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Factors Affecting the Nutritional Quality of Cottonseed Meals

SUMMARY

Eighteen samples of cottonseed oil meals from different countries in Central America were analyzed for proximate composition, free and total gossypol, epsilon-amino lysine groups and lysine, methionine, and threonine content. The meals were fed to weanling rats at a 10% protein level for 8 weeks, and protein efficiency ratios were determined at 4 and 8 weeks. The results showed a significant positive correlation between epsilon-amino groups of lysine content and PER and a negative correlation between weight gain and total gossypol content as well as between residual oil content and PER, or weight gain. L-lysine supplementation alone did not improve a poor-quality meal, while exhaustive extraction of the oil did increase the PER. The possibility of heat damage to the residual oil during the process of extraction was discussed.

Cottonseed oil meal is recognized as a potential source of good-quality protein for human consumption, especially in areas where the intake of both animal and vegetable protein does not meet the daily requirements of the population INCAP Vegetable Mixture 9 (Bressani *et al*, 1961) has been introduced successfully as a protein supplement into several populations in Latin America whose animal protein intake is low This product has a biological value slightly lower than milk (Scrimshaw *et al*, 1961), and its main source of protein is high-quality cottonseed oil meal Cottonseed meals, however, vary greatly in composition and nutritional quality with the variety of cotton, the time of harvest, and, perhaps more important, the industrial processes to which the seed is subjected during the extraction of the oil (Altschul, 1958)

In the experiments reported here, variation in the chemical composition of 18 samples of cottonseed oil meals from different countries in Central America was determined by analyses of proximate composition as well as of free and total gossypol, epsilon-amino groups of lysine and total lysine, methionine, and threonine content to continue the studies on Central American cottonseed flours (Bressani *et al*, 1964) Biological trials were also carried out with rats

MATERIALS AND METHODS

The different meals were stored at 4°C until analyzed for moisture, ash, fat, crude fiber, and protein The epsilon-amino groups of lysine were determined by the method of Conkerton and Frampton (1959), free and total gossypol by the method of the AOCS (1950), and lysine, methionine, and threonine by microbiological techniques (Bressani and Rios, 1962) Urea fractionation of the residual fat was done according to the method of Crampton *et al* (1953) Gas chromatography was also carried out on the methyl esters of the residual oils of some of the meals with a Perkin-Elmer fractometer, Model 154, an adipate polyester column, and helium as a carrier gas Of the 18 samples analyzed, 7 (samples Nos 4996, 5130, 5263, 5268, 5269, 5535, 5986) were produced by the prepressed solvent extraction method and 11 by the screw-press process

For the biological trials, each meal was fed for 8 weeks at the protein level of 10% to 8 female and 8 male rats of the Wistar strain The rats were housed in individual cages with raised screen bottoms in a constant-temperature room Feed and water were supplied *ad libitum,* and the animals were weighed every week. Weekly records of feed consumption were kept, and protein efficiency ratios were calculated at the end of 4 and 8 weeks The basal diet consisted of, in g per 100 g 4 0 Hegsted mineral mixture (Hegsted *et al*, 1941), 5 0 cottonseed oil, 1 0 cod liver oil, 38 0 dextrose, 52 0 cornstarch, and vitamin solution as recommended by Manna and Hauge (1953) Cottonseed meals replaced starch in sufficient amounts to provide 10% protein in the diet

When L-lysine supplementation was studied, the amount of supplementary L-lysine was calculated taking into account only the epsilon-amino lysine group content of the meal This was then increased to 3 6 g per 16 g of nitrogen in the meal with L-lysine so that all supplemented diets contained the same amount of available lysine, namely, 0 6% of the diet

Residual fat in the meal was removed with hexane in a continuous extracting apparatus

Table 1. Proximate composition (g%) of cottonseed oil meals.

