involved in oil removal. It remains to be determined whether other factors besides free gossypol content, such as the nature of bound gossypol, affect the nutritive value of the meal for poultry and swine.

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The Detoxication of Tung Meal

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THE tung oil industry of the Gulf coastal area has grown remarkably in the last two decades. The industry produces as by-product large quantities of a press-cake which is quite toxic. This toxicity has restricted the use of the cake to that of a fertilizer or fertilizer extender. The problem of up-grading the cake to a feedstuff has attracted the attention of several laboratories in the area without seemingly producing a clear-cut answer.

Erickson and Brown (4) found a commercial press cake to be non-toxic to rats and reported that oil-free meal prepared in the laboratory could be detoxified by heat alone. Preliminary moistening aided this detoxication. Rusoff, Mehrhof, and McKinney (6) reported that a press cake extracted with naphtha, steamed, and fermented in storage had little, if any, toxicity and small value as a feedstuff. Later Davis, Mehrhof, and McKinney (2) reported that a very toxic press-cake could not be detoxified by heat alone. Chicks were used as experimental animals in both cases. The commercial cakes used by Emmel (3) had a relatively low level of toxicity which decreased during storage. These he converted into non-toxic meals by first extracting with ether and alcohol or acetone and alcohol and then hydrolyzing the residue by autoclaving in the presence of dilute hydrochloric acid. Both solvents were necessary to detoxify the material completely. Emmel concluded that two toxic principles were present: a saponin and a second alcohol soluble material. Chicks were the experimental animals used. In a brief summary devoid of all details Bryan (1) stated that the toxic principle is an albumin and that tung meal may be detoxified quite readily to provide a nutritious feed-stuff for chicks, mice, and cattle

The literature based on research in the Far East does not seem to clarify the above picture. We hope to do so in this report.

Experimental

General Procedure. Two commercial press-cakes were obtained. A solvent extracted meal was also obtained from the Tung Oil Laboratory of the United States Department of Agriculture. This meal had been prepared by cracking and flaking cleaned tung kernels and then extracting the oil without heat by forcing Skellysolve B through the flakes. The final meal was air-dried.

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Preliminary observations indicated that albino rats do not relish diets containing tung products. There were also indications that rats are more resistant to the toxicity than chicks. Chicks readily consumed even the most toxic tung products and showed no resistance to the property. Week-old New Hampshire chicks were accordingly used as test animals. They were fed the rations detailed in Table I. It

TABLE I

Composition of Rations

Component	Stock	Experimental
	%	%
Milo	25.0	10.0
Wheat bran	18.0	18.0
Wheat shorts	18.2	18.2
Soybean oil meal	17.0	12.0
Meat scraps	5.0	5.0
Liver meal	2.5	2.5
Alfalfa leaf meal	10.0	10.0
Oyster shell	1.0	1.0
Bone meal	2.0	2.0
Salt	1.0	1.0
Delsterol	0.3	0.3
Tung meal or cake	.....	20.0

should be stressed that the experimental ration containing 20% of tung meal is not an economic ration but was designed to give good growth, irrespective of the quality of the tung protein, provided the tung fraction was non-toxic. Extracts of the meals were stripped of solvent and fed in capsules to chicks consuming the stock ration. Feed and water were continually available. Experimental and control groups contained 10-12 chicks unless otherwise specified. The fowls were weighed every fourth day over the experimental period of 24 days.

Toxicity of Meals Used. The cakes and meal were tested as soon as possible after receipt and at intervals thereafter. The results are summarized in Table II. The drop in toxicity of the press-cake is in marked contrast to the stable level of toxicity of the extracted meal. The decrease in toxicity coincided with a change in appearance of the cake from an oily to a dry solid. No further decrease in toxicity was noted thereafter. It seems probable that the initial rapid decrease in toxicity is coupled with oxidation of the residual tung oil, a reaction initiated by the expeller process.

Action of Solvents. Portions of the Skellysolve extracted meal were placed in a Soxhlet type extractor

TABLE II

Toxicity of Untreated Press Cakes and Meal

	On receipt	Mortality	
		Aged 3-5 months	Aged 12-15 months
	%	%	%
Press cake A	100	20	50
Press cake B	100	50	60
Extracted[a] meal	100	100	100

[a] Extracted with Skellysolve B without heat.

and continuously extracted under vacuum for a 48-hour period with various solvents. The residual meals were air-dried, incorporated into the test diet, and fed to groups of 4-6 chicks. All meals were still lethal. The stripped extracts were semi-solid, and all were toxic with the exception of those prepared with petroleum ether fractions and dioxane. The solvents which extracted lethal material, in decreasing order of their apparent effectiveness, were carbon tetrachloride, 95% ethanol, ethyl acetate, ethyl ether, benzene, chloroform, acetone, and trichloroethylene.

