in press).
 Vix, H. L. E., Pollard, E. F., Spadaro, J. J., and Gastrock, E. A., Ind. Eng. Chem., Anal. Ed., 38, 635-42 (1946).
 Williams, P. A., Boatner, C. H., Hall, C. M., O'Connor, R. T., and Castillon, L. E., J. Am. Oil Chemists' Soc., 24, 362-69 (1947).
 Williams, P. A., Haddon, R. P., Hall, C. M., Castillon, L. E., Guice, W. A., O'Connor, R. T., and Boatner, C. H., J. Am. Oil Chemists' Soc., 26, 28-34 (1949).

[Received September 13, 1954]

# The Nutritional Value of Prepress-Solvent **Cottonseed Meals**

WAN-YUIN CHANG, J. R. COUCH, and CARL M. LYMAN, Texas Agricultural Experiment Station, College Station, Texas; WILLIAM L. HUNTER, VAN P. ENTWISTLE, and WILLIAM C. GREEN, Bureau of Field Crops, State of California, Sacramento, California; A. B. WATTS and C. W. POPE, Poultry Industry Department, Louisiana State University, Baton Rouge, Louisiana; C. A. CABELL and I. P. EARLE, Animal and Poultry Husbandry Research Branch, United States Department of Agriculture, Beltsville, Maryland

<sup>¬</sup>HE purpose of this paper is to report the results of a coordinated study of the nutritional value of prepress-solvent cottonseed meals. The feeding trials were conducted in four separate laboratories. No attempt was made to standardize on a single type of test, and each investigator used a method of his choice. The report of each laboratory constitutes a section of this paper.

Meals produced in commercial mills under varied but carefully controlled conditions were used. They were manufactured during a study of prepress-solvent mill operation by Pons, Thurber, and Hoffpauir (1), and the effects of processing conditions on the physical and chemical characteristics of the samples have been described by these authors.

It is also the purpose of this paper to evaluate further the relationship of the chemical and physical characteristics of cottonseed meals to the nutritional value of the protein. The free gossypol content of all meals used in this investigation (0.024-0.063%) was much lower than the minimum level which will result in gossypol toxicity in chicks (2). The types of nutritional tests conducted by the different laboratories varied considerably, but all were designed to measure protein quality.

If it can be established that certain physical and chemical characteristics of cottonseed meal are closely related to the nutritional value of the product, then analyses of the meals for these characteristics become extremely useful guides in the production of superior

TABLE I Evaluation of Protein Quality in Prepress-Solvent Cottonseed Meals by Different Laboratories