Sample no.	Moisture	Ether extract	Crude fiber	Crude protein [a]	Ash
4996	6.2	1.7	5.0	51.9	7.3
5130	9.4	2.7	4.5	49.4	7.7
5259	10.6	6.8	4.5	45.7	6.9
5260	8.6	5.4	9.1	43.6	7.0
5261	9.0	8.1	4.9	46.1	7.4
5262	8.4	6.4	7.7	46.1	7.5
5263	10.5	0.7	4.2	46.6	7.7
5264	7.2	8.3	9.9	40.8	7.0
5265	9.8	9.7	4.4	42.1	6.5
5266	6.9	5.2	7.8	46.9	7.6
5267	10.3	6.2	3.2	53.0	6.2
5268	7.1	3.1	4.1	49.8	7.9
5269	6.8	3.7	4.4	51.1	7.8
5270	8.8	6.3	9.9	40.0	7.0
5535	5.8	2.2	4.7	50.0	8.2
5986	7.3	1.7	4.4	52.0	7.9
5987	7.6	10.4	8.7	40.8	6.2
6003	3.1	5.4	3.3	52.8	6.6
Mean	8.0±	5.2±	5.8±	47.2±	7.2±
standard deviation	1.9	2.8	2.4	4.4	0.6

[a] N content × 6.25.

RESULTS

Table 1 shows the results of the proximate analysis of the meals.

Table 2 gives the epsilon-amino groups of lysine, free and total gossypol and free lysine, and threonine and methionine content of the different meals.

Table 3 shows the weight gain and protein efficiency ratios in rats fed the different meals. The results are presented separately for males and females at 4 and 8 weeks of age. It is evident that the different meals differed greatly in these parameters.

Fig. 1 illustrates the correlation between epsilon-amino groups of lysine and PER, calculated at 4 weeks of age; there was a positive correlation between these two variables, with a correlation coefficient of 0.62, significant at the 1% level.

Fig. 2 demonstrates the negative correlation between total gossypol content and weight gain, calculated at 4 weeks of age. The correlation coefficient was –0.65, which was significant at the 1% level.

Fig. 3 shows the correlation between residual fat content in the meals and protein efficiency ratio at four weeks of age. Again, the correlation was negative, with a coefficient of –0.62, significant at the 1% level.

Fig. 4 shows the correlation between residual oil content in the meals and weight gain at four weeks of age. There was a negative correlation between these two variables, with a correlation coefficient of –0.77, significant at the 1% level. Sample No. 5265 was omitted from this calculation; when included in the analysis, the correlation coefficient was –0.52, which was significant at the 5% level. Results at 8 weeks showed the same trends as those obtained at 4 weeks.

A multiple-regression approach indicated that the cottonseed meal content of epsilon-amino lysine had a significant effect on both weight gain and PER, while a significant effect of total gossypol content could be shown only in the case of weight gain.

Table 4 shows the effect of lysine supplementation and of defatting of two cottonseed meals, one of which gave a low-PER (*KH*) and the other a high-PER (*B*). As can be seen from the table, lysine alone could not raise either the weight gain or the protein efficiency ratio of a low-PER meal to the level obtained with the high-PER meal supplemented with the same level of free lysine. Removal of residual fat from a cottonseed meal of good quality (high-PER) and a cottonseed meal of poor quality (low-PER) slightly increased the PER, but more so in the poor-quality meal than in the good-quality meal.

Table 5 shows the percentage of urea adducts in two samples of cottonseed oil, and the gas chromatography findings. As can be seen, the largest percentage of the oil did not react with urea; however, the products that did not react had a higher iodine