Ethanol (95%) was selected for further trial. A sample of the same meal was extracted continuously in the Soxhlet at atmospheric pressure for 96 hours and then batch-extracted for a week with overnight soaking periods. Both extract and residue were lethal. It should be noted that the meal was subjected to some heat as it remained at the boiling point of the solvent or slightly below for the 96-hour period.

Heat Treatment. The Skellysolve extracted meal was autoclaved for 6 hours at 15 lbs. pressure. No marked decrease in toxicity was observed.

Production of a Non-Toxic Meal. The Skellysolve extracted meal was extracted with ethanol (95%) in a Soxhlet at atmospheric pressure for 48 hours, air-dried, and then steamed at 100°C. The resulting meal was non-toxic, provided it had been moistened with water before heating and the steaming period was at least two hours. See Table III. The presence of water was definitely desirable. This same process was effective with both press-cakes and steaming time could be reduced to one hour. This data is not reproduced as it parallels that given. It should be emphasized that prior extraction of the press-cakes with a solvent other than ethanol was not necessary.

TABLE III

Effect of Various Treatments on Toxicity of the Solvent Extracted Meal[a]

Treatment	Average gain[b]	Mortality	LT_{50}[c]
	g.	%	*days*
1. None	....	100	3
2. Ethanol extracted 48 hrs., 1 atm	....	100	17
3. Autoclaved 6 hrs., 15 lbs. pressure	....	100	6
4. No. 2 moistened, steamed 100°C., ½ hour	171	0	....
5. No. 2 moistened, steamed 100°C., 1 hour	181	0	....
6. No. 2 moistened, steamed 100°C., 1½ hours	201	0	....
7. No. 2 moistened, steamed 100°C., 2 hours	260	0	....
8. No. 2 dry, steamed 100°C., 2 hours	143	0	....
9. Stock ration[d]	246	0	....

[a] Press-cakes yielded similar data.
[b] Over a 24-day period.
[c] Days required for 50% mortality.
[d] Average gain from six trials. Range = 235-257 g.

Quality of Tung Protein. Oil meals as feedstuffs are primarily of value as protein concentrates which supplement the standard cereals. The results given so far indicate only that detoxified meals are digestible and may act as sources of energy. We therefore made no attempt to pin-point the conditions necessary for detoxication but tested the ability of our non-toxic meals to supplement the cereals by the method of Heiman, Carver, and Cook (5). This method utilizes chicks as test animals and casein as the reference protein. Protein concentrates are fed at a level which will increase the protein content of a basal cereal ration by 3%. The increase in growth caused by such supplementation is compared with the increase brought about by casein. The results shown in Table IV indicate that the tung protein had practically no supplementary value for the basal cereal ration. It was far inferior to the sample of soybean oil meal run at the same time. Although different species have different requirements, we feel that this casts serious doubt on the value of detoxified tung meals as protein concentrates.

TABLE IV

Relative Nutritional Values of the Tung Protein

	Protein (N×6.25)	Average[a] gain	Relative[b] value
	%	*g.*	
Basal cereal	8.0	27	0
Casein[c]	....	77	100
Soybean oil meal	45.7	55	56
Expeller meal A	28.8	37	20
Expeller meal B	31.9	32	10
Extracted meal	42.1	33	12

[a] Over a 14-day period following a 14-day depletion period on the basal cereal ration.
[b] Casein assigned a value of 100.
[c] Acid washed, alcohol extracted.

Discussion

The simplest explanation of the necessity for both heat and alcohol extraction is that two toxic principles are present in tung meals. One is heat-stable and soluble in alcohol and the lipid solvents listed. We (7) have concentrated this material by solvent partition and chromatography. The other is insoluble in lipid solvents and destroyed by heat in the presence of water. This is the saponin of Emmel or the albumin of Bryan.