		Chemic	al Ohara	cteristics				Chick Gro	owth Rat	9		Prot	ein Effic Feedir	iency in ( 1g Tests	Chick	Protein Reple- tion tests with rats	
Meal no. <sup>b</sup>						Texas. A	.gr. Expt	. Station	Californ	nia Dept	of Agr.	Louisia Un	na State iv,	Calif, of 4		U.S. D.A., Belts- ville	Av. index values
	Nitro solubil		.Free gossy-	Total gossy- pol	Chemi- cal	Av. gain in wt. of chicks	Feed/ gain	Growth	Av. gain in wt. of chicks	Feed/ gain	Growth	Grams gain/ Grams	Index	Grams gain/ Grams	Index	Index	
	0.02 N NaOH	0.5 M NaCl	pol	content	Index	at 4 weeks	ratio	index	at 8 days	ratio	Index	protein		protein		Index	ļ
	%	%	%	%		gm.			gm.							1	
Std. 1	88.2 ª	78.5ª	0.006	0.20	103.8	409.4	2.42	100.0	80.0	2.22	100.0	2.08-	100	0.07	100.0	100.0	100.0
Std. 2	83.1 ª	64.5 a	0.021	0.15	97.8	396.3	2.40	96.8				2.14ª		2.25	100.0	100.0	100.0
5-B	83.4 80.1	$rac{47.8}{45.7}$	$\begin{array}{c} 0.028 \\ 0.032 \end{array}$	$0.71 \\ 0.75$	$98.1 \\ 94.2$	$295.7 \\ 297.6$	$3.09 \\ 3.10$	72.2	74.0	2.66	92.5	1.87	90	1.88	83.5		81.9
10-B 5-A	80.1	45.7	0.032 0.024	$0.75 \\ 0.72$	94.2 93.8	297.6 319.8	2.73	$72.6 \\ 78.1$	$78.0 \\ 78.0$	$\substack{2.42\\2.58}$	97.5 91.2	$1.73 \\ 1.94$	83 93	$2.07 \\ 1.94$	$92.0 \\ 86.2$	76	80.9 86.3
9-A	79.0	42.4	0.058	0.86	91.7	305.9	2.91	74.7	69.0	$2.50 \\ 2.73$	86.2	$1.54 \\ 1.73$	83	1.83	81.3	84	80.8
9-C	77.4	43.6	0.051	0.82	91.1	302.5	3.06	73.9	75.0	2.64	93.7	1.43*	67*	1.90	84.4	84	77.3
9-B	$76.5 \\ 76.1$	$40.1 \\ 46.2$	0.055	0.84	$90.0 \\ 89.5$	$306.7 \\ 294.1$	$3.12 \\ 3.16$	75.2	68.0	2.82	85.0	2.00	96	1.77	78.7		83.3
10-A 6-A	74.8	$\frac{45.2}{40.9}$	$0.025 \\ 0.035$	0.76	89.5	294.1	$3.10 \\ 3.34$	$71.8 \\ 66.3$	$68.0 \\ 69.0$	$\begin{array}{c} 2.78 \\ 2.67 \end{array}$	$     85.0 \\     86.3 $	$1.91 \\ 2.28$	$^{92}_{110}$	$1.80 \\ 1.88$	$\begin{array}{c} 80.0\\ 83.5\end{array}$	91 80	83.7 85.0
10-C	74.7	43.1	0.024	0.69	87.8	325.8	2,80	79.5	77.0	2.45	96.2	1.59*	74*	2.04	90.7		81.4
6-B	74.2	37.8	0.036	0.83	87.3	300.9	3,15	73.5	70.0	2.71	87.5	2.01	96	1.85	82.2	74	81.4
6-C 7-C	$73.2 \\ 72.5$	40.7 37.9	$\begin{array}{c} 0.037 \\ 0.048 \end{array}$	$0.92 \\ 1.00$	79.6	$281.4 \\ 265.3$	$3.18 \\ 3.19$	$68.7 \\ 64.8$	$\begin{array}{c} 70.0 \\ 61.0 \end{array}$	2.74	87.5	1.38*	64*	1.83	$\frac{81.3}{70.0}$		71.3
11-A	72.3 72.3	37.9 32.7	0.048	0.66	$72.5 \\ 85.1$	306.8	3.19	64.8 74.9	70.0	$\frac{3.00}{2.65}$	$76.2 \\ 87.5$	1.63* 1.96	76* 94	$1.66 \\ 1.88$	$\begin{array}{c} 73.8\\ 83.5\end{array}$	72	$71.5 \\ 81.1$
$2 \cdot C$	69.9	36.7	0.054	1.02	68.5	262.8	3.21	64.2	60.0	3.00	75.0	$1.34^{*}$	63*	1.67	74.2	75	69.1
$8 \cdot B$	69.5	37.1	0.052	1.26	55.2	187.5	3.73	45.8	49.0	3.44	61.2	1.71	82	1.46	64.9		64.2
11-B 7-B	69.2 68.9	$\frac{30.9}{36.8}$	$0.058 \\ 0.048$	$0.86 \\ 1.07$	$80.5 \\ 64.4$	$285.2 \\ 279.1$	$\frac{3.19}{3.23}$	$\begin{array}{c} 69.4 \\ 68.1 \end{array}$	66.0 58.0	$2.77 \\ 3.10$	$     82.5 \\     72.5 $	1.49	$\frac{72}{76}$	$1.80 \\ 1.61$	80.0	65	73.8
8-0	68.7	37.9	$0.048 \\ 0.042$	1.28	53.7	204.3	3.70	49.9	58.0 64.0	$\frac{3.10}{2.79}$	$\begin{array}{c c} 72.5 \\ 80.0 \end{array}$	$1.59 \\ 1.89^*$	76 88*	$1.61 \\ 1.79$	$71.5 \\ 79.5$	65	70.2
1-A	68.5	30.8	0.032	0.86	79.6	213.1	4.50	52.1	65.0	3.32	70.0	1.71	82	1.50	66.7	76	69.2
11-C	68.4	33.3	0.063	0.92	74.3	290.9	3.16	71.0	66.0	2.71	82.5	 1.59		1.85	82.2	81	78.1
$^{8-A}_{2-B}$	$\begin{array}{c} 68.2 \\ 67.1 \end{array}$	$\begin{array}{c} 35.0\\ 32.8 \end{array}$	$0.058 \\ 0.050$	$1.14 \\ 1.05$	$59.8 \\ 63.9$	$243.6 \\ 263.4$	$3.46 \\ 3.23$	$59.6 \\ 64.3$	$55.0 \\ 63.0$	$^{3.25}_{2.93}$	68.7 78.8	$1.59 \\ 1.29$	$\begin{array}{c} 76 \\ 61 \end{array}$	$1.54 \\ 1.70$		82 56	$71.5 \\ 64.2$
2-A	66.8	37.0	0.055	1.12	59.6	203.4 209.9	3.85	51.2	58.0	$\frac{2.95}{3.11}$	72.5	$1.29 \\ 1.89$	91	1.60	71.1		71.1
3-A	66.1	34.0	0.057	1.05	62.9	237.8	3.36	59.1	55.0	3.21	68.7	1.65	79	1.55	68.9	51	64.5
7-A 4-A	$     \begin{array}{c}       66.1 \\       65.4     \end{array} $	$\begin{array}{c} 34.6 \\ 25.5 \end{array}$	$\begin{array}{c} 0.039 \\ 0.034 \end{array}$	$1.07 \\ 0.99$	$\begin{array}{c} 61.8\\ 66.1 \end{array}$	$\begin{array}{r} 249.4 \\ 204.8 \end{array}$	3.47 4.10	60.1 50.0	$59.0 \\ 52.0$	$3.04 \\ 3.38$	73.7	1.73	83 80	$1.64 \\ 1.48$	$72.9 \\ 65.8$	75 68	$72.8 \\ 66.0$
		ding tris					· · · · · · · · · · · · · · · · · · ·			·····	65.0	1.66	80	1.40	05.8	1 00	00.0