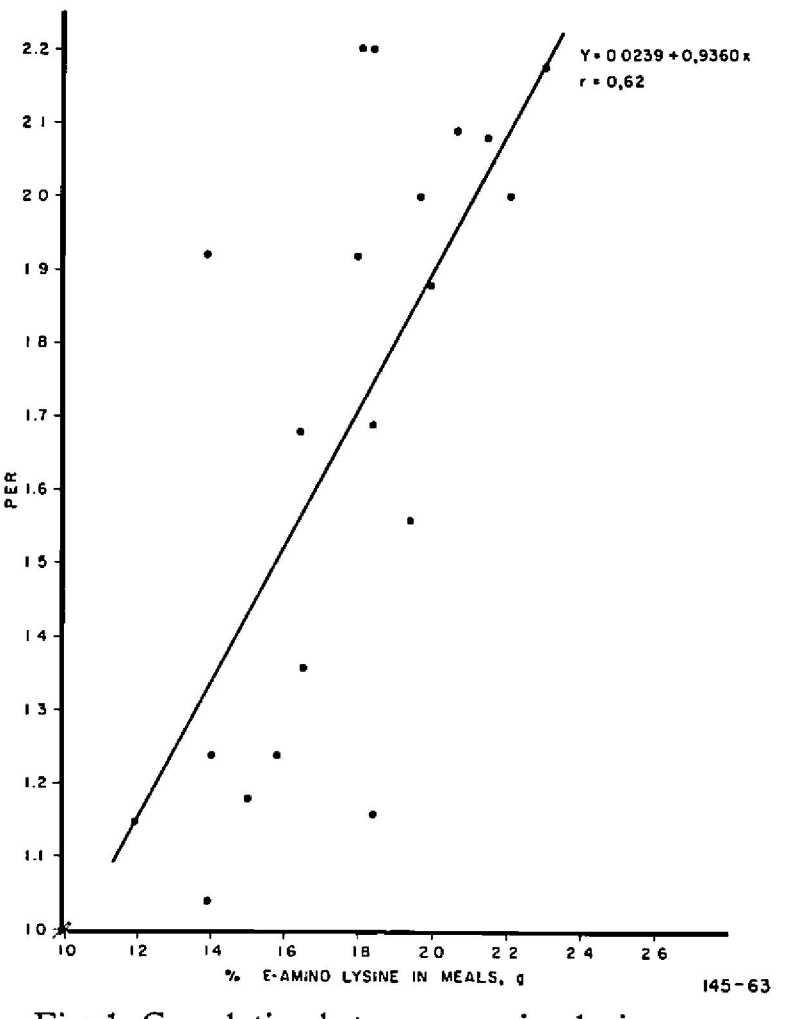

Fig. 1. Correlation between ε-amino lysine groups in cottonseed meals and PER in rats.

Table 2. Epsilon-amino lysine, free and total gossypol, and total threonine, lysine, and methionine content of cottonseed oil meals.

Sample no.	ε-Amino lysine (g%)	ε-Amino groups of lysine (g/16 g N)	Free gossypol (mg%)	Total gossypol (g%)	Threonine (g%)	Lysine (g%)	Methionine (g%)
4996	2.217	4.27	79.0	1.092	1.92	2.71	0.70
5130	2.161	4.38	80.9	1.058	1.77	2.08	0.74
5259	1.839	4.02	41.9	0.940	1.66	2.27	0.71
5260	1.652	3.48	51.4	1.223	1.66	2.26	0.66
5261	1.844	4.06	79.4	1.250	1.70	2.28	0.70
5262	1.390	3.01	84.1	1.224	1.64	2.19	0.71
5263	1.800	3.87	48.8	0.888	1.82	2.71	0.70
5264	1.500	3.67	56.2	1.264	1.70	2.15	0.63
5265	1.844	4.38	73.9	0.937	1.86	2.45	0.70
5266	1.585	3.38	50.5	1.184	1.65	2.10	0.65
5267	1.940	3.66	40.1	0.738	1.51	2.84	0.62
5268	1.992	4.00	95.6	1.155	1.88	2.47	0.67
5269	1.969	3.85	128.2	1.036	1.70	2.89	0.71
5270	1.398	3.49	62.2	1.172	1.51	2.16	0.68
5535	2.068	4.13	72.8	1.042	1.61	2.71	0.76
5986	1.806	3.47	67.4	0.880	1.76	2.90	0.56
5987	1.392	3.41	99.9	1.085	1.59	2.33	0.46
6003	1.640	3.10	61.0	1.071	1.69	2.66	0.48
Mean	1.780±	3.76±	70.7±	1.069±	1.70±	2.45±	0.66±
standard deviation	0.259	0.41	22.5	0.146	0.12	0.29	0.08

Table 3. Weight gain and PER of rats fed cottonseed oil meals.