It seems apparent that the rather wide variability in reported toxicities of press cakes for the same species of test animal can be explained on the basis of variable heat treatment during pressing and on the time elapsing between pressing and feeding. In the case of Erickson and Brown, who used a stored meal, the use of a somewhat resistant animal, and failure to observe growth also enters the picture.

The synergistic effect of heat and moisture noted by Erickson and Brown seems beyond question. The dilute acid treatment of Emmel is not necessary; water alone is sufficient. We are inclined to believe that moisture hastens the coagulation of the albumin of Bryan rather than functions as a hydrolytic agent.

There seems to be no doubt that petroleum ethers or naphthas are ineffective as solvents for the toxic materials. Ethanol is a good solvent for the material present in cakes or oil-free meals. The dual solvent

treatment of Emmel is not necessary. His extraction time with ethanol was apparently of too short duration. Indeed, if this is not the case, his data lead to the conclusion that three toxic materials are present.

There seems to be agreement that the detoxified meals are digestible and may be used as sources of energy. The literature does not contain data on the quality of the protein in detoxified meals. Bryan implies, but certainly does not state, that his detoxified meals were the equal of soybean oil meal. Certainly our three meals were inferior to soybean oil meal. They were comparable to the cereals but no better. We feel that in the presence of adequate supplies of cereal grains the detoxication of tung meals or cakes solely for the production of a feedstuff would probably be an uneconomic process.

Conclusions

1. Commercial press-cakes and petroleum ether-extracted tung meals are toxic.
2. The toxicity of press-cake decreases markedly in common storage. The toxicity of oil-free meals does not decrease under such storage.
3. Many lipid solvents remove toxic material from tung products but cannot be used alone to produce a non-toxic meal.
4. Heat alone cannot detoxify tung meal or cake.
5. Moistening increases the effectiveness of the heat treatment.
6. Tung cake and meal may be detoxified by combining the moist heat treatment with solvent extraction.
7. Two toxic materials are present in tung nuts.
8. The biological value of tung protein is low insofar as the chick is concerned.
9. It seems improbable that the value of the detoxified meal as a feedstuff would justify the expense of detoxication.

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The Relative Rates of Destruction of Propyl Gallate and Butylated Hydroxyanisole in Oxidizing Lard[1]

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UNTIL comparatively recently there has been little information recorded in the literature on the kinetics of the destruction of phenolic antioxidants in oils and fats. This lack of data has been attributed in part, at least, to the scarcity of satisfactory analytical procedures. However with the development of improved methods a number of studies have been reported on individual antioxidants.

Filer and co-workers (2) have studied the oxidative destruction of gallic acid in commercially refined cottonseed oil, aerated at 110°C., and reported that the rate of loss of gallic acid was approximately constant and was virtually independent of the initial concentration. They concluded that the destruction of gallic acid exhibited characteristics of a zero order reaction.

Lundberg *et al.* (7) investigated the rates of destruction of four antioxidants added to lard held at 100°C. under a stream of oxygen and simultaneously followed the change in peroxide value. They studied hydroquinone, catechol, nordihydroguaiaretic acid, and gallic acid, added separately to lard, at concentrations of 0.02, 0.10, and 0.50% by weight. These workers concluded that the deterioration of these phenolic antioxidants in oxidizing lard did not occur as a single low order reaction but was complicated by the products formed from the fat and/or the antioxidants during the oxidation. They observed a positive catalytic effect upon peroxide formation during the early stages of the oxidation of lard which accompanied the use of the higher concentrations of these antioxidants.

In 1949 Kraybill and co-workers (4) reported the development of a new antioxidant preparation designated as "AMIF-72," which consisted of 20% butylated hydroxyanisole (BHA), 6% propyl gallate, and 4% anhydrous citric acid in 70% propylene glycol. This antioxidant preparation is now widely used for the stabilization of edible fats. At that time no satisfactory methods were available for the determination of mixtures of these antioxidants. However a procedure which permits the determination of combinations of propyl gallate, butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherols in lard and shortening has now been reported by Mahon and Chapman (8). These workers have also developed a method for the estimation of the 2-tert-butyl-4-hydroxyanisole (2-BHA) and 3-tert-butyl-4-hydroxyanisole (3-BHA) isomers in lard and shortening (10). Since there were no data in the literature on the destruction of propyl gallate and butylated hydroxyanisole when combined in a fat or oil, it was considered of interest to apply the foregoing procedures to a study of the relative rates of destruction of these phenolic antioxidants in oxidizing lard.

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