quality meals. Olcott and Fontaine (3) suggested that the solubility of the protein in 0.5 M sodium chloride was related to the nutritional value of the meal. This test has been used in connection with studies on mill operations and meal characteristics by a number of investigators, Haddon et al. (4), Milligan and Bird (5), and Reuther *et al.* (6). Lyman and associates (7) reported that many meals with low nitrogen solubility in 0.5 M of sodium chloride still had high nutritional value. These authors found a relationship between the solubility of the protein in dilute sodium hydroxide solution and chick growth rate. A relationship between total gossypol and the quality of the protein has also been reported (7) (8). Lyman and associates (7) preposed an empirical chemical index as a measure of protein quality. This chemical index takes into account the relationship of both total gossypol and nitrogen solubility to nutritional value.

### General Methods

Analyses of the samples for physical and chemical characteristics were made at the Southern Regional Research Laboratory. Details of the methods used have been described previously (1).

In all of the nutritional tests a standard cottonseed meal prepared at the Southern Regional Research Laboratory was used for comparison with the mill samples. This standard cottonseed meal was prepared by hexane extraction, followed by extraction with butanone to remove gossypol. The minimum amount of heat necessary to remove all traces of solvent was used. Such a procedure avoids denaturation of the protein and gives a product with high nitrogen solubility.

In this publication the term index value as applied to the nutritional tests is used to indicate the relative value of a meal as compared to the standard, expressed as percentage. It is calculated as follows:

 $\frac{\text{Value obtained with experimental meal}}{\text{Value obtained with standard meal}} \times 100$ 

#### Chick Feeding Tests at the Texas Agricultural Experiment Station

*Experimental.* Five hundred sixty straight run New Hampshire day-old chicks were distributed at random into 28 groups. Each group of 20 chicks was used for testing one meal. The chicks were fed in electrically heated batteries with raised screen floors. Feed and water were supplied *ad libitum*. The chicks were weighed initially and at weekly intervals thereafter. Records of feed consumption were kept throughout the experimental period of four weeks.

The basal diet used in this study contained 5% salts IV, Wesson oil in the amount necessary to make the total fat content of the diet 4%, about 50% cottonseed meal, and 40% cerelose. The percentage of cottonseed meal was adjusted so that all diets contained 21% protein, and the cerelose content was adjusted to make a total of 100% for all ingredients. In addition, the diet was supplemented with the following vitamins in milligrams per kilograms: riboflavin 6, calcium pantothenate 15, niacin 100, vitamin  $B_6$  3, thiamine 4, biotin 0.2, folacin 2, inositol 1,000, paraaminobenzoic acid 100, menadione 0.5, choline 2,000, and alpha-tocopherol 6. Vitamin  $B_{12}$  was also added (50  $\mu$ g. per kilogram). Six hundred I.U. of vitamin  $D_{3}$ , and 10,000 I.U. of vitamin A were added per kilogram of diet.

Nitrogen solubility in 0.02 normal sodium hydroxide was determined according to the method of Lyman, Chang, and Couch (7). The values given in

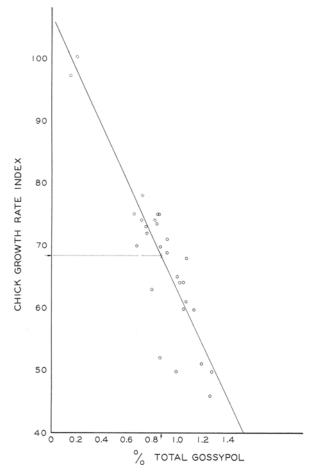


FIG. 1. The relationship of total gossypol to protein quality in cottonseed meal.

Table I were obtained at the Southern Regional Research Laboratory. They were rechecked at this laboratory immediately before the feeding tests. Good checks were obtained in all instances.