Sample no.	Av. initial wt. (g)	Weight gain [a] (g)				PER [a]			
		4 weeks		8 weeks		4 weeks		8 weeks	
		Males	Females	Males	Females	Males	Females	Males	Females
4996	47	84	90	180	156	1.96	2.03	1.71	1.58
5130	48	83	86	171	162	2.02	2.13	1.77	1.79
5259	46	76	80	166	152	1.69	1.68	1.55	1.45
5260	50	51	55	94	117	1.36	1.35	1.16	1.27
5261	49	42	45	89	96	1.10	1.23	1.09	1.20
5262	46	34	34	62	66	1.11	0.96	0.94	0.88
5263	46	109	100	220	172	1.98	1.87	1.71	1.48
5264	47	45	48	92	97	1.21	1.14	1.08	1.05
5265	46	114	101	141	123	2.25	2.16	2.11	2.05
5266	55	44	56	96	112	1.14	1.34	1.11	1.20
5267	45	72	66	142	129	1.62	1.51	1.43	1.32
5268	47	92	90	194	153	1.92	1.84	1.64	1.43
5269	44	112	94	232	167	2.11	1.88	1.83	1.54
5270	44	54	54	112	110	1.23	1.24	1.10	1.09
5535	42	97	94	199	183	2.18	2.00	1.90	1.74
5986	48	96	92	200	178	2.25	2.15	1.96	1.84
5987	48	55	66	116	126	1.84	1.99	1.68	1.64
6003	44	63	62	150	142	1.69	1.67	1.64	1.53
Mean	47±	74±	73±	148±	136±	1.70±	1.68±	1.52±	1.45±
standard deviation	3	26	21	51	32	0.41	0.39	0.36	0.30

[a] 8 males and 8 females per group. PER: weight gain/protein consumed.

Table 4. Effect on the performance of rats of lysine supplementation and of defatting a high-PER (B) (CN) and a low-PER (KH) cottonseed oil meal.

Treatment of cottonseed meal	Av. initial wt. (g)	Weight gain [a] (g)	S.D.	PER [a]	S.D.	Total gossypol (mg%)	ε-amino lysine (g%)
B	50	94	11	1.90	0.13	1062	1.97
B + lysine	50	122	17	2.40	0.21		
KH	50	45	9	1.24	0.21	1186	1.22
KH + lysine	51	78	16	1.93	0.20		
CN	47	102	16	2.08	0.23	896	1.37
CN defatted	47	107	10	2.12	0.08	914	1.55
KH	48	52	10	1.34	0.15	1186	1.22
KH defatted	48	57	11	1.48	0.15	1211	1.27

[a] 16 rats per group (8 males and 8 females).