The results of the chick feeding test designed to measure protein quality are given in Table I, in which the samples have been arranged in the order of their nitrogen solubility in 0.02 N NaOH. Considerable variation in the quality of the protein of the samples is shown by the four-weeks chick-growth rate test. None of the meals proved to be as good as the two standards, both of which were prepared without heat and contained only small amounts of total gossypol. The results of chemical analyses are included in this table in order that direct comparisons may be made between nutritional value and the various chemical characteristics. In all but a few cases both nitrogen solubility in 0.02 N NaOH and chemical index values (7) proved to be reliable for predicting the results of the actual feeding trials. The relationship between total gossypol and protein quality previously reported is even more apparent in the present work.

In order to determine which of the various chemical characteristics would be most satisfactory for predicting the results of the feeding trials, correlation coefficients between the chick-growth rate index and the various chemical characteristics have been calculated. The correlation between growth rate and nitrogen solubility in 0.02 N NaOH was 0.818. The corresponding value for nitrogen solubility in 0.5 M NaCl was +0.787, for total gossypol -0.899; for chemical index value +0.829. In the present investigation, with the exception of the standards, all meals were manufactured by the prepress-solvent method. Under this limitation the results reported would indicate that total gossypol content may be at least as good an indication of protein quality as any of the other measurements studied in this investigation.

In Figure 1 the total gossypol content of the meals has been plotted against the chick-growth rate index. The curve has been drawn with the slope calculated from the experimental values for the different samples. In a previous report (7) it was concluded that the relationship between total gossypol content and chick-growth rate did not extend to the lower range of gossypol content. In the present investigation the two standards with total gossypol content, 0.20 and 0.15 per cent, respectively, fall very close to the curve, one on one side and one on the other. This would suggest that if a suitable manufacturing method can be developed to produce meals containing only small amounts of both total and free gossypol, these would probably be meals of exceptionally high nutritional value, provided that the solubility of the protein remained high.

Comparison of Figure 1 with Figure 2, which shows the relationship between nitrogen solubility in 0.02 N

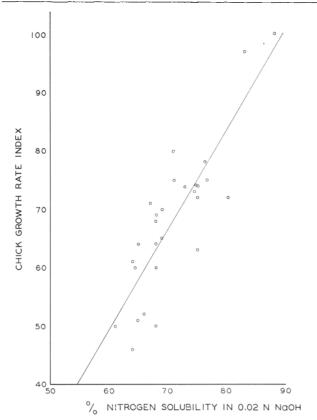


FIG. 2. The relationship of nitrogen solubility in 0.02 N NaOH to protein quality in cottonseed meal.

NaOH and the chick-growth rate indices, suggests that nitrogen solubility in 0.02 N NaOH and total gossypol are not completely independent factors. The correlation coefficient between these two measurements was 0.83. From this finding and previous concepts concerning the nature of bound gossypol it might be postulated that when gossypol is converted to the bound form, a gossypol protein complex is formed which is insoluble in 0.02 normal sodium hydroxide. In connection with this postulate it was of interest to determine whether the protein extracted by 0.02 N NaOH contained any bound gossypol. Free gossypol was removed from a sample of cottonseed meal containing 1% bound gossypol by extraction with 80% acetone. The soluble protein was then extracted with 0.02 N NaOH, and after removal of solid materials by centrifugation and filtration the protein was precipitated with methyl ethyl ketone. Only 0.002% total gossypol was found in the protein preparation. This is a negligible amount and indicates that if indeed bound gossypol is gossypol in the form of a protein gossypol complex, then the complex is not extracted with 0.02 N NaOH.

The correlation coefficients indicate that nitrogen solubility in 0.02 N NaOH is a better measure of protein quality than solubility in 0.5 M NaCl. However the latter measurement did prove to be more closely related to nutritional value than in previous studies in this laboratory.

Although excellent correlations between several chemical characteristics and nutritional value have been shown, there are a few meals which definitely do not fit the picture. Meals number 6A and 1A did not show the growth rate which might have been predicted. These meals were tested in a second feeding trial against the same standard, and the chick-growth rate index values were not very different from those obtained in the first experiment shown in Table I. Undoubtedly some factor affects the nutritional value of cottonseed protein, which we have not as yet been able to measure chemically. Work is in progress on this problem at the present time.

In view of the fact that a few meals were found in the series which do not fit the typical pattern with respect to the relationship between chemical characteristics and nutritional value, the question arises as to the practical value of the determination of nitrogen solubility in 0.02 N NaOH as a guide in controlling mill operations. In order to answer this question, average values for the performance of the different mills are given in Table II. It will be seen that mills D, H, and I produced meals with nitrogen solubility

		TABLE II	
Protein	Quality	and Chemical Characteristics o Meals from Different Mills	of Cottonseed

Mill desig- nation	No. of samples	Chick growth rate index	Total gossy- pol	Nitrogen solubil- ity in 0.02 N NaOH	Chemical index	Gossypol content of cot- tonseed kernels <sup>a</sup>
D H I E J	2 3 3 3 3	75.274.674.669.5	% 0.72 0.84 0.73 0.85	% 81.6 77.6 77.0 74.1	96.0 91.3 90.6 87.2	$\binom{\%}{0.65} \\ 0.74 \\ 0.67 \\ 0$
J A F B	3 1 3 2 2 3	$\begin{array}{c c} 71.8 \\ 52.1 \\ 64.3 \\ 59.9 \end{array}$	$\begin{array}{c} 0.81 \\ 0.86 \\ 1.05 \\ 1.06 \end{array}$	70.0 68.5 69.2 67.9	$82.4 \\ 79.7 \\ 65.9 \\ 64.1$	0.69 0.69 0.81 0.91
Ğ G	$\frac{2}{3}$	$54.6 \\ 51.8$	$1.02 \\ 1.23$	65.8 68.8	64.5 55.9	0.76

\* Rolled cottonseed meats used in the preparation of the meals.

averages ranging between 77.0 and 81.6% and that these were the meals with the highest average nutritional value.

Mills producing meals with average total gossypol content of 1% or greater did not produce meals with average nutritional values in the upper range. The variables which contribute to the production of meals with low total gossypol content are therefore of interest. The report of Pons and associates (1) shows that there are two major factors; one, the content of gossypol in the original cottonseed, and the other the loss of gossypol during processing where it may be dissolved in the oil to be removed later during refining. For example, the cottonseed kernels used by mills H and C had an average gossypol content of 0.74 and 0.76% respectively (Table II). In the case of mill H the final average gossypol content of the meals was 0.84%. In the case of mill C the corresponding value was 1.02, indicating considerable difference in the loss of gossypol during processing.

#### Chick Feeding Test at the Bureau of Field Crops, Department of Agriculture, State of California

*Experimental.* Day-old Single-Comb White Leghorn cockerel chicks were grown to two weeks of age on a practical chick-starter ration. At the end of the pre-experimental period the chicks were weighed and assigned to groups of 14 each on a weight basis. The average weight of all groups was about the same at the beginning of the experimental period. After the initial weighing the birds were weighed every two days during the experimental period of eight days.

Electrically heated battery brooders having raised wire floors were used for both the preliminary and experimental periods. Feed and water were provided *ad libitum*.

The basal diet used in this study is listed in Table III. The cottonseed meals used in this study varied

Composition of the Basal Diet a	Per cent
Cottonseed meal	To a 20% protein
Vitamin Mix No. 33	diet 5.0
Vitamin Mix No. 31	0,3
Salt Mix No. 34	6.42
Choline Chloride	0.2
Cottonseed Oil, Wesson	5.0
Sodium Chloride, iodized	0.5
Cerelose to 100%	

from 39.7% to about 58% protein. The percentage of cottonseed meal in the diet was adjusted so that the diet contained 20% protein. Cerelose was the adjustable ingredient making the diet up to 100%.

The figures listed in the following mixes are percentage of diet: Mix No. 33 thiamine hydrochloride, 0.001, riboflavin 0.001, pyridoxine hydrochloride 0.003, folic acid 0.006, calcium (d) pantothenate 0.003, folic acid 0.001, biotin 0.00001, 2-methyl-1, 4naphthohydroquinone diacetate 0.001, and cerelose to 5.00. Mix No. 31 feeding oil (2250A-300D) 0.25, mixed tocopherols (340 mg/gm) 0.05. Mix No. 34 Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 2.00, CaCO<sub>3</sub> 1.80, KCl 0.60, MnSO<sub>4</sub>·H<sub>2</sub>O 0.03, Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O 0.20, ferric citrate 0.074, CuSO<sub>4</sub> (Anhydrous) 0.005, ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.005, cobalt acetate (4H<sub>2</sub>O) 0.002, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.600, Al<sub>2</sub> (SO<sub>4</sub>)·18H<sub>2</sub>O 0.100, NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O. Results and Discussions. The protein efficiency index numbers are given in Table I. They range from 64.9 to 92.0, indicating considerable variation in the quality of the protein in the different samples. In addition to the tests on the experimental meals listed in Table I, one group of chicks was fed a soybean oil meal. The protein efficiency on this sample was 84.0. Although none of the prepressed-solvent cottonseed meals were quite as good as the standard cottonseed meal, four of them had index values higher than the soybean oil meal and three others with index values of 83.5 were only very slightly below. A sample of herring fish meal had a protein efficiency index number of 111.1, indicating that it was slightly better than the standard cottonseed meal.

Correlation coefficients between protein efficiency index numbers and three chemical characteristics, namely nitrogen solubility in 0.02 N NaOH, nitrogen solubility in 0.5 M NaCl, and total gossypol content were +0.807, +0.739, -0.805, respectively. These values, all highly significant, would indicate that nitrogen solubility in 0.02 NaOH is a better measure of protein quality than nitrogen solubility in 0.5 M NaCl and that total gossypol content is just as good an indicator as nitrogen solubility.