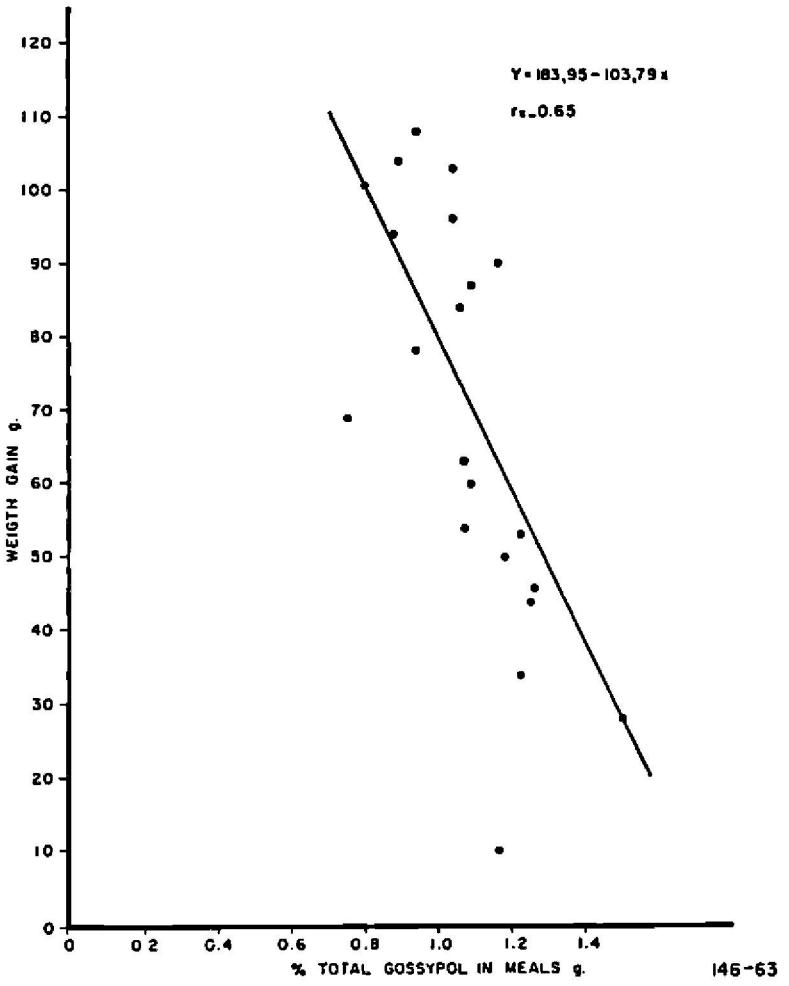

Fig. 2. Correlation between total gossypol in cottonseed meals and weight gain of rats.

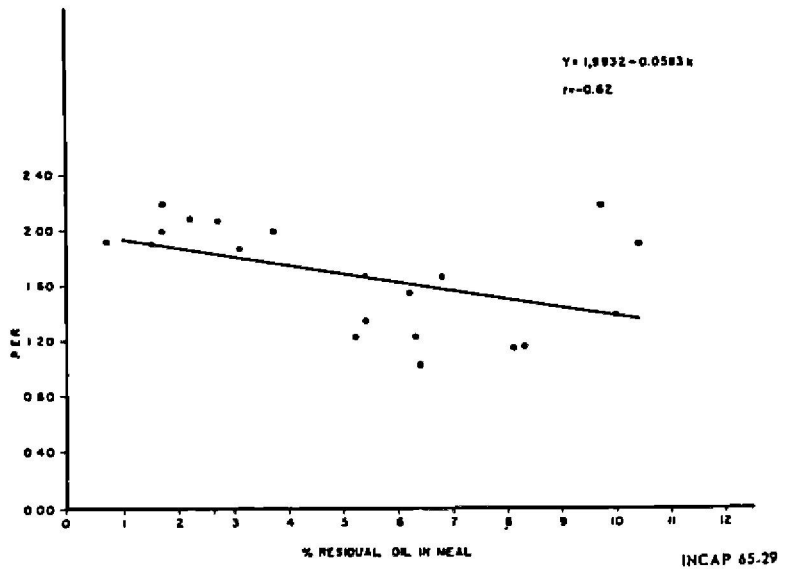

Fig. 3. Correlation between PER and fat content of cottonseed oil meals.

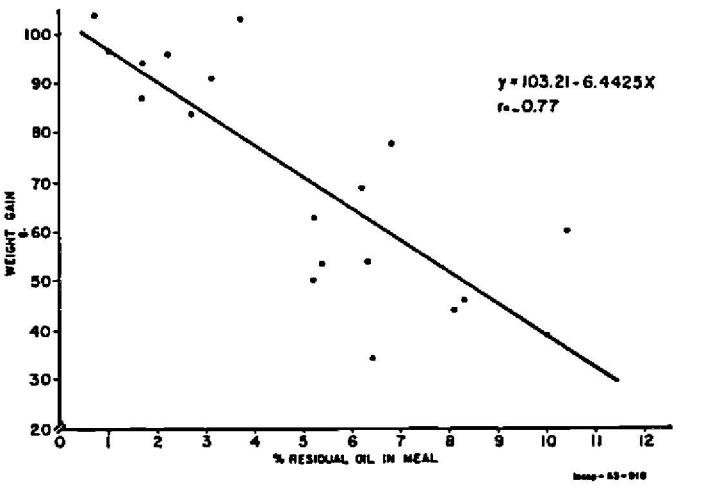

Fig. 4. Correlation between weight gain in rats and residual oil in cottonseed oil meals.

Table 5. Fatty acid gas chromatography and urea adducts of samples of residual cottonseed oil.

	% in cottonseed oil			
Fatty acid	Refined	KH	CN	B
C^{12}	0.21	0.11	0.08	0.07
C^{14}	0.98	0.99	0.91	0.83
C^{16}	25.82	26.94	26.23	27.90
$C^{16:1}$	0.81	1.25	1.57	1.50
C^{18}	2.59	2.65	2.98	2.90
$C^{18:1}$	16.97	16.59	15.87	16.50
$C^{18:2}$	52.60	50.90	51.54	49.50
Urea adducts	14.00	9.71	5.48	—
Urea non-adducts	61.34	74.00	75.98	—
IN [a] urea adducts	41.3	36.8	45.9	—
IN [a] urea non-adducts	101.6	82.2	92.2	—

[a] Iodine number.

number than those capable of forming urea adducts.

The gas chromatography findings of the oil from a good- and a poor-quality cottonseed meal and that of refined cottonseed oil, used as control, showed that the residual fats did not differ in fatty acid composition from those in refined cottonseed oil.

Table 6 shows the gossypol content expressed as bound gossypol percent and as percent of total gossypol. The meals were grouped according to the

Table 6. Free epsilon-amino groups, gossypol, and ether extract of cottonseed meals and their effect on rat growth and PER.

Meal no.	Free ε-amino groups of lysine (g%)	Free ε-amino groups of lysine (g/16 g N)	Gossypol Free (mg%)	Gossypol Total (g%)	Gossypol Bound (g%)	Gossypol Bound (% of total)	Ether extract (g%)	Rat (4 weeks) Wt. gain (g)	Rat (4 weeks) PER
4996 P	2.22	4.27	79.0	1.09	1.01	92.7	1.7	87	2.00
5130 P	2.16	4.38	80.9	1.06	0.98	92.4	2.7	84	2.08
5263 P	1.80	3.87	48.8	0.89	0.84	94.4	0.7	104	1.92
5268 P	1.99	4.00	95.6	1.16	1.06	91.4	3.1	91	1.88
5269 P	1.97	3.85	128.2	1.04	0.91	87.5	3.7	103	2.00
5535 P	2.07	4.13	72.8	1.04	0.97	93.3	2.2	96	2.09
5986 P	1.81	3.47	67.4	0.88	0.81	92.0	1.7	54	2.20
Av.	2.00 ± 0.16	4.00 ± 0.30	81.8 ± 24.9	1.02 ± 0.10	0.94 ± 0.09	92.0 ± 2.2	2.2 ± 1.0	88 ± 17	1.88 ± 0.11
5259 SP	1.84	4.02	41.9	0.94	0.90	95.7	6.8	78	1.68
5260 SP	1.65	3.48	51.4	1.22	1.17	95.9	5.4	53	1.36
5261 SP	1.84	4.06	79.4	1.25	1.17	93.6	8.1	44	1.16
5262 SP	1.39	3.01	84.1	1.22	1.14	93.4	6.4	34	1.04
5264 SP	1.50	3.67	56.2	1.26	1.20	95.2	8.3	46	1.18
5265 SP	1.84	4.38	73.9	0.94	0.87	92.6	9.7	108	2.20
5266 SP	1.58	3.38	50.5	1.18	1.13	95.8	5.2	50	1.24
5267 SP	1.94	3.66	40.1	0.74	0.70	94.6	6.2	69	1.56
5270 SP	1.40	3.49	62.2	1.17	1.11	94.9	6.3	54	1.24
5987 SP	1.39	3.41	99.9	1.08	0.98	90.7	10.4	60	1.92
6003 SP	1.64	3.10	61.0	1.07	1.01	94.4	5.4	62	1.68
Av.	1.64 ± 0.20 [a]	3.60 ± 0.41	63.7 ± 18.7	1.10 ± 0.16	1.03 ± 0.16	94.2 ± 1.6	7.1 ± 1.8	60 ± 20	1.48 ± 0.36