#### Chick Feeding Trials at the Louisiana Agricultural Experiment Station, Louisiana State University

Experimental. The protein quality indices of the meals were determined by feeding trials on groups of 10-day-old chicks. Except for several minor modifications, the method used for the evaluation of protein quality was that of Heiman et al. (9). Immediately after hatching, broiler strain New Hampshire chicks were placed on a standardization ration composed of yellow corn meal fortified with vitamins and minerals. This ration was fed for 10 days. At the end of the 10-day period the chicks were weighed individually and divided into groups according to weight. The chicks in each weight group did not differ in weight more than 5 g. In distributing the chicks into experimental lots, the same number of chicks for each weight group was placed in each experimental lot. This technique reduced considerably the individual variation in each experimental lot.

Two of the experimental lots were fed each of the test rations. These test rations were formulated to supply 12% protein  $(N \times 6.25)$ ; 6% protein was supplied by yellow corn meal, and 6% protein was supplied by the cottonseed meal under investigation. Vitamins and minerals were added, and equal parts of starch and sugar were used to make the ration up to 100 parts. The composition of a typical ration, in parts by weight, was: yellow corn meal, 65.0; cottonseed meal, 12.8; starch, 9.0; sugar, 9.1; steamed bone meal, 2.0; oyster shell flour, 1.0; salt, 0.5; and 0.6 parts supplied by a mixture of vitamins that fortified the ration with all known vitamins, according to the NRC recommended allowances. The chicks were fed this ration for a period of two weeks. At the end of this time the chicks were weighed individually, and group feed consumption was determined. The total two weeks gain for each lot was divided by the total protein consumed by each lot and the result expressed as gain per gram of protein consumed or protein efficiency. The quality index was obtained by dividing the gain per gram of protein consumed for each cottonseed meal by that of the standard cottonseed meal.

Results and Discussion. Some of the prepress-solvent cottonseed meals compared very favorably with the standard cottonseed meal in respect to protein quality. Approximately one-third of the meals had protein quality indices of 90 or more. One-third of the meals had indices of 80 or below. It appears that many prepress-solvent cottonseed meals are being manufactured in such a manner that the protein quality is kept high. However some are produced that are lacking considerably in respect to quality of protein. The correlation coefficients between the chemical characteristics and protein efficiency index are given in Table V. The negative correlation between protein quality index and total gossypol and the positive correlation between protein quality index and nitrogen solubility in 0.02 N NaOH are both significant at the 5% level.

#### Rat Repletion Tests at Beltsville, U.S.D.A.

Experimental. Eighteen meals were evaluated by rat protein repletion tests using a modification of the Cannon (10) technique. The rats were depleted by feeding a low protein diet containing 0.1% of protamone for a period of three weeks. The protamone was then discontinued, and the low protein diet was supplemented daily with cottonseed meal in an amount necessary to supply 0.16 g. of nitrogen per rat. The repletion period was continued for 10 days, and the gain in weight during this period was used as a measure of the quality of the protein. Further details of the procedure as applied to the study of cottonseed meals and an evaluation of the reliability of this method for such studies have been reported by Cabell and Earle (8).

Results and Discussion. The protein quality index numbers, as shown in Table I, varied from 51 to 91, indicating considerable variations in the quality of the protein in the different samples. This range is somewhat greater than that of the values for the chick-growth rate index shown in Table I by the data from the Texas and Louisiana laboratories; namely, 49.9 to 78.1 and 65.0 to 97.5, respectively, for the same meals. A more critical comparison of the values obtained by the rat-repletion method and chick-growth rate index for these laboratories is indicated by the correlation coefficients of 0.49 and 0.48, both of which are statistically significant at the 5% level. This shows a higher correlation between rat-repletion and chickgrowth than indicated by the results reported by Cabell and Earle (8) on other cottonseed meal samples but is probably due to the larger number of samples tested in this group. None of the prepresssolvent meals were quite as good as the standard meal which had been prepared without heat. The correlation coefficients between protein quality and the chemical characteristics, total gossypol content, nitrogen solubility in 0.02 N NaOH, and nitrogen solubility in 0.5 M NaCl are all highly significant (Table V). Of these the highest correlation, namely 0.75, is between protein quality index and nitrogen solubility in 0.02 N NaOH. The findings indicate that this solubility measurement is a good indicator of protein quality in cottonseed meal required for repletion of body protein of rats. All of the correlation coefficients calculated for rat-repletion, including the values of -0.68 for total gossypol and +0.68 for nitrogen solubility in 0.5 M NaCl, show that rat-repletion is in

general agreement with the other measures of protein quality of these meals.

#### Comparison of the Results Obtained by Different Laboratories

Keeping in mind that the conditions of the experiments and even the type of nutritional tests carried out by the different laboratories are different, it is of interest to note that the Louisiana group found 8 meals with protein-efficiency numbers which were at least 90% of the standard while the California group found only 2 and the Beltsville group using the ratrepletion test found only one. On the basis of chickgrowth rate the Texas group did not find any of the meals with values as high as 90. Undoubtedly these differences are due in part to the type of test performed. For example, in the chick-feeding tests at Louisiana State University, a 12% protein diet was used and half of the protein was supplied by corn. In the Texas and California tests the protein content of the diets was 21 and 20%, respectively, and cottonseed meal constituted the major source of protein.

The finding of the California group that four of the cottonseed meals had index values higher than their sample of soybean meal indicates that the better meals are excellent protein supplements. At the same time the possibilities for improvement in prepresssolvent cottonseed meals is clearly indicated.

For the purpose of evaluating mill performance, average values obtained by the different laboratories for the meals from the various mills are given in Table IV. Two general types of nutritional tests are

	TABLE IV		7
Nutritional	Prepress Solvent rent Laboratories	Meals	by

Mill	Nitrogen solubil-	Chick-gr	owth rate	index a	Protein efficiency index b				
desig- nation	ity in 0.2 N NaOH	Califor- nia	Texas	Av.	Louisi- ana	Califor- nia	Av.		
D	81.6	91.9	75.2	83.6	92.5	84.9	88.7		
н	77.6	88.3	74.6	81.5	82.0	81.5	81.8		
I	77.0	92.9	74.6	83.8	83.0	87.6	85.3		
$\mathbf{E}$	74.1	87.1	69.5	78.3	90.0	82.3	86.2		
$_{ m F}^{ m J}$	70.0	84.2	71.8	78.0	83.0	81.9	82.5		
$\mathbf{F}$	69.2	74.1	64.3	69.2	78.3	74.6	76.5		
$\mathbf{G}$	68.8	70.0	51.8	60.9	82.0	70.9	76.5		
в	67.9	75.4	59.9	67.7	72.3	73.6	73.0		
С	65.8	66.9	54.6	60.8	79.5	67.4	73.5		

<sup>a</sup> Growth rate expressed as per cent of rate obtained with standard cottonseed meal. <sup>b</sup> Sample protein efficiency  $\times 100$ 

Standard meal protein efficiency  $\times$  100.

summarized. The first of these is based on rate of growth of chicks. The results obtained by the Texas and the California groups are generally in good agreement with respect to determining which are the better meals and which are the poorer ones. When the index values obtained by the two laboratories are averaged, a clear-cut relationship between protein quality and nitrogen solubility in 0.02 N NaOH is apparent. The agreement between the results obtained by the Louisiana and California groups based on protein efficiency index number is less satisfactory. However it will be noted that when the protein efficiency index numbers obtained by the two laboratories are averaged, all mills producing meals with nitrogen solubility in 0.02 N NaOH of 70%, or higher, also produced meals with protein efficiency index numbers above 80. In every case average protein efficiency index numbers below 80 were obtained when nitrogen solubility dropped below 70%. Because the Beltsville group tested only part of the meals, their results have been omitted in the above comparison.

Two laboratories using the chick as the assay animal and one laboratory using the rat found a highly significant negative correlation between their nutritional index value and the total gossypol content of the meals (Table V). The third laboratory using the chick

	Corre	TABLE V elation Coef				
Chemical		rowth rate dex	Protein index	Rat reple-		
character- istics	Texas	Califor- nia	Louisi- ana	Califor- nia	U.S.D.A.	
Total gossypol content	-0.899 b	-0.791 b	-0.40 ¤	-0.805 b	-0.682 b	
Nitrogen solubility in 0.02 N NaOH	0.818 b	0.826 b	0.41 ª	0.807 b	0.749 <sup>b</sup>	
Nitrogen solubility in 0.5 M NaCl	0.787 b	0.665 <sup>b</sup>	0.37	0.739 <sup>b</sup>	0.680 b	

<sup>b</sup> Significant at the 1% level.

obtained data in which this correlation was significant but only at the 5% level. This laboratory used an experimental ration in which half of the protein was supplied by cottonseed meal and the other half by corn. This difference in the type of test performed may possibly account for the difference in results.

The correlation coefficients obtained by all of the four laboratories indicate that nitrogen solubility in 0.02 N NaOH is a better measure of protein quality in cottonseed meal than the solubility test with dilute NaCl. Comparison of the results obtained by the four laboratories would indicate that total gossypol content and nitrogen solubility in 0.02 N NaOH have approximately equal value as indicators of protein quality in prepress-solvent cottonseed meals. Interpretation of the finding concerning total gossypol should not be extended to other types of cottonseed meals without experimental evidence beyond that presented in this paper. Further investigations will be necessary before the relationship of total gossypol to protein quality can be fully understood.

#### Acknowledgments

The investigations at the Louisiana Agricultural Experiment Station were supported in part by a grant-in-aid from The National Cottonseed Products Association.