[a] Standard deviation. P = prepressed solvent extracted. SP = screwpress or expeller.

procedure used for extraction of the oil. As can be seen, the bound gossypol shows little correlation with the free epsilon-amino groups, which suggests that free epsilon-amino lysine groups are not the only active compounds which can bind gossypol.

DISCUSSION

From the data, it is evident that several factors affect the nutritive value of cottonseed oil meals. The main factors seem to be free epsilon-amino lysine groups, total gossypol, and residual fat content. The relation of free epsilon-amino groups of lysine to PER was expected, since it is known that cottonseed protein is limiting in lysine and that, during processing, free gossypol and the effect of heat bind certain groups like the epsilon-amino group of lysine, which renders them resistant to enzymatic hydrolysis. The correlation is, however, not as high as that reported by others (Frampton, 1960), suggesting that other amino acids are also possibly affected.

Total gossypol has also been known to affect weight gain in animals (Baliga and Lyman, 1957; Smith *et al.*, 1958), as it did in this study. Since total gossypol is the sum of bound and free gossypol and since free gossypol represents only a rather small quantity of the total, the negative relation between weight gain and total gossypol is really between weight gain and bound gossypol. This reasoning is logical since more bound gossypol would mean less available lysine, and less bound gossypol would mean more available lysine. This would in turn mean more or less gain in weight.

No significant relation was found between free gossypol and weight gain or PER. This is not surprising since the rat is not as susceptible to free gossypol toxicity as other animals, such as swine. Furthermore, the amounts present in the diet are relatively small. On the other hand, more free gossypol means more available lysine, which would possibly be more important to the young, growing rat and to PER determinations.

The effect of residual oil in the meal on PER is probably due to the fact that this oil has been overexposed to heat and oxidation during the industrial process of extraction. Sample No. 5265 was an exception, however. This sample had a high free lysine content with a low total gossypol, and a relatively higher amount of threonine than the other samples. It is evident that processing results in a significant decrease in lysine. It is also likely that lysine is not the only amino acid affected, since when a poor-quality meal was supplemented with free lysine to give 3.6 g of free lysine/16 g N, the improvement in weight gain and PER was far below that resulting from a good-quality meal with the same amount of free lysine.

Previous studies (Braham *et al.*, 1962) at this laboratory with swine fed rations adequate in all nutrients, but with different levels of cottonseed meal, showed that as the level of cottonseed meal in the ration was increased, certain symptoms of toxicity appeared in the experimental animals. These symptoms were characterized mainly by diarrhea, rough hair coats, and skin lesions, which had not previously been reported as symptoms of gossypol toxicity but rather as a result of a low fat intake, and especially as a deficiency of essential fatty acids (Hanson *et al.*, 1958). Since, in those studies, fat intake was adequate, and since, in the experiments reported here, all diets were supplemented with pure cottonseed oil, a deficiency either of fat or of essential fatty acids cannot be indicted as a cause of the gross symptoms observed in swine and, even less, of the negative correlation found between protein efficiency ratio and residual oil in rats.

Certain fractions of overheated edible oils were reported by Crampton *et al.* (1953) to be toxic for rats, probably due to the polymerization of fatty acids. Although the temperature used in their study can be easily attained in some of the industrial oil-extraction processes, in cottonseed meal the exposure time is much shorter. It is possible that the damage by heat and subsequent oxidation during storage would account, at least in part, for the negative correlation found between PER and residual oil content in rats. Further support for this speculation seems to be the lack of effect of lysine supplementation on the PER and the increase, though slight, of the PER when the residual oil was extracted from the meals.

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