The investigations at the Texas Agricultural Experiment Station were supported in part by grantsin-aid from The Cotton Research Committee of Texas and The National Cottonseed Products Association. Inositol and cerelose were supplied through the courtesy of Corn Products Refining Company, Argo, Ill. Folic acid was supplied by Lederle Laboratories, Pearl River, N. Y.; biotin by Hoffman-LaRoche Inc., Nutley, N. J.; vitamin A by Stabilized Vitamins Inc., Brooklyn, N. Y.; vitamin D<sub>3</sub> by Bowman Feed Products Inc., Holland, Mich.; and the remainder of the vitamins by Merck and Company, Rahway, N. J.

The reports from the various laboratories which form parts of this paper were assembled by Carl M. Lyman.

#### REFERENCES

1. Pons, W. A., Thurber, F. H., and Hoffpauir, C. L., J. Am. Oil
Chemists' Soc. (in press).
2. Couch, J. R., Chang, W. Y., and Lyman, C. M., Poultry Science
(in press).
3. Olcott, H. S., and Fontaine, T. D., Ind. Eng. Chem., 34, 714
(1942).
4. Haddon, R. P., Schwartz, A. H., Williams, P. A., Thurber, F. H.,
Karon, M. L., Dechary, J., Guice, W., Kupperman, R., O'Connor, R. T.,
and Altschul, A. M., Cotton Gin & Oil Mill Press, 51, No. 9, 18-20
(1950).
5. Milligan, J. L., and Bird, H. B., Poultry Science, 30, 651 (1951).

Reuther, C. G. Jr., LeBlanc, M. F. H. Jr., and Batson, D. M.,
 J. Am. Oil Chemists' Soc., 30, 28 (1953).
 Lyman, C. M., Chang, W. Y., and Couch, J. R., J. Nutrition, 49, 679 (1953).
 R. Cabell, C. A., and Earle, I. P., J. Agr. and Food Chem., 2, 787 (1954).
 Heiman, V. J., Carver, J. S., and Cook, J. W., Poultry Science, 18, 464 (1939).
 Cannon, P. R. Humphreys, E. M., Wissler, R. W., and Frazier, L. E., J. Clin. Invest., 23, 601 (1944).

[Received September 29, 1954]

## Nomograph for Determining Percentage Extraction Efficiency

ANGELO V. GRACI JR.,<sup>1</sup> Southern Regional Research Laboratory,<sup>2</sup> New Orleans, Louisiana

THIS alignment chart enables convenient and accurate determinations to be made of vegetable oil processing extraction efficiencies. Percentage extraction efficiency as used here is defined as

 $100 imes rac{\text{oil removed by processing}}{\text{oil available in feed material}}$ 

The range of the variable "% lipids in the feed material" has been extended to cover the oil contents of all oleaginous materials, including low-oil content press cakes destined for subsequent solvent extraction. The range of the variable "% lipids in the extracted meal or cake" has been extended to cover solvent extraction, screw and hydraulic press operations. The percentages of lipids and solids, indicated on the chart, are both to be used on a "wet" or "as received" basis.

The use of the chart can be illustrated in the following example. A certain feed material before extraction contained 30% oil and 8% moisture (62%solids). The meal or cake after extraction contained 2% residual lipids and 6% moisture (92% solids). What extraction efficiency was obtained?

Using the above values, the percentage extraction efficiency is determined as follows: A line is drawn connecting % lipids and % solids in the feed material. A second line is drawn connecting % lipids and % solids in the extracted meal. Each of these two lines intersects a reference line. A third line joining the two intersection points crosses the % extraction efficiency line at a value of 95.5%.

The equation on which this alignment chart is based is

$$Z = 100 \left(1 - \frac{BC}{AD}\right), \text{ where}$$

$$A = \% \text{ lipids in feed material}$$

$$B = \% \text{ solids in feed material}$$

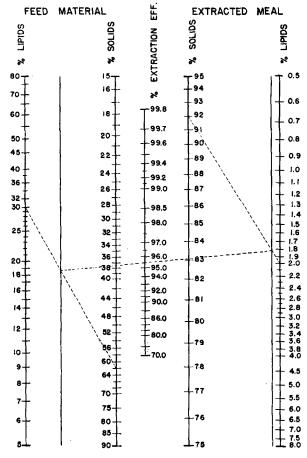
$$C = \% \text{ lipids in extracted meal or cake}$$

$$D = \% \text{ solids in extracted meal or cake}$$

$$Z = \% \text{ extraction efficiency.}$$

### EXTRACTION EFFICIENCY

CHART



[Received July 28, 1954]

<sup>&</sup>lt;sup>1</sup> Present address: Wurster and Sanger Inc., 5201 South Kenwood Avenue, Chicago, Ill.

<sup>&</sup>lt;sup>2</sup> One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